Seafood Processing

Adding Value Through Quick Freezing,
Retortable Packaging, and Cook-Chilling

V. Venugopal

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To My Parents

Foreword

The international seafood industry is faced with interesting complexities and many contradictions, such as the following:

Seafood is among the most internationally traded food product categories, but it is also among the most perishable and requires flawless distribution chain management.

Value addition through processing is the key to success, but often the least prepared seafood — fresh or even live from the sea — fetches the highest price.

Seafood is a valuable part of a healthy diet but consumers lack the skills and confidence to prepare it.

Health authorities advise increased fish consumption but production is constrained by sustainability issues in the capture fisheries and environmental concerns in aquaculture.

How does the seafood industry operate within these apparent contradictions and constraints? There is no simple answer, except to say that it is driven by the market pull that is created by increasingly health conscious consumers, sound technology in processing, modern logistics, and not the least by across-the-world cooperation of production and marketing people who face the everyday challenges of the business with enthusiasm and expertise.

Value addition is an important term for the modern food business. Traditionally, it has been thought of as a processing term, that is, adding value to basic foods by means of coating, combination of ingredients, processing, and convenient presentation. The driving force for value is, however, created in the market by the consumer who finds the product to have added value as per his or her circumstances. It is therefore appropriate to think of value primarily as a marketing term and in each instance based on consumer perception.

Worldwide, marketing people agree that the trends driving the demand for seafood in years to come and in the major markets are closely tied to trends in lifestyles, which seem remarkably similar in many areas of the world. Across the globe, these driving forces originate in the need and desire of the human race when it is presented with a wide choice of food and a reasonable income, and they are manifested in the quest for convenience in preparation, product quality, and the healthy image that seafood has gained in terms of nutritive value, and even special health benefits.

This book brings together a wealth of information on seafood processing and consumption. It provides an overview for the global fisheries production and consumption pattern, highlights the nutritional importance of fishery products, and

also discusses perishability and the biohazards associated with seafood as well. It gives a thorough description of processing technologies for quick freezing, cook-chilling, and retort pouch packaging, among others with a briefer look at smoking and canning.

In short, this book will greatly help to explain how the seafood industry operates quite successfully in spite of the contradictions mentioned above, but primarily it will serve as an expert source on modern processing technology for seafood.

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Preface

With changes in life styles, consumers' perception of processed food is also changing. As a consequence there is an increasing demand for convenient, nutritive, and safe foods all over the world. In addition, consumers are aware of the nutritive value of food and the effects of processing on it. These changes have caused more and more agricultural products to enter international trade in processed form rather than as raw commodities. This scenario indicates prospects for novel techniques of value addition that can add convenience, novelty, and marketability to products without causing significant losses in their nutritive value. Minimal processing techniques including nonthermal methods and techniques that are less harsh in their thermal effects are gaining importance to satisfy the needs of modern consumers.

Seafoods, which traditionally were traded in bulk consignments without much serious processing, can attract novel processing techniques. The time has arrived for the fishery sector to take advantage of the outlook of the modern consumer to capture wider markets through process-diversification techniques. The 1980s saw seafood items getting increasing media attention as a source of valuable nutrients. Greater demands for fishery products, diminishing marine landings, and depletion of certain fish stocks have resulted in rapid rise in fish production by farming techniques. However, currently most farmed items are marketed with negligible levels of value addition. As observed recently by Prof. J.M. Regenstein, Cornell University (*Food Technol.*, 58, 28, 2004), if the fishery industry is to compete with other food industries more creativity will be needed in their processing and marketing. Because of its diversity in composition, seafood offers scope for a wide range of product forms having diverse flavors. Success of the seafood industry in the coming years lies in the judicious application of value addition methodologies to develop products that are nutritive, tasty, appealing, and stable for extended storage, supported by marketing strategies. Developments in minimal processing, nonthermal methods, or methods that are less harsh allow processing of seafood without much adverse impact on their flavors and contents of essential nutrients. These methods, which rely heavily on principles of physics, chemistry, and microbiology, include irradiation, high hydrostatic pressure, antimicrobials, ultrasound, pulsed electric light, and oscillatory magnetic fields. In this book, an attempt has been made to focus the upcoming technologies for value addition of marine and aquacultured fishery products. The introductory chapter is devoted to briefly discuss the current global status of seafoods, consumption pattern, and to highlight prospects for value addition. This is followed by two chapters, one that discusses perishability and biohazards associated with the commodity, and the other discusses the bulk

handling and chilling of fishery products. The remaining part of the book discusses different process technologies for value addition. Conventional techniques such as smoking and canning have not been discussed separately; but briefly dwelt upon in relevant chapters. At the end, a chapter is devoted to highlighting the nutritional importance of fishery products including the influence of processing on its nutritive value. An appendix provides some information related to seafood processing.

Contents

1 Availability, Consumption Pattern, Trade, and Need for Value Addition

1.1 INTRODUCTION

The term "seafood" generally refers to a variety of groups of biologically divergent animals consisting not only of fish, whether of freshwater, estuarine, or marine habitats, but also of shellfish which include crustacea and mollusks. The crustacea comprises of crayfish, crab, shrimp, and lobster, while the mollusks could be bivalves such as mussel, oyster, and scallop, univalve creatures such as abalone, snail, and conch, and cephalopods which include squid, cuttlefish, and octopus. Fisheries provide a vital source of food, employment, trade, and economic well-being for people throughout the world. In the early 1970s fish was considered as a resource against hunger in the world. Presently, fish has gained importance as a health food, because several species have been identified as rich in easily digestible proteins containing all the essential amino acids, therapeutically important polyunsaturated fatty acids, in addition to calcium, iodine, vitamins, and many other nutrients. Fishery products constitute a major portion of international trade, which is a valuable source of foreign exchange to many developing countries. Fisheries sector enjoys some advantages over other animal production systems. First, fish is the cheapest source of animal protein and a health food. Second, high fecundity (up to 1 million eggs) and fast growth rate of fishes have no parallel among other animal protein sources, like livestock including poultry. These biological advantages offer considerable benefits to fish as a tool to achieve nutritional and social security. While contribution of agriculture to gross domestic product (GDP) is decreasing all over the world, that of fisheries is increasing in most countries.

1.2 AVAILABILITY OF FISHERY PRODUCTS

Fish makes a very significant contribution to the diets of many communities in both the developed and developing worlds. According to the State of World Fisheries and Aquaculture, 2002, published by the Food and Agriculture Organization (FAO) of the United Nations, more than one billion people worldwide rely on fish as an important source of animal proteins, deriving at least 20% of protein from fish.[1] This share could exceed 25% in poor countries and could be much higher in isolated

TABLE 1.1

Capture Production (in tons) by Some Major Groups of Species During the Years 2000 and 2002

Species group	2000	2002
Carps, barbels, and other cyprinids	570,965	592,962
Tilapia and other cichlids	680,066	682,639
Salmons, trouts, smelts	805,139	806,998
Flounders, halibuts, soles	1,008,471	918,840
Cods, hakes, haddocks	8,673,042	8,392,479
Miscellaneous coastal fishes	6,039,972	6,471,124
Herring, sardines, anchovies	20,628,706	22,472,563
Tunas, bonitos, billifishes	5,828,375	6,088,337
Sharks, rays, chimaeras	857,749	818,542
Freshwater crustaceans	568,469	818,993
Lobsters, spiny-rock lobsters	222,052	222,132
Shrimps, prawns	2,949,714	2,979,336
Oysters	249,647	186,699
Mussels	276,276	264,101
Scallops	660,700	747,516
Clams	798,339	825,651
Squids, cuttlefish, octopuses	3,660,404	3,173,272
Freshwater mollusks	595,286	633,561
Miscellaneous marine mollusks	1,497,351	1,491,849

Source: Adapted from FAO, *FAO Yearbook, Fishery Statistics, Capture Production*, Vol. 94/1. Food and Agriculture Organization, United Nations, Rome, Italy, 2002. With permission.

parts of coastal or inland areas in some countries. For example, the proportion of animal protein derived from marine products in the diet of the population in West Africa is as high as 63% in Ghana, 62% in Gambia, and 47% in Senegal.[2] However, in the course of the last four decades, the share of fish proteins to animal proteins has exhibited a slight negative trend due to a faster growth in consumption of other animal products.[1]

1.2.1 Capture Fisheries

The fish landings in the world has increased from 39.2 mt in 1961 to 122.1 mt in 1997 (at an annual growth rate of 3.6%), while food fish supply has grown from 27.6 mt to nearly 94 mt during the same period.[1] The landings were 130.9 mt and 132.9 mt in 2000 and 2002, respectively.[1] World fish production of finfish remained relatively stagnant at around 130 mt for the last few years.[1] Table 1.1 indicates world landings of major groups of fishery products in recent years. Two thirds of the total food fish supply is from marine and inland water fisheries.[1] The

irregular and dwindling supply of capture fish is adversely affecting seafood industries in several countries. Decline in capture fisheries is likely to have a serious impact on food security, nutrition, and income levels for people in the developing countries in the coming years. Unlike processors of other food commodities, the seafood processor is limited in his choice of raw materials to what is available with respect to species, size, and quality at a given time. In order to meet the protein requirements of the world population, which is likely to increase to 8.5 billion in the next 25 years, fish production has to double during this period. While efforts are needed to maintain sustainable fish production to satisfy the demand, growth in capture fisheries has not been promising. The FAO has estimated that 19% of the world's major fishing grounds have either reached or exceeded their natural limits and that at least nine fishing areas, about 69% of the world's fisheries, are either fully exploited, overexploited, depleted, or slowly recovering from the effects of overfishing.[1] Major reasons for overfishing are use of sophisticated techniques, which adversely affect breeding of the species and large government subsidies in this sector facilitating overexploitation and limitation of resources. Furthermore, pollution and overfishing have severely depleted fish population affecting several maritime countries. It has been estimated that approximately only 100 mt of conventional species can be fished from the ocean on a sustainable basis.[3]

There are specific examples for diminishing fish stocks. Several years ago, Canada had to close down many processing plants that depended on a few selected species such as cod and salmon.[3,4] The recent decline in salmon stocks in that country has led to conservation measures, which included selective harvesting, improvements in handling, augmentation through aquaculture, and development of value-added products.[4] Another example is that of white pomfret, a highly preferred fish in the Indian subcontinent. According to the Central Marine Fisheries Research Institute, Mumbai, India, the landing of the fish in the western coast of India has fallen alarmingly, from 19,000 t in 1983 to 4,500 t in 1995. Similarly, the landing of Bombay duck, the third largest fish resource in India after sardines and mackerel, has declined from 3,500 in 1981 to 700 t in 1993. Sand lobster has almost completely disappeared in the waters near Mumbai, India.

Introduction of mechanized bottom trawling in the late 1950s resulted in a 2.7-fold increase in the catch of demersal fish from the Indian Ocean. However, as a result of intensive trawling and introduction of gears such as purse seines, resources in the 0 to 50 m depth zone were significantly exploited. Some of the exploited species were sciaenids, silver bellies, elasmobranchs, pink perch, lizardfish, goatfish, threadfins, and eels, while resources such as catfish, the whitefish, ghol, and flatheads declined in production. This was chiefly due to overfishing, which caused destruction of juveniles as well as the trampling of the bottom habitat.[5] Another example is of tuna stocks in the Indian Ocean, which provides over 1.5 mt, or a nearly a third of world total, dominated by yellowfin and skipjack tuna. Of these, while skipjack stocks appear to be unaffected, yellowfin and big eye tuna stocks are fully exploited.[6]

Depletion of fish stocks has been felt in other parts of the world too. In the United States, most capture-fishery stocks are fully exploited, or, in the case of

TABLE 1.2
U.S. Supply of Fishery Products

| | Domestic commercial landings (1000 t) | | |
Year	Edible fishery products	Industrial fishery products	Total
1992	7,618	2,019	9,637
1993	8,214	2,253	10,467
1994	7,936	2,525	19,461
1995	7,667	2,121	9,788
1996	7,474	2,091	9,565
1998	7,173	2,021	9,194
1999	6,832	2,507	9,339
2000	6,912	2,157	9,069
2001	7,314	2,178	9,492

Source: MPEDA Newsletter, 7, 7, 2000, Marine Products Export Development Authority (India). With permission.

Atlantic cod, it is even overexploited. The availability of edible and industrial fishery products in the United States is given in Table 1.2. It can be seen that the availability of fish was almost stagnant during the last decade. Diminishing catches in countries belonging to the European Union have also resulted in heavy seafood imports. In Australia, out of a total of 67 target species, 11 species are classified as overfished. These species include southern blue fin tuna, brown tiger shrimp, grooved tiger shrimp, southern scallop, tropical rock lobster, and orange roughy among others.[7] India ranks third in fish production, after China and Japan, where production has increased more than 10 times in recent years, reaching a value of 6.39 mt during 2003, a growth arguably one of the highest in the food-production sector in the country. Nevertheless, marine fisheries may not be in a position to meet the projected demand of 10% annual increase.[8] The 2004 Tsunami disaster has dealt a severe blow to capture fisheries in India and some other Asian countries.

Growing concerns on overfishing and environmental impact of fishery activities has led to a series of international initiatives such as identification of maximum sustainable yields and introduction of quota systems for several species.[9] The United Nations Agreement on Straddling Fish Stocks, the Agreement to Promote Compliance with International Conservation and Management Measures by Fishing Vessels on the High Seas, and the Code of Conduct for Responsible Fisheries are three such measures. The Kyoto Declaration for "Sustainable Contribution of Fisheries to Food Security" set an *Action Plan* either directly or in cooperation with other states or through the FAO, which set ten major goals.[10] These include assessment and monitoring the present and future levels of fish production, enhancement of cooperation among countries for straddling fish stocks,

promotion of aquaculture, control of postharvest losses, and optimum use of unexploited or underexploited resources.[10] Since the most important environmental impact of capture fisheries is overfishing and by-catches, a need has been felt for the production methods to be able to protect the environment. Labeling in general, and es pecially environmental labeling, is increasingly becoming an important marketing tool.

An analysis by the Malaysia based World Fish Center (WFC) and the International Food Policy Research Institute cautioned that within the next 20 years, fish, which currently accounts for about 7% of global food supplies, will deprive one billion people in developing countries of their source of protein.[11] The Center fears that some fish species will disappear from markets and the quality of seafood will decline. Almost three-quarters of the 130 mt landed in 2000 came from fish stocks already depleted, overfished, or fully exploited. The situation is alarming particularly due to the annual increase of about 90 million in world population as well as increasing consumer interests in fishery products. It was observed that only appreciable growth in fish farming could save the world from a critical situation of shortage of fishery products.[11]

Although supply of several commercially important fish species is dwindling, a significant amount of the available fish remains underutilized. These fish species consist mostly of the by-catch of fishing operations of targeted species such as shrimp. In addition, several varieties of pelagic, demersal, and unconventional fish species are not fully utilized for human food. Out of a total production of 22.5 mt of demersal and 37.6 mt of pelagic fish, only 13.7 and 18.8 mt, respectively, are used for human consumption. The rest are reduced to fish meal or discarded in the ocean.[12] Many of the currently underutilized fish having potential as human food, and therefore, have been arranged roughly in the order of their possible food value. These fish include anchovy, barracuda, Bombay duck, catfish, croaker, flying fish, garfish, grey mullet, hake, herring, horse mackerel, jewfish, leatherjacket, mackerel, pony fish, ray, rock cod, sardine, scad, Spanish mackerel, spotted bat, and tilapia, among others.[13] The underutilized bottom-water species include blue ling, roundnose, grenadier, black scabbard, and various small sharks. The global trend in low-cost fish catch and need for their better utilization for human consumption have been discussed extensively.[13–19]

1.2.2 Aquaculture

The interest in aquaculture stemmed from stagnating capture fisheries, which failed to meet the rising demand for fish. Fish farming is being considered the best option to make preferred fish species available to the consumers. Fish production by this method has reached 38 mt worth US$55.7 billion in 2001.[20,21] Thus, while capture fisheries remained more or less the same from 91.6 mt in 1995 to 89.0 mt in 2000, aquaculture production increased from 24.5 to 33.3 mt during the same period. In 1999, Asia produced about 91% of the world's total cultured fish, with China, India, Japan, Republic of Korea, Philippines, Indonesia, and Thailand topping the list.[1] As many as 39 and 52 species are cultured in China and Korea, respectively.

Freshwater aquaculture is a major source of growth in world fisheries. In general, freshwater fish is cheaper and is an indispensable source of animal protein, as it is preferred among the lower income groups in the Asian countries. Among the Asian countries, China ranks first in fish farming, producing 26 mt (worth US$26 billion) of fish and shellfish in 2001. Other major producing countries in 2001 were India (2.2 mt), Indonesia (864,000 t), Japan (802,000 t), Indonesia (864,000 t), and Thailand 724,000 t).[21]

Finfish, with a share of 23 mt, ranked first in the total aquaculture output in 2000, and accounted for about 65% of the total production. The major share was carps (68%), consumed mostly in the producing countries, mainly, China and India. Because of its diminishing wild stock, Atlantic salmon (*Salmo salar)* is important among various species cultured worldwide, with a contribution 1 mt or 2.39% to the total aquaculture production.[4,20] Norway, Chile, the United Kingdom, and the United States are the major producers of farmed salmon. Consumer demand for white, easy-to-prepare fillets was the reason for rapid rise in farming of catfish and tilapia in the United States.

Shrimp farming is one of the most rapidly developing areas of the international seafood industries. Since 1990, black tiger (*Penaeus monodon*) is the main shrimp farmed. The shellfish can grow up to 13 inches, but the average harvest size is between 9 and 11 inches in length. Farmed black tiger has a mild or almost bland flavor, compared with the prominent taste of its marine counterpart. Important suppliers include Thailand, Bangladesh, India, Indonesia, and Malaysia. Thailand farmed about 300,000 t of this shellfish, out of a total world production close to 570,000 t, in 2000. There is a large variety of black tiger shrimp products in the world markets, predominated by individually quick-frozen (IQF) or block-frozen headless shrimp. The shellfish is often an ingredient in combination dishes with fish, and is often used in pasta dishes with vegetables. During the last decade, the white spot virus caused dramatic drop in production of shrimp making significant financial losses to Asian farmers.[1] Table 1.3 shows world production of some major aquacultured fishery items in 2000.

1.3 TRADE IN FISHERY PRODUCTS

Depletion of fish stocks and diminishing catch of preferred species have contributed to an imbalance in supply and demand for fishery products in several countries, which has promoted international trade in seafood. More than one third of global fisheries production moves into international markets. World exports of fish and fishery products were 5.6 mt in 1967, which grew to 24.7 mt in 1997. The present value of globally traded fishery products is approximately US$57.[21] For many developing countries in Asia, Africa, and Latin America fishery products have become an important foreign exchange earner and their market share in terms of value is just over 50%.[22]

Shrimp accounts for only about 3% by weight of internationally traded seafood, but in monetary terms, the shellfish trade is worth approximately 20%. About 80% of shrimp consumed in the United States are being met through imports.[1]

TABLE 1.3
Production of Some Major Fishery Items through Aquaculture in the Year 2000

Species	Production (1000 t)
Freshwater fish	19,801
Salmon	1,000
Catfish	280
Trout	140
Mollusks	10,732
Aquatic plants	10,130
Diadromous fishes	2,257
Crustacea	1,648
Marine fishes	1,010

Source: Adapted from *State of World Fisheries and Aquaculture*, 2002, Food and Agriculture Organization of the United Nations, Rome, Italy, With permission.

Japan, Spain, France, and the United Kingdom are other major shrimp importing countries.[22,23] The international shrimp suppliers annually serve the United States, Japan, and Europe with more than 600,000 t of the shellfish from aquaculture operations. Over the past years, increasing environmental awareness all over the world has resulted in demand for quality products and services. The farmed shrimp has received a negative image in Europe because of occasional presence of antibiotic residues and other hazardous substances. As a consequence, exporters to Europe have been required to comply with environmental legislation and regulations in order to be able to export to Europe.

Some of the other major internationally traded seafood items include Alaska pollock, cod, dogfish, haddock, hake, tuna, salmon, sea bass, sea bream, shark, tilapia, trout, cephalopods, and mollusks. During the last few years, prices of aquatic products have increased in all the countries. Developed countries accounted for more than 80% of total fish imports with Japan accounting for 26% of global total import.[1] Notwithstanding huge imports, developed countries also process substantial amounts of seafood. The United States, in addition to being the world's fourth largest exporting country, is the second largest importer, particularly of shrimp. Southern bluefin tuna, orange roughy, rock lobster, swimming crabs, squid, oysters, and scallops are the major seafood of trade in New Zealand, whereas, frozen fish and other value-added products are the important items traded in the United Kingdom.[24,25] The sea bream and sea bass industry has grown strongly in Europe, with a 120,000 t production of these species in 2001.[25] In France, where per capita fish consumption is approximately 28 kg,

700,000 to 750,000 t, worth about US$5 billion, raw, chilled, delicatessen, canned, and frozen seafood were processed in 2000.[26]

Fish production in China has reached about 30% world production, which exported seafood and aquacultured products worth US$3.7 billion in the year 2000. The aqucultured products exported consisted of eel, shrimp and other shellfish, oysters, crabs, and tilapia.[25,26] China also reprocesses imported raw material for export, creating a strong value addition in the process. The main thrust in Chinese seafood industry in recent years has been export of live fish species, individual quick-freezing and retail packaging, and downstream processing. Thailand is another major exporting country, which exported products worth US$4.4 billion in 2000. The disadvantages faced by developing countries in increasing the exports to European countries include tariffs for value-added products, and the necessity to prove their ability to deliver quality products on time and at stable prices.

Seafood industry is showing signs of some development in other countries of the world too. Cephalopods are utilized as an important food item in various countries, especially in Asia. The Japanese consumes some of the species raw. There is a huge potential for processing and marketing of cephalopod products in Asia.[27] In the Arab countries, at present the industry is limited to employing simple and traditional methods, despite developments in some fish canning and processing projects. Most Arab countries depend on fish imports, especially canned products.[28] Fisheries development issues and their impact on the livelihood of fishing communities in West Africa have been discussed recently.[2] Latin American countries have an abundant source of unexploited or underexploited aquatic resources, where there is also an urgent need to increase the consumption of aquatic protein in order to alleviate the problem of malnutrition. India is a major exporter of fishery products, worth above one billion US$ per year. The share of seafood exports in India is about 16% of the total exports of agriculture products.[29−31]

Most international trade in fishery products is limited to items as bulk frozen or chilled forms. Trade in consumer friendly, value-added products is very limited, except a few items, according to the Yearbook of Fishery Statistics, published by the FAO, Rome, Italy.[1] In the year 2002, the major traded value-added products were canned shrimp (276,282 t), imitation crabsticks (20,102 t), marinated and spiced fish (7,809 t), small amounts of fish pastes (3,201 t), fish cakes (5,590 t), and fish sausages (49 t). The Southeast Asian Fisheries Development Center has compiled data on several value-added products developed in seven ASEAN countries, which have potential for export to destinations throughout the world including the United States and the European Union.[32] Lack of sufficient raw material and concerns about the quality of processed products are some of the problems facing the current international seafood industry. Other related issues include environmental concerns regarding aquaculture, changes in quality and safety control measures particularly adoption of Hazard Analysis Critical Control Point (HACCP)-based strategy, the concepts of risk assessment, traceability in major markets, third-country processing, and eco-labeling.[1] The subcommittee on Fish Trade of the Food and Agriculture Organization, in its ninth Session

in Bremen, Germany, identified major requirements for a healthy future in global trade in fishery products. These included need for science-based safety-monitoring and eco-labelling systems for seafood products, improving the accuracy of catch reporting by the fishing sector, and measures to help developing countries and small-scale fishermen increase their access to international markets.[21]

1.4 PER CAPITA AVAILABILITY AND CONSUMPTION PATTERN

The per capita availability of fish and fishery products has nearly doubled in the last 40 years. The share of animal protein intake of human population derived from fish, crustaceans, and mollusks increased from 13.7% in 1961 to 16.1% in 1996 and then showed a decline to 15.8% in 1999.[1] The value is higher against per capita consumption of 11 kg recommended by the World Health Organization for nutritional security. The quantity of fish consumed and the composition of the species vary with respect to countries and regions. The values for various regions were as follows: Oceania (22.5 kg), Europe (19.1 kg), Asia (excluding China) (13.7 kg), China (25.1 kg), North and Central America (16.8 kg), South America (8.5 kg), and Africa (8.0 kg).[1] The reasons behind wide variations in consumption level include movement of people to urban area away from the coastal zones, disparity in income level, and religious beliefs.[29] Per capita consumption by continents and economic groupings in 1999 is given in Table 1.4. The world average per capita fish consumption is expected to rise between 19 and 21 kg by the year 2030.[33]

A survey of human fish consumption pattern showed that fresh fish (53.7%) was the most preferred item, followed by frozen (25.7%), canned (11.0%), and cured fish (9%).[1] An amount of 45 mt of marine finfish accounted for 75% of the per capita fish consumed in 1997. Shellfish (crustaceans, mollusks, and cephalopods) shared the remaining 25%. Demersal fish are highly preferred in North Europe and North America. In these countries, as much as 60% of all fish consumed is either fillet or value-added product. Cephalopods are consumed in certain Mediterranean and Asian countries, and to a much lesser extent in other continents. Crustaceans are highly priced commodities and their consumption is mostly in the affluent countries.[32]

1.4.1 Changing Consumer Trends Toward Processed Foods

Modern consumers prefer processed foods that are more convenient to handle, store, and prepare. The consumers insist that such products also possess high quality, freshness, nutrition, and health. They would also appreciate flavorsome food items produced by more ethical methods, including environmentally friendly processes and economically acceptable behavior.[27,33] The changes in consumer lifestyles have resulted in increased demands for two distinct types of seafood products. The first type includes *fresh*, *chilled* products that are conveniently

TABLE 1.4
Total Fish and Shellfish Supply and Annual Per Capita Consumption by Continents and Economic Groupings in 1999

Location	Total supply (mt, live weight)	Annual per capita consumption (kg)
World	95.5	16.1
World excluding China	64.3	13.6
Africa	6.2	8.0
North and Central America	8.1	16.8
United States	—	21.3
South America	2.9	8.5
China	31.2	25.1
Asia (excluding China)	32.5	13.7
Europe	13.9	19.1
United Kingdom	—	20.2
Germany	—	12.2
Portugal	—	57.0
Norway	—	50.0
Oceania	0.7	22.5
Industrialized countries	25.4	28.3
Economies in transition	3.7	12.7

Source: From FAO, *State of World Fisheries and Aquaculture*, Vol. 95. Food and Agriculture Organization of the United Nations, Rome, Italy, 2002. Courtesy, *Infofish*, Kuala Lumpur, Malaysia. With permission.

packaged, processed, and ready-to-cook, such as salmon steaks or hoki loin fillets. The second group consists of *processed*, *chilled*, *ready-to-eat* seafood products, such as cold smoked salmon or hot smoked mussels. In both types, a need for convenience and easy handling has been focussed. These demands can lead to development of novel techniques to extend the shelf life and add convenience to seafood.[34] The emergence and growth of supermarkets also facilitate a greater penetration of value-added seafood products such as salmon in regions that are far from the sea.[23,33]

The major reasons for changing consumer trends have been identified. The shrinking family size and more women entering the work force have resulted in less leisure time and increased purchasing power, which have made a demand for processed, convenient, ready-to-eat, or ready-to-prepare products. The second important trend is the increase in awarness of the importance of eating healthy. Consumers are becoming health conscious and are aware of the protective role of diet in the control of problems such as obesity, cancer, diabetics, and coronary heart diseases. Food items that are low in calorie, fat, sugar, and sodium are now recognized as health protecting foods. Furthermore, modern consumers

TABLE 1.5
Classification of Some Seafood According to Their Flavor

Mild	Cod, crab, flounder, grouper, haddock, hake, halibut, lobster, monkfish, orange roughy, pollock, scallop, seer fish, sole, see bass, shrimp, snapper, squid, tilapia, tilefish, white pomfret, wolffish
Moderate	Black pomfret, butterfish, catfish, cray fish, lake perch, lobster, mahi-mahi, octopus, shark, sturgeon, orange roughy, shrimp, tilapia, tuna, whitefish, whiting
Strong	Blue fish, clams, Indian salmon, mackerel, marlin, mussel, oyster, salmon, sardine, swordfish

are also aware of health hazards associated with food, such as the presence of pathogenic microorganisms, parasites, viruses, and industrial pollutants. These trends exert a considerable influence on food product development and marketing. Consumers expect a positive assurance that the food product including seafood is safe, tasty, easy, and quick to prepare, light in calories, easy to digest, and nutritive. Modern trends in seafood technology are essentially aiming to address the changing consumer interests.[35]

Generally, consumers relate freshness of fishery products to the inherent quality of the freshly caught fish. They consider that, if the time lapse after harvest is short, the product retains its original characteristics. This may not always be correct, since eating quality is a subjective attribute. The flavor of cooked cod, for example, has the strongest intrinsic characteristics after 2 days storage in melting ice. Many fatty species such as salmon, ocean perch, and halibut, improve much in flavor, taste, and texture during the first 2 to 4 days in ice. This is mainly due to the redistribution of fat, and the development of flavor components such as amino acids, nucleotides, or sugars, produced by the autolytic processes occurring during rigor mortis.[36] Butterfish, cod, crab, flounder, haddock, hake, lake perch, mussel, oyster, pollock, scallop, sole, whitefish, and whiting have delicate texture. Bluefish, crayfish, lobster, mackerel, orange roughy, salmon, sardine, shrimp, and tilapia are species having moderate texture. Clams, catfish, grouper, halibut, mahi-mahi, marlin, monkfish, octopus, salmon, see bass, seer, shark, snapper, squid, swordfish, tilefish, tuna, and wolffish are characterized by hard texture. Table 1.5 present tentative classifications of some seafood according to their flavor. In affluent countries, a significant amount of fish is consumed outside the home in the form of ready-to-eat products. Vast majority of the population in these countries has the means to purchase adequate food. Retailing of fish in these countries is not merely a question of satisfying a hungry consumer at a competitive price. Marketing campaigns launched for some fish products tend to affirm that consumption of fish is an appropriate means of satisfying the consumer's need for variety as well as for nutritious, tasty, healthy, and fashionable foods.

Shrimp being the major traded seafood, a detailed survey on the quality attributes of the shellfish that influence consumers has been conducted.[37] Quality

attributes that influence consumers in buying the product are freshness, color, size, texture, taste, and other aesthetic and eating characteristics. Live prawns are the most preferred items, followed by chilled or cooked samples. Headless peeled prawn with or without tail is a highly preferred product form, followed by headless breaded form. Value-added breaded shrimp products fetch premium price. Aquacultured black tiger shrimp is the most preferred species, followed by sea-caught banana prawn. Consumers in Europe, particularly France, for example, favor shrimp products that are preprocessed and ready for consumption such as peeled shrimp, marinated shell-on or shell-off shrimp, shrimp on skewer, etc. The perceived value of a product can increase due to attractive packaging in bright colors and large windows.[23] The consumer opinion could be a road map for processors to develop products that can command high marketability.[35] In marketing, one of the trends predicted for the new millennium is the elimination of the seafood source counter in the grocery store. Self-service counters can help offer more opportunity for branding, packaging, and consumer education.[38]

A number of recent surveys by professional bodies have indicated the trends in seafood consumption in the United States.[38–41] According to the U.S. National Oceanic and Atmospheric Administration, overall seafood consumption in 2002 was 7.1% of total food consumption, with an annual per head purchase of 5 kg fish, consisting mostly of fresh and frozen items. A survey by the National Fisheries Institute showed that elderly people preferred seafood to red meat, since these people were aware of the nutritive merits of seafood. People in the age group of 50–64 are 71% more likely to eat fish; while those above the age of 65 ate 41 times a year. It was observed that the per capita consumption of fish might reach about 27–31 kg in the next 15 years in the United States.[40] Consumers preferences for value-added seafood products were shown in another survey. Grilled seafoods were more popular, particularly, preseasoned, ready-to-grill items, while boil-in-bag products were preferred less. Fried products attracted poor support, while boneless fillets were highly popular. Traditional battered and breaded items, which once formed 70–80% of the products consumed, decreased to a consumption level of 50%. Minimally processed products like salmon portions made up the rest.[38] Consumers liked an increase in sturdiness of the seafood packages with inclusion of recipes on the label. Zip-lock and vacuum-sealed packaging as well as see-through packaging were preferred.

Convenience is the driving force behind daily food choices in the United States and Japan.[44,45] Majority of food shoppers (69%) preferred *heat-and-eat*, *packaged for on-the-go* eating, even if their prices were 2 to 3 times more than their unprocessed counterparts.[42] Some of the criteria in food selection included shelf stability, minimal packaging, not soiling the hands, single-serve, bite-size, resealable, portion control, portable but safe at room temperature, and requirement of no extra utensils among others. The top three seafood items presently consumed in United States are shrimp, tuna, and salmon, followed by lobster, pollock, catfish, crab, clams, and tilapia.[40] Buying salmon was mainly due to recognized health benefits associated with consumption of the fish; taste and flavor were secondary in this respect. Trout is another preferred species, fresh fish commanding increased

TABLE 1.6
Preference of Seafood Among Consumers in the United States

Species	Mean rating (Maximum rating 7)
Lobster	6.25
Shrimp	5.85
Crab	5.46
Clams	4.82
Swordfish	4.71
Salmon	4.32
Cod	4.30
Sole	4.25
Flounder	4.15
Trout	4.09
Tuna	4.02
Haddock	3.96
Oyster	3.95
Mussel	3.91
Catfish	3.50
Shark	3.39
Snapper	3.36
Herring	3.15
Eel	2.13
Anchovy	1.48

Source: Reprinted from Dholakia, N. and Jain, K., *Infofish Int.*, 2, 21, 1992. With permission from *Infofish*.

acceptance than frozen samples.[43] There was an overall downward trend in the U.S. per capita seafood consumption from the mid-1980s due to reasons such as insufficient supply, lack of convenient products, high prices, lack of perceived value, and lack of general promotion campaigns. However, it has been predicted that seafood consumption could equalize muscle food in the next 30 years.[44] Seafood will be eaten as mince, *surimi*, soup, flavoring, and specialty, red-meat poultry seafood. Recently ready-to-eat shrimp in six different flavors have appeared in market.[44] Table 1.6 indicates preference of seafood among consumers and Table 1.7 gives per capita fish consumption in terms of species in the United States.

During 1965 to 1998, demand for fish in Japan paralleled the increase in average income. While elderly people favored *sashimi* or *sushi* products, younger generation preferred cooked or grilled steaks and fillets of tuna. Quantities of fish consumed in restaurants as ready-to-eat products also increased substantially in

TABLE 1.7
Per Capita Fish Consumption in 2003 by
Species in the United States

Species	Per capita consumption (pounds)
Shrimp	4.00
Tuna (canned)	3.40
Salmon	2.22
Pollock	1.70
Catfish	1.13
Cod	0.64
Tilapia	0.54
Clam	0.53
Scallop	0.34

Source: Adapted from National Fisheries Institute, Washington, D.C., USA.

the country.[45] Consumption of fish and seafood in Europe is predicted to increase in all major European markets. This is attributed to a number of factors including consumers' attitudes and lifestyles, recent scare over meat safety and increased "add-value" opportunities for fish and fish products. It was shown that the main species presently consumed in Europe are mussel and cod followed by tuna, herring, cephalopods, sardines, salmon, shrimp, and trout.[36] Recently, the European Union has backed a collaborative seafood project, entitled "SEAFOODplus" to investigate the benefits of seafood for the consumer as well as related issues in aquaculture, the environment, and the economy.[46] Total fish production and consumption profiles in the 13 new countries joining the European Union are also available.[22]

1.5   NECESSITY AND IMPORTANCE OF VALUE ADDITION OF FISHERY PRODUCTS

Value is among the most important modern marketing concepts. Value is most often seen as a combination of quality, service (convenience), and price, which is often called the customer value triad.[47] In the case of food, the most basic benefits of value addition include the functional and emotional benefits related to quality and nutrition, convenience in preparation, and sensory appeal at a reasonable cost. Value addition in seafood is regarded as any activity along the supply chain that increases the usability, culinary attributes, or economic viability of a fish or shellfish item. The primary activities in value addition of fishery products include procurement of the material, processing (primary and further

processing), transportation, and marketing. The secondary activities include procurement of ingredients, technology development, human resource, and company management.[47]

The changing market demands are generally favoring attempts to add value to basic agricultural commodities. According to a press release by the World Trade Organization,[48] pattern in agricultural trade during 1985 to 2003 has shifted away from commodities to processed foods. The reasons are changes in consumer attitudes emphasizing convenience in the handling of food products. In view of these changing trends, there is a need for diversification of conventional seafood-processing techniques to prepare consumer friendly products from shellfish, cephalopods, and finfish. In addition, emphasis on good nutrition is another factor that can work in favor of fish. Possibilities of preparation of several such value-added consumable product from fish and shellfish have been recently pointed out.[27,49–51]

Market studies describing trends, methodology, and results of innovative work in the seafood industry, with particular emphasis on Western markets, have been compiled in a recent publication. The study, which examined philosophy, processes, and marketing strategies of three major areas of value addition, namely, fillets, *surimi* seafood, and aquacultured products, also included 20 worldwide case studies on popular products. It was concluded that the reasons that will drive the demand for seafood in the coming years will be closely related to trends in lifestyles coupled with demand for products having convenience in preparation, quality, and health benefits.[47] It was recently pointed out that if the fishery industry has to compete with other center-of-the-plate foods and gain the environmental benefits more creativity in the use of seafood would be needed.[50]

Several factors are critical in the development of new food products. These include involvement of the consumer early in the process, real-life study of consumer liking for the food product, multidisciplinary cross-functional teams, cost reduction, and support of the management. The best recipe for success has been designated as consistency in quality, reliability in supply, consumer understanding, and constant improvement and innovation in all aspects of the business.[47] According to a recent report, a grilled hake fillet, squid ring salad, or a lobster tail with rosemary sauce is not to be "consumed" but to be "enjoyed."[52] With respect to appearance, the flesh can be red like a tuna loin, orange like a slice of salmon, white as sole fillet, or almost transparent. Seafood, fresh or frozen, whole or filleted, crude or cooked has its own typical flavor, more or less marine, which changes with cooking. The sensory properties can be enriched or minimized according to individual taste with a series of condiments. Improvement of the sensuality of the product to suite the consumer's choice represents value addition, which in turn, helps per capita seafood consumption. Table 1.8 shows classification of new products according to their degree of value addition.

Novel methods of value addition can be a boost to the rising aquaculture industry. Shrimp from aquaculture can be a major raw material for the purpose, because of its regular availability in adequate quantity. Further, the shellfish

TABLE 1.8
Classification of New Products

- Classically innovative products. These involve high cost and risk to the company, but can be highly beneficial, if successful
- Product line extensions. These new products supplement the market through variations in package, recipes, etc.
- Improved and revised existing products. New products that provide improved performance and greater perceived value
- Repositioned products. These are existing products that are targeted for new markets or market segments
- Cost-reduced products. New products that provide similar performance at lower cost than the original product

Source: Adapted from Moller, A.B. Studies on Seafood Value Addition, Fishery Industry Division, FAO/GLOBEFISH, Special Market Study, Food and Agriculture Organization, Rome, Italy, 2003, p. 93. With permission.

exhibits significant amenability for value addition and packaging in retail pouches or window-packs.[49] The value-added fishery products may be lightly salted, smoked, acidified, irradiated, high-pressure treated or heated, which can be packaged in a modified atmosphere or "sous vide."[51,53,54] Developments in technology can be highly beneficial in these efforts. For example, the traditional canning process, which is dependent on expensive metallic cans and large storage space, can be adapted to retort pouch packaging of ready-to-eat items. There is also scope to make use of the experience of chefs in these ventures. A fusion of culinary/chef skills and technical/scientific skills has shown initial success in development of attractive products from West Coast albacore tuna.[55] The modern seafood processors are generally willing to changing market requirements. In recent years, the processors have committed themselves to HACCP-based production, planning, processing control, and packing and labeling requirements.[49] Some of the value-added products currently traded commercially are summarized in Table 1.9. The traded product forms from bivalves are also given in Table 1.10.

Value addition of fishery products can benefit particularly developing countries. Some of the products that are currently produced for trade in ASEAN countries are shown in Table 1.11. There are immense possibilities for these countries to tap a growing European market for value-added fish and seafood products, particularly in view of their low-cost manpower and availability of raw material.[22,25,56,57] Nevertheless, the industry, in developing countries needs to demonstrate their capability to deliver quality products continuously, on time and at stable prices. A Concept Paper, "Fish for All" prepared by the WFC presented an overview of the characteristics and trends of the global fishery industry over the next two decades with respect to liberalization of trade and globalization of markets.[11] The study cautioned growing wises and controversies

TABLE 1.9
Currently Available Value-Added Products from Major Seafood Groups

Crustacea	Mollusk	Finfish
Live	*Mussel*: live, fresh: half shell, shucked meat	Live
Fresh/frozen: (IQF/block frozen)	Frozen: whole, half shell, shucked meat, cooked/uncooked	Fresh: whole/dressed
Peeled tail-on shrimp	Value added: smoked, canned meat/soup, breaded fritters, entries	Frozen: Bulk/IQF
Peeled tail on stretched (Nobashi)	*Squid*: peeled, double skinned, frozen blocks or IQF, head-on in boil-in-bag, breaded, all vacuum skin tray-packed.	Fish fillets, vacuum packed, canned
Butterfly tail-on shrimp		Composite fillets from mince
Head-on, tail-on, shrimp	*Cuttlefish*: fillets, frozen, boil-in-bag	Raw steaks
Accelerated freeze dried	*Scallop*: live, fresh, IQF, block-frozen with or without roe, shucked meat in sauce, smoked, breaded, battered	Cooked tuna light meat
Breaded butterfly shrimp		Fish burger
Breaded round shrimp		Seafood stew
Shrimp delights	*Clam*: live, IQF, with shell/half shell, steamed meat, chopped or minced, stuffed, breaded, fried, juice, chowders, cakes etc.	Seafood salad
Cooked head-on shell-on shrimp		Grilled steaks
Cooked, peeled, deveined tail-on shrimp		Cooked light meat
Cooked peeled deveined tail-off	*Oyster*: live, fresh: shell on, half shell, shucked meat, cooked or uncooked, smoked, canned, soups, breaded, fritters	
Shrimp rings		
Shrimp-based meals		
Breaded baby clams		
Cooked lobster		

are plaguing the seafood industry with increase in global fish consumption. While the world's oceans are being overfished, fish farming is leading to pollution of waters. The WFC studies, however, highlighted a necessity of value addition because of increasing consumer demand and rising markets and for better food security and value of the catch. The other advantages are total utilization of the catch including by-catch and nonconventional species, diversification of traditional technologies, better marketability, and by-product recovery.[58–61] In conclusion, development of value-added fishery is an exciting and rewarding task for the seafood industry. Innovations in technologies and improvised policies in both developed and developing countries can help create a thriving and sustainable fish industry.

TABLE 1.10
Main Product Forms of Bivalves in International Trade

Mussel
    Live   Shell on
    Fresh   Half shell, shucked meat
    Frozen   Whole, half shell, shucked meat, cooked and uncooked meat
    Value added Smoked, canned, soup and stew, breaded, fritters, entrees
Oysters
    Live   Shell on
    Fresh   Half shell, shucked meat
    Frozen   Whole, half shell, shucked meat, cooked and uncooked
    Value added Smoked, canned meat, soups, stews, breaded, fritters,
                   entrees
Scallop
    Live   Rinsed, shell on
    Fresh   Meat
    Frozen   IQF, block frozen without shells, with or without roe
    Value added Smoked meat, breaded and battered (frozen), shucked meat
                   in sauce
Clams
    Live   Rinsed, shell on
    Fresh   Meat, whole steamed
    Frozen   IQF half shell, whole with shell meat, raw and steamed
    Value added Canned meat, chopped or minced, stuffed, breaded, fried,
                   prefried strips, clam juice, chowders, biscuits, cakes

TABLE 1.11
Major Fish and Shellfish Product Forms Traded by ASEAN Countries

Canned products   Anchovy, baby clam, crab meat, fish (mackerel) in tomato
                        sauce, milk fish in oil, milk fish in tomato sauce, sardine in
                        tomato sauce, canned shrimp, squid, cuttlefish, octopus,
                        canned tuna in oil
Comminuted products Comminuted breaded fish finger, breaded squid ring, cuttlefish
                        ball, comminuted cuttlefish sausage, comminuted fish noodle,
                        fish ball, fish cake, minced fish, prawn burger
Dried products   Several fish and shellfish, dried under varied conditions
Fermented products   Fermented sauce, muscle, pickled prawn, fish paste, shrimp
                        paste
Frozen products   Several products including IQF fish fillets, eel, shrimp,
                        cuttlefish, squid ring

Source: Adapted from Chng, N.M., Hoon, C.G., and Kwang, L.H., *Southeast Asian Fish Products*, 3rd ed. Southeast Asian Fisheries Development Center, Singapore, 1996. With permission.

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2 Postharvest Quality Changes and Safety Hazards

2.1 INTRODUCTION

Freshness and *quality* are perceived differently by consumers, processors, regulatory officials, and scientists. This is particularly so with respect to seafood, the freshness and quality of which are subject to wide interpretations. Maintenance of the quality of both wild and aquacultured fish is more difficult than in the case of other muscle foods. Unlike all other major food supplies, the production of seafood cannot be directly controlled, enhanced, or accurately predicted. There is an unusual diversity in the seafood industry depending on the types of harvest, fishing techniques, types of products, production volumes, and location. In addition, the inherent nature of seafood makes them more susceptible to food-borne hazards.[1,2]

The quality of fishery products is influenced by both intrinsic and extrinsic factors. Species, size, sex, composition, spawning, the presence of parasites, toxins, contamination with pollutants, and cultivation conditions are the factors responsible for changes in intrinsic quality.[3,4] The biochemical characteristics of fish muscle such as low collagen, comparatively higher contents of unsaturated lipids as well as soluble nitrogen compounds influence autolysis, rapid microbial proliferation, and spoilage. Fatty fish such as sardines and herrings deteriorate more quickly than lean fish. Small fish that have been feeding heavily prior to being caught may undergo tissue softening and break easily after death due to autolysis.[4] Larger fish have higher marketability and value because of the higher yield of edible material and longer shelf life.

The extrinsic factors influencing the quality of harvested fish are: the location of catch, season, methods of catch (gill net, handline, longline, or trap, etc.), on-board handling, hygienic conditions on the fishing vessel, processing, and storage conditions[5] (see Chapter 3). Developing high quality seafood products begins with the consideration of the condition of the animal in water, the impact of environmental stresses, nutritional deficiencies, or seasonal changes on the intrinsic quality and the effect of the method of capture on the natural state.[6]
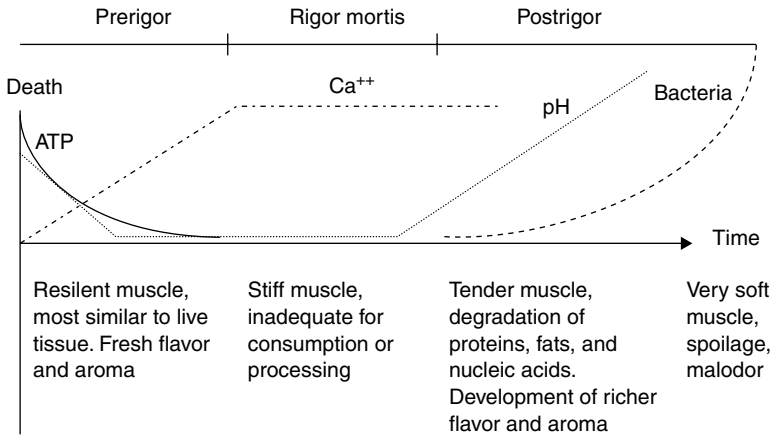
FIGURE 2.1   Schematic representation of the postmortem events occurring in fish muscle (Reprinted from Martinez et al., Outlook on Agriculture 26, 197, 1997, with permission)

2.2 SPOILAGE OF FRESH FISH

2.2.1 Postmortem Changes

Live fish muscle is relaxed and elastic. Immediately after death rigor mortis sets in, then the whole body becomes inflexible and rigid. The onset of rigor depends upon the temperature of the fish, particularly on the difference of temperatures between that of water and storage. The greater the difference, the shorter the time from death to rigor and vice versa. Aerobic respiration ceases and anaerobic oxidation of glycogen leads to accumulation of lactic acid, resulting in a drop in the muscle pH from about 6.8–6.5. Most teleost fish and crustaceans, however, have a lower carbohydrate content, whereas its content is higher in bivalve and molluskan shellfish. The final pH depends upon the species and composition of the animal. During rigor, the loss of adenosine triphosphate (ATP) due to autolytic degradation (about 1 μM/g tissue) results in a stiffening of the muscle as a result of the irreversible association of myosin and actin molecules. Slime is formed in certain cells of fish skin and the process becomes very active just after death. This is particularly so in most freshwater fish, which secrete slime to the extent of 2–3% of the fish mass that creates problems in processing. Slime contains large amounts of nitrogenous compounds and these provide good nourishment for microorganisms contaminating the fish from the environments. Resolution of the rigor is a slow process essentially due to the low pH-favored hydrolysis of actomyosin by acid proteases such as cathepsins that are present in the muscle.[4] Figure 2.1 depicts a schematic representation of the postmortem events that occurs in fish muscle.

Rigor mortis of fish has technological significance since the process influences the quality of fillets. Ideally, fish should be filleted postrigor. Fillets prepared in rigor will be stiff with poor yields. If the fillets are removed from the bone
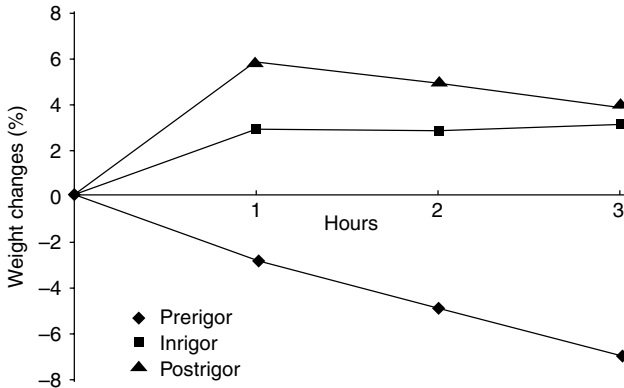
FIGURE 2.2 Average weight changes of pre-, in-, and postrigor cod fillets during brining for 1, 2, and 3 h (Reprinted from Elvevoll, E.O. et al., *Meat Sci.*, 43, S265, 1996. With permission from Elsevier)

prerigor, the muscle can contract freely and the fillets will shorten following the onset of rigor, and this phenomenon is called gaping. Further, the behavior of the fillets during processing also differs depending upon whether they are collected from prerigor or postrigor fish.[4,7] The influence of rigor on the salt absorption behavior of salmon and cod fillets have been reported. It has been shown that when the postrigor fish, was immersed in saturated brine, the fillets absorbed 3% salt showing a 6.5% increase in weight within a period of 1 h. On the other hand, the prerigor fillets lost 7% in weight in 3 h and absorbed the same amount of salt.[8] The texture may also become firm and dry if stressed fish is processed before rigor mortis.[4] Figure 2.2 depicts the influence of brining on pre-, in-, and postrigor conditions cod fillets. Fillets also undergo changes in length depending upon rigor state and storage temperature. Figure 2.3 shows reduction in the length of prerigor salmon fillets during storage at 0, 10, and 20°C. Maximum contraction of salmon fillets was attained after 12 h of storage at 20°C, while at 0 and 10°C a maximum contraction was attained after about 40 h of storage. The contracted fillets never regained its original length during different handling conditions. Furthermore, the shortened fillets were less shiny and hence had a poor appearance as compared with the postrigor filleted product.[8]

The biochemical changes during fish spoilage and the role of intrinsic and extrinsic factors on the phenomenon have been reviewed by several scientists.[4,9–16] Immediately after death, the initial biochemical quality of the muscle is prone to rapid changes due to cessation of respiration, breakdown of cellular ATP, autolytic action of proteolytic enzymes on the muscle, oxidation of lipids, and the metabolic activities of microorganisms. The ATP is sequentially degraded to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), and inosine (HxR) by autolytic enzymes as shown below:

$$ATP \rightarrow ADP \rightarrow AMP \rightarrow IMP \rightarrow HxR \rightarrow Hx \rightarrow X \rightarrow U$$
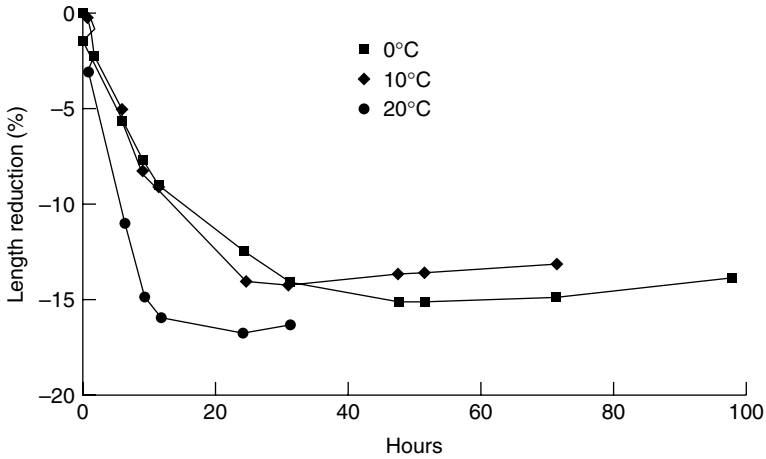
FIGURE 2.3   Reduction in the length of single fillets of Atlantic salmon stored at different temperatures as a function of time (Reprinted from Elvevoll, E.O. et al., *Meat Sci.*, 43, S265, 1996. With permission from Elsevier)

The first four steps of the reaction sequence proceed at a relatively faster rate in fish and shellfish. Oxidation of HxR to hypoxanthine (Hx) is much slower and is the result of microbial enzyme activity. A strong correlation has been observed between nucleotide catabolism and the loss of freshness of fish.[11,17] It was proposed that the freshness of fish could be determined in terms of "*K*-value," by estimating the contents of autolytic degradation products of ATP,[18] as given below:

$$K\text{-value} = \frac{[\text{HxR}] + [\text{Hx}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}] + [\text{IMP}] + [\text{HxR}] + [\text{Hx}]}$$

where [ATP], [ADP], [AMP], [IMP], [HxR], and [Hx] represent the relative concentrations of ATP, ADP, AMP, IMP, HxR, and Hx, respectively, at any given time during the storage of the fish. As the *K*-value increases, the freshness of the fish decreases. The enzymatic reactions involved in the oxidation of HxR to Hx to xanthine (X) and finally to uric acid (U) are:

Inosine + Pi → Hypoxanthine + ribose − Pi (Enzyme:nucleotide phosphorylase)

Hypoxanthine + oxygen → Xanthine + hydrogen peroxide (Xanthine oxidase)

Xanthine + oxygen → uric acid + hydrogen peroxide (Xanthine oxidase)
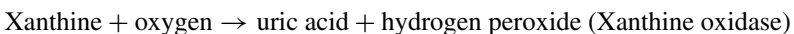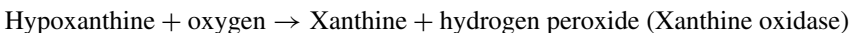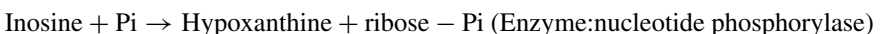
Inosine monophosphate is known to contribute to fresh fish flavor, and its loss due to breakdown by bacterial nucleoside phosphorylase causes loss of flavor of fish flesh. Since the concentrations of ATP, ADP, and AMP significantly change within

the first day of death, a simplified K_i-value has been employed, as:

$$K_i\text{-value} = \frac{[\text{HxR}] + [\text{Hx}]}{[\text{IMP}] + [\text{HxR}] + [\text{Hx}]}$$

The K_i-value has been found suitable for several fish species, although it has limitations for species such as cod.[7] Instead of K-value, a modified K_p-value, as a ratio of concentration of Hx to adenine has been found suitable for the evaluation of the freshness of certain shellfish. The K-value of threadfin bream stored in ice increased from 9 to 40% after storage for 12 days.[19]

2.2.2 Action of Muscle Proteases

Fish muscle contains several proteases including cathepsins, trypsin, chymotrypsin, and peptidases that also act on the muscle during postmortem storage.[7,19,20] The changes in the fish muscle as a result of these reactions may provide favorable conditions for bacterial proliferation. However, the reactions *per se* do not cause significant spoilage of the muscle.[21] The autolytic reactions nevertheless can support invasion of the muscle by organisms present in the intestines.[22] The contribution of autolysis and microbial reactions in change of nonprotein-nitrogen compounds of Antarctic krill stored at 3 and 20°C has been studied.[3] It was found that nonprotein-nitrogen formation as an indicator of autolytic reactions varied with respect to the harvesting season of the fish, suggesting the role of microorganisms in spoilage. Rough handling can damage cellular structures that result in the release of autolytic enzymes including proteases, that result in the enhancement of spoilage. One of the most adverse effects of autolytic proteolysis is the belly bursting of pelagic species such as herring and capelin.[11] The spoilage of cephalopods is dominated more by autolysis. Presence of a cathepsin D like proteinase of lysosomal origin can play a vital role in the degradation of squid mantle muscle. The result is an increase in the level of muscle-derived nitrogen, favoring proliferation of degenerative microflora and hence a shorter shelf life.[23]

2.2.3 Lipid Oxidation

Lipid oxidation is associated with early postmortem changes in the fish tissue. The process is initiated by removal of a proton from the central carbon of the unsaturated fatty acid, usually a pentadiene moiety of the fatty acid, and formation of a lipid radical. The latter reacts quickly with atmospheric oxygen making a peroxy-radical (LOO$^{\cdot}$). The chain reaction involving the peroxy-radical results in the formation of hydroperoxides that are readily broken down and catalyzed by heavy metal ions to secondary products. The reactions are favored by activation of haemoproteins and increase in free iron, while antioxidants negatively influence the oxidation.[24] Lipid oxidation is comparatively more during frozen storage than during chilling (0 to 2°C) storage, and can be both non-enzymatic as well as enzymatic. Enzymes such as lipoxygenase, peroxidase,

and microsomal enzymes from animal tissues can also potentially initiate lipid peroxidation producing hydroperoxides. The breakdown of hydroperoxides into aldehydes, ketones, and alcohols causes development of off-flavors. Fish lipids, rich in n-3 polyunsaturated fatty acids, are very susceptible to oxidation, giving rise to n-3 aldehydes that cause distinctive oxidative off-flavors. These compounds include *cis*-4-heptenal, *trans*-2-heptenal, *trans*-2-*cis*-4-heptadienal, etc. and also 1,5-octadien-3-ol, 1-octen-3-ol, and hexanal.[24–27]

Lipoxygenases are concentrated in the skin tissue and remain active for upto 48 h of chilled storage. Lipoxygenase-dependent oxidative activity has been detected during chilled storage of fatty fish species such as sardine (*Sardina pilchardus*) and herring (*Clupea harengus*).[24,25,27] The pro-oxidative activity due to haem proteins continued longer than that due to lipoxygenase. The most abundant degradation products of the hydroperoxides formed from arachidonic and docosahexaenoic acids are 12- and 16-hydroxy acids.[24,25] The formation of fluorescent compounds resulting from the interaction between lipid oxidation products and biological amino constituents has been noticed during chilled storage.[27] The extent of lipid oxidation can be reduced by glutathione peroxidase, which reduces unstable lipid hydroperoxides to nonradical, stable products, which are inactive in the oxidative chain propagating mechanism. Other enzymes useful in this respect are superoxide dismutase and catalase, which remove superoxides from the peroxidation mechanism.[24] Fish lipids are also prone to hydrolysis by lipases with the formation of free fatty acids. Lipid hydrolysis is more in ungutted than in gutted fish, probably due to the involvement of lipases present in the digestive enzymes. Cellular phospholipases are known to hydrolyze the lipids, particularly, phospholipids that leads to increased oxidation of the hydrolyzed lipids.[7]

2.2.4 Microbial Spoilage

It has been estimated that about one-third of the world's food production is lost annually due to microbial spoilage.[28] Microbial activity is responsible for spoilage of most fresh and several lightly preserved seafood. Microorganisms associated with fishery products generally reflect the microbial population in their aquatic environment.[11,29,30] The fish muscle is sterile at the time of catch, but becomes quickly contaminated by surface as well as intestinal bacteria, and bacteria from water, equipment, and humans during handling and processing. Microorganisms are found on the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The microflora of fish from temperate waters is dominated by psychrotrophic, aerobic or facultative anaerobic Gram negative, rod-shaped bacteria and in particular *Psuedomonas*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrio*, *Photobacterium*, and *Aeromonas* spp. The Gram positive organisms that are isolated from fish include *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus*, *Coryneforms*, and *Brochothrix*.[7,30] In polluted waters, high numbers of Enterobacteriaceae may be found. During chilled storage, there is a shift in bacterial types, with psychrotrophic *Pseudomonas* and *Shewanella* spp. dominating after 1 to 2 weeks of storage. At higher storage temperatures, for example,

20°C, the microflora, which will ultimately grow on the products, are mesophilic ones including *Bacillus* and *Micrococcus* spp.[30]

Although the level of bacterial contamination influences fish spoilage, all the contaminant organisms are not equally responsible in the spoilage process. Therefore, total viable count can be a poor indicator of both the quality and the remaining shelf life of chilled fish. Instead, a count of organisms producing hydrogen sulfide (H_2S) has been found to be a better indicator of fish quality.[31] Although most of the data on microbial spoilage that have been collected are on fish of marine origin, the spoilage pattern of both marine and freshwater fish species are comparable.[32] However, depending upon the nature of flora, variations in spoilage rates are possible. The spoilage potential of individual bacterial isolates from fish have been determined by assessing the extent of spoilage they cause during growth in sterile fish muscle.[7,33,34] These studies showed that bacterial spoilage of fish was essentially caused by organisms belonging to *Pseudomonas* and *Alteromonas* spp., *Aeromonas*, and *Proteus* spp. were found to cause proteolysis in shrimp.[35] The role of *Aeromonas hydrophila* in the spoilage of tropical fish has been recognized.[33,36] Some of the other major microbial spoilers include *Pseudomonas fluorescens*, *P. perolens*, and *Alteromonas putrefaciens*.[37]

Bacteria initially grow at the expense of soluble, low molecular weight compounds present in the fish muscle and slime that serve as nutrients. Organisms belonging to *Pseudomonas* spp. grow more rapidly in the protein-free fraction of fish press juice containing soluble components than in the protein fraction devoid of soluble compounds.[38] Initially bacterial growth is on the surface, and when slime builds up, conditions become more favorable for the growth of anaerobes. With the exhaustion of nutrients, protein catabolism becomes essential for the survival of microflora. Therefore, at terminal spoilage, only those organisms that secrete proteases can survive in the fish muscle, which leads to a predominance of proteolytic bacteria in spoiling protein foods including fish.[39] The role of extracellular bacterial proteases in fish spoilage is well understood.[40]

The bacterial spoilage reactions in chilled fish have been elucidated.[29] The contaminant organisms initially utilize lactic acid and nonprotein-nitrogen compounds, particularly trimethylamine-oxide (TMAO) with the liberation of trimethylamine (TMA), dimethylamine (DMA), ammonia, and other volatile compounds. During 4 to 7 days of storage of fish in ice, a sharp reduction in the content of free amino acids can be noticed without any significant extent of proteolysis. Thereafter, the bacterial counts increase to above 10^8 colony forming units (cfu) per cm^2 skin surface or per g tissue, causing breakdown of proteins accompanied by increase in amino acids and volatile sulfur compounds, such as mercaptans and H_2S. This stage is also marked by adverse changes in the organoleptic quality of the fish. The number of H_2S producing bacteria could, thus, be taken as a general index of the extent of proteolysis and hence, spoilage.

Trimethylamine-oxide is a natural nontoxic compound, generally associated with the osmoregulatory function of marine fish. After the death of the fish some of the bacterial species such as *Alteromonas*, *Proteus*, *Photobacterium*, *Vibrio*, and *S. putrefaciens*, and also intestinal bacteria of the Enterobacteriaceae present

are able to carry out anaerobic respiration by using TMAO as an electron acceptor. The bacterial enzyme TMAO-reductase reduces TMAO to TMA. Formation of TMA depends primarily on the content of TMAO in the fish. Most marine animals contain TMAO in appreciable quantities, with elasmobranchs and deep-sea fish species containing higher levels. Many freshwater fish do not contain TMAO but some species like the Nile perch and tilapia contain TMAO. Therefore, apprehensions have been raised on the use of TMAO as a reliable indicator for marine origin.[41] Bacterial reduction of TMAO results in accumulation of about 10 to 25 mg TMA per 100 g muscle of spoiling fish, contributing to the characteristic spoiled fish odor. TMA, in addition to ammonia and other volatile amines, is a major component of the total volatile basic nitrogen (TVBN) compounds in the spoiled fish. TMA production in many fish species is also paralleled by bacterial production of Hx. Many spoilage-causing bacteria such as *Shewanella putrefaciens* and *Vibrio* spp. also produce off-smelling volatile sulfur compounds such as H_2S methyl mercaptan, and dimethylsulfide, from sulfur containing amino acids.

Shewanella putrefaciens and *Psuedomonas* spp. have been recognized as the *specific spoilage organisms* (SSOs) in iced fresh fish such as cod.[30,38] During the storage of seafood at particular conditions of temperature, atmospheres, salt, a_w, preservatives, etc. these SSOs grow faster than the remaining microflora, and eventually produce the metabolites responsible for off-flavors and sensory product rejection. Modified atmosphere-stored marine fish from temperate waters are spoiled by the CO_2-resistant *Photobacterium phosphoreum*. The bacterium has also been identified as the main spoiler in vacuum-packed cod and ice stored squid.[42,43] Fish products with high salt contents may spoil due to the growth of halophilic bacteria, anaerobic bacteria, and yeast, whereas in lightly salted fish, spoilage could be due to lactic acid bacteria and certain Enterobacteriacea.[7,30] Consequently, the numbers of SSOs and the concentration of their metabolites can be used as objective quality indices for shelf life determination in seafood. With SSOs responsible for spoilage, a close relationship between log-numbers of SSOs and the remaining shelf life has been suggested.[7,38,42] This also helps to predict shelf life of seafood based on knowledge about initial numbers and growth of SSOs. The number of four different SSOs, that is, *S. putrefaciens*, *P. phosphoreum*, *Brochothrix thermosphacta* and lactic acid bacteria have been shown to correlate significantly with the remaining shelf life of the product.[7,30] Table 2.2 shows examples of SSOs responsible for the spoilage of some seafood.

2.2.5 Flavor Changes

The flavor of fish significantly influences its consumer acceptability. Fresh marine fish are nearly odorless, because they contain only a small quantity of volatiles. Immediately after harvest the product is considered to retain its original characteristics. However, the lower quantity of volatiles need not be related with freshness of fish as perceived by the consumer, since eating quality is a subjective experience. In fact, most fish gourmets prefer "matured" postrigor fish. The ripening period, like the onset, intensity, and duration of rigor mortis, differs from species to species. The flavor of cooked cod, for example, has the strongest intrinsic

characteristics after 2 days in melting ice. Many fatty species such as salmon, ocean perch or halibut, improve much in flavor, taste, and texture during the first 2 to 4 days in ice. This is mainly due to the redistribution of fat and the development of flavor components, such as amino acids, IMP, and sugars, produced by the autolytic processes occurring in the postrigor muscle.[44] During storage, the action of endogenous enzymes in the postmortem tissue contributes to flavor changes. Rapid oxidation of large amounts of unsaturated lipids in fish is a major reason for changes in smell, taste, color, texture, and nutritional value. Enzymatic as well as nonenzymatic oxidation of polyunsaturated fatty acids of fish muscle produce a variety of carbonyls, alcohols, etc. that are responsible for flavor changes in fish. Associated with the lipid oxidation compounds, formation of several volatile compounds due to microbial action results in significant loss of fresh fish flavor.[45] In the case of elasmobranchs like sharks and rays, ammonia generated by the action of endogenous enzyme urease on urea influences the flavor. Similarly, rapid formation of ammonia in shrimp is correlated with adenosine deaminase activities on nucleotides, arginase-catalyzed formation of urea and its conversion to ammonia.[7] The factors influencing the flavor of farm-raised fish have been discussed in detail.[46]

2.2.6 Changes in Texture

Texture is an important quality parameter of muscle foods including fish. Fish is generally softer than red meat because of its low content of connective tissue and lower degree of cross-linking. Fish can be grouped into three types according to their texture (Chapter 1). The textural deterioration of fish takes place essentially due to degradation of the connective tissue by endogenous proteases.[20] Tenderization or softening of the flesh is associated with the disappearance of Z-disks in the muscle cell with the release of α-actinin, dissociation of the actomyosin complex, destruction and general denaturation of the connective tissue.[47] Proteolytic digestion of sarcolema that links the major structural units together is a major reason for tenderization.[48] Muscle proteases including cathepsin D and cathepsin L, calcium-activated proteases (calpains), trypsin, chymotrypsin, alkaline proteases, and collagenases are all involved in the softening of fish tissue during storage.[2,20] Softening of tissues of cephalopods during cold storage is a major problem. The involvement of proteolytic enzymes has been implicated in the phenomenon.[23] The major source of hydrolytic activity in Bombay duck, a marine fish, is concentrated essentially in the drip, while in the freshwater fish, tilapia, it is in the skin. Removal of cathepsin-D by removing drip or skinning tilapia suppressed enzyme-mediated softening.[49] Collagenases have been found to be involved in the development of mushiness in prawns stored in ice.[50]

As a result of proteolysis during storage, the gel strength (compressive force) of red hake (*Urophycis chuss*) was reduced to 46% after being stored in ice for 3 days, and to 63% when stored in chilled seawater.[51] During ice storage of threadfin bream up to 6 days, significant reduction in breaking force of washed mince has been reported.[19] These changes can influence the *surimi* quality made from fish. Similarly, a decrease in the breaking force and deformation of *surimi* has been observed in the big eye snapper stored in ice.[52] Changes were observed in

the octopus arm muscle during the early stage of chilled storage as a result of the autolysis of myosin heavy chain and also paramyosin. Therefore, care should be taken to control the loss of the quality of octopus during processing to ensure the functionality of myosin.[23] Proteolysis can also adversely affect water holding capacity (WHC) of fish muscle during storage. Fresh muscle is able to retain water, a functionality, which is of importance both to the industry and consumers.[53,54] Decreased WHC of muscle has often been described as the result of structural alterations in the muscle postmortem such as detachment of sarcolemma, gaps in the extracellular matrix, increased myofibrillar space and transverse shrinkage.

2.2.7   Discoloration

Another quality problem facing the seafood industry is the discoloration of products. The pink/red color of the skin of most fish fade during iced/chilled storage due to oxidation of the carotenoid pigments. The extent of the loss of color depends upon the fish, availability of oxygen, and the storage temperature.[55] Color is a very important quality parameter for salmonid fish. Wild salmon and trout flesh are naturally reddish due to the presence of carotenoid pigments. Generally astaxanthin and other carotenoid pigments are added to the diets of aquacultured salmon to improve appearance of the fish fillets. Fading of carotenoid color may take place due to (i) autooxidation of the conjugated double bonds, (ii) free radicals released during lipid oxidation that combine with carotenoids to form lipid hydroperoxides, and (iii) enzyme activity.[56] The activities of the enzymes involved in the oxidation of carotenoids are influenced by hydrogen peroxide and halides, particularly bromides and iodides. Bleaching of β-carotene could be due to one of the above three mechanisms.[57] Oxidation of myoglobins in the muscle is another reason for color change in fish. Oxidation of bright red myoglobin to brown metmyoglobin can occur through both nonenzymatic and enzymatic routes. An enzyme, metmyoglobin reductase, has been isolated from the skeletal muscle of dolphins and bluefin tuna.[25]

2.2.8   Melanosis

Development of black spots or melanosis is a problem found in most commercial shrimp, lobster, and other crustacea, that can cause a negative impact on the commercial value and consumer acceptance of the product.[58] Melanosis is triggered by a biochemical mechanism by which phenols are oxidized to quinones by the enzyme polyphenol oxidase (PPO). The quinones are highly reactive and undergo nonenzymatic oxidation and polymerization giving rise to dark pigments having high molecular weight. PPO is found in the zymogen form on the exoskeleton, chiefly on the shell of the cephalothorax of the crustaceans. The zymogen is converted to active enzyme by the action of a serine protease that exhibits trypsin-like activity. The zymogen can also be activated by nonphysiological agents such as detergents, organic solvents, gamma radiation, and heat or by immune response to microbial invasion.[58] The enzyme remains active during ice storage and

post-freezing thawing of the crustacea. Dipping the fresh shellfish in an aqueous solution of sodium bisulfite has traditionally controlled black discoloration on the shrimp shell. A 1 min dip in a 1.25% solution of the chemical is generally practised, which gives an average of 80 ppm sulfite in edible product. Freezing the treated shellfish can result in up to 17% reduction in the sulfite content, whereas, ice storage for 6 days can reduce the content below 10 ppm.[59] Recently, the safety of sulfiting agents has been questioned in the clinical literature and by consumer groups. The United States Federal Regulation insists that the residues of bisulfite above 10 ppm must be labelled to indicate the presence of the substance, since the presence of excess bisulfite can cause respiratory disorders in consumers. Laboratory and field trials have shown that, instead of sulfite, another compound, 4-hexyl-resorcinol, can be used as an efficient inhibitor of black spot formation in shrimp.[60] A commercial product, *Everfresh* that inhibits melanosis in shrimp has been marked as generally recognized as safe (GRAS) processing aid, in the United States since 1991.

2.2.9 Other Changes

Ice storage has been shown to result in changes in adenosine triphosphatase (ATPase) activities in threadfin bream. Total sulfhydryl content of the fish actomyosin increased after 3 days of ice storage, and then decreased. Surface hydrophobicity gradually increased within 6 days. Protein loss was also observed.[19] A similar decrease in the ATPase activity of the big eye snapper during ice storage has been reported.[52] Extractability of natural actomyosin (NAM) increased over the first 2 to 3 days of storage of fillets of Atlantic salmon at 0 and 4°C. Water binding capacity of the fish muscle and apparent viscosity of NAM decreased during this period. However, these parameters increased during further storage. Reactive sulfyhydryl content and surface hydrophobicity were lowest after 3 days, whereas disulfide content is highest at 2 and 14 days of storage. Viscosity, hydrophobicity, and sulfhydryl content were generally lower in fillets stored at 0° than at 4°C.[61] In the case of shark meat, ice storage for a period of 12 days reduced total nitrogen from 4.27 to 3.9%, while nonprotein-nitrogen was reduced only marginally. TVBN increased twofold during this period. Dynamic viscoelastic measurements showed that sharks have the excellent ability to form gel that was reduced only marginally during ice storage, unlike the case of several other fish species.[54]

2.3 DETERMINATION OF FRESHNESS

Freshness of fishery products deteriorates fast, and hence its determination is of utmost importance for development of products of acceptable quality. Apart from traditional sensory methods, numerous microbiological, physical, biochemical, as well as instrumental methods have been developed for evaluation of fish freshness. Biochemical indicators can be divided into three categories, namely, (i) protein changes like extractability, hydrophobicity, viscosity,

and electrophoresis; (ii) changes in the activities of enzymes such as ATPase, aldolase, malic enzyme, α-glycerophosphate dehydrogenase, acid phosphatase, 5′-nucleotidase, and phospholipase (particularly as a result of frozen storage); and (iii) accumulation of metabolic products such as TMA, free fatty acid, peroxide value, 2-thiobarbituric acid value, etc. Physical measurements include WHC, cooking loss and texture measurement.[10,61–65] However, no single method is universally applicable for assessment of freshness. The methods depend upon the nature of raw material, processing treatments, and storage conditions. Some of the quality indices used to assess freshness of fish may not suitable for cephalopods such as squid, because of differences in some of the metabolic pathways.[53]

2.3.1 Sensory Methods

Sensory evaluation is the rapid method for food quality assessment and is defined as the scientific discipline used to evoke, measure, analyze, and interpret characteristics of food perceived by the senses of sight, smell, taste, touch, and hearing.[62] Sensory tests can be divided into three groups: discriminative, descriptive, and affective tests.[7] Discriminative testing (triangle test, ranking test) is used to determine if a difference exists between samples. Descriptive tests are used to determine the nature and intensity of the differences (profiling and quality tests). The affective test (market test) is based on a measure of preference or acceptance by the consumer. Sensory methods are commonly used for quality assessment by inspection services in the fishing industry. However, being subjective, the data are subject to variation. This could be reduced by employing trained and experienced panelists.

In Europe, the most commonly accepted method is the European Union scheme, introduced in 1976 and updated in 1996.[66] The method includes four descriptive levels for grading the freshness of whole and ungutted fish from "E" (extra), "A" (acceptable), and "B" (poor) to "C" unacceptable for human consumption). The "E" quality denotes fish, which is very fresh having a sweet, seaweedy, and delicate taste. In cod, haddock, whiting, and flounder, the sweet taste is maintained up to 3 days after catch. "A" grade fish are characterized by some loss of odor and taste. The flesh becomes neutral but has no off-flavors. "B" grade denotes a sign of spoilage and presence of a range of volatiles, including TMA and other unpleasant smelling substances. During the later stages sickly sweet, cabbage-like, ammoniacal, sulfurous, and rancid smells develop. "C" grade fish are characterized by spoilage and putrid odors. However, suitability of the method has been questioned because the general parameters do not consider specific differences among species.

An alternative to the above scheme is the quality index method (QIM) for evaluation of raw fish based on sensory parameters. The QIM, originally developed in Australia, is based on significant, well-defined characteristic changes of outer appearance attributes. The fish quality is evaluated in sequence by sight, smell, and touch. Each attribute is scored from 0 to 3 (typically) by novice or experienced assessors with lower scores indicating better quality, and a score of 3 indicating spoiled fish. For example, the signs of stale mackerel are sunken, cloudy and discolored (red or brown) eyes, loss of bloom of the skin, dark red or brown gills

TABLE 2.1
Quality Indices for Cod (*Gadus morhua*)

Quality parameter	Point
General appearance	
Surface appearance	0–3
Skin	0–1
Slime	0–3
Stiffness	0–1
Eyes	
Clarity	0–2
Shape of pupil	0–2
Gills	
Color	0–2
Smell	0–3
Slime	0–2
Flesh color	
Open surfaces	0–2
Blood	
In throat cut	0–2
Sum of demerit points	23

Source: From Dalgaard, P., *Handbook of Food Science Technology and Engineering*, Hui, Y.H., Ed., 2005. With permission from CRC Press, Boca Raton, FL.

with a dark blood red mucus oozing from the gills, and sweety or sour odor. The sum of all attribute scores is called demerit points, or QIM index points, which increases linearly with storage time for a given fish. A typical example for cod is given in Table 2.1. Computer programs based on these evaluations have also been developed to predict the overall quality and hence, the value of the fish at any given point of ice storage.[67] A straight line relationship between storage time and QIM score has been determined for many species when stored at 0°C.

Quality index method schemes are now available for fish species including anchovy, brill, cod (fresh and frozen), haddock, flounder, herring, hoki, plaice, redfish, saithe, salmon, sardines, sole, spotted trevalla, turbot, and whiting. Based on the QIM scheme, whole octopus was found to have a shorter shelf life of 8 days, as compared with finfish. The shelf life as determined by QIM compared well with quality measured using radio-frequency (RF) freshtester.[68] QIM is primarily used in the evaluation of whole and gutted fish and is difficult to use for fish fillets. Schemes for lightly preserved seafood are not yet available. The QIM method is finding increasing applications in the seafood industry, particularly in European countries. Software for QIM has been developed.[69] Further studies are, however, required to determine if QIM schemes can be used in general to predict remaining shelf life of products when stored at different temperatures.

2.3.2 Objective Methods

2.3.2.1 Total viable counts

The content of microorganims in terms of total viable counts (TVC), aerobic plate counts (APC), or cfu has been used in mandatory seafood standards in some European Countries, Japan, and USA. Newly caught fish have TVC in the range of 10^2–10^6 cfu/g or cm^2 surface. At the point of sensory rejection, TVC of fish products are typically 10^7–10^8 cfu/g or cm^2 surface. However, since only a small fraction of the microorganisms present on newly processed seafood is actually of importance in product spoilage, TVCs correlate poorly with the degree of freshness or remaining shelf life of fish products. SSOs, which are better correlated with product quality, could be determined to assess product quality, depending upon the processing conditions employed. Table 2.2 indicates SSOs in different seafoods.

The conventional methods for microbiological enumeration are slow, with results being available only after a few days. Modern rapid microbiological methods, such as those relying on the polymerase chain reaction (PCR), oligonucleotide probes, or antibody techniques can provide responses within one day. However, these methods are unlikely to be used for routine seafood evaluation in the near future due to lack of sensitivity when applied in food, qualitative or semiquantitative responses, and high cost.

2.3.2.2 *K*-value

In most fish, *K*-values increase linearly during the first day of chilled storage and hence it can serve as an index of freshness. Nevertheless, the *K*-value has limitation in general as an index of spoilage because of its poor correlation with sensory data in the case of some fish species.[7] In addition, extensive sophisticated analyses of

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TABLE 2.2
SSOs in Seafood Stored under Different Conditions

Product	SSO
Fresh chilled fish stored in air	*S. putrefaciens*, *Pseudomonas* spp.[a]
	S. putrefaciens, *Pseudomonas* spp.[b]
Fresh chilled fish stored in vacuum or MAP	*P. phosphoreum*[a]
MAP fish stored at 0°C	*S. putrefaciens*, Lactic acid bacteria[b]
Fresh fish stored at >10–15°C in air	Vibrionaceae, Enterobacteriaceae
Cooked, brined shrimps and possibly several other lightly packed and preserved seafoods stored at 15–25°C	*Enterococcus faecalis*
Warm water species particularly with little or no TMAO	Lactic acid bacteria, *Brochothrix thermosphata*

[a] Typical of marine, temperate-water fish.
[b] Typical of freshwater fish and fish from warmer waters.

Source: Adapted From Dalgaard, P., *Handbook of Food Science Technology and Engineering*, Hui, Y.H., Ed. 2005, With permission from CRC Press, Boca Raton, FL.

nucleotide concentrations are required for K-value estimations. Development of biosensors can help simple and rapid determinations of adenine nucleotides and also other chemical indices of freshness. Such sensors may soon be available for practical use in process control, seafood inspection, and aquaculture.[70]

2.3.2.3   TMA and TVBN

Since TMA is produced only during bacterial spoilage of fish, its content can only be used as an index of spoilage and not as an index of freshness.[63] TMA concentration at sensory rejection is dependent on the products and storage conditions. For example, in modified atmosphere packed cod fillets, where spoilage is caused by *P. phosphoreum*, 30 mg TMA nitrogen per 100 g was detected at the time of sensory rejection, whereas in aerobically stored cod, spoiled by *S. putrefaciens*, lower levels of TMA were present at the time of sensory rejection.[42] Since many fish may not contain TMA-O, TVBN can be a better quality index. There is a good correlation between TVBN values and sensory data.[7] An amount of 10–15 mg TMA nitrogen or 30–40 mg TVB nitrogen per 100 g is usually regarded as the limits, beyond which whole chilled fish can be considered too spoiled for most uses.[7]

The European Commission as per the Council Regulation No. 95/149/EEC of March 1995 has permitted the use of TVBN for freshness determination of the freshness of fish. Critical limits of TVBN ranging from 25 to 35 mg per 100 g were established for different groups of fish species. In processed, lightly or semipreserved seafood, levels of TVBN at sensory rejection are more variable. An amount of 30–40 mg TVBN per 100 g has been found at sensory rejection of sliced vacuum packed cold-smoked salmon but lower levels of 10–20 mg of the compounds per 100 g have been found in modified atmosphere packaged (MAP), cooked, and brined shrimps. However, as much as 75 mg TVBN per 100 g was present in sugar-salted herring products of acceptable sensory quality. The variability of TVBN contents makes it useful as an index for determination of the freshness of fish only at the terminal storage life.[10]

In seafood having high contents of free amino acids, such as squid, crustaceans, and some dark fleshed fish like herring, substantial amounts of ammonia can be formed during chilled storage. The ammonia content of squid correlates significantly with TVBN and hence is an accepted index of squid quality. An enzymatic method for determining ammonia has been reported.[7] The formation of DMA from TMAO is due to the action of indigenous enzyme TMAO dimethylase. Although this is a slow process, DMA can be a useful index of spoilage, for example, in frozen hake. The identification of the off-flavor in squid (*Dosidicus gigas*) meat and the partial characterization of the responsible compounds have been recently reported. It was found that certain amino acids and low molecular weight water soluble peptides were responsible for the off-flavor.[71]

2.3.2.4   Biogenic amines

Biogenic amines provide a chemical indication of spoilage in fish. Consumption of food containing these amines above a threshold level can cause neurological

problems in the consumer. Histamine formed by bacterial decarboxylation of the amino acid, histidine, is responsible for "scombroid poisoning" because the so called scombroid fish such as mackerel, tuna, bonito, etc., have a high content of histidine.[72,73] Decarboxylation of amino acids arginine, lysine, and tyrosine, also produces respective amines, namely, agmatine, cadaverine, and tyramine. Other biogenic amines include putrescines, sperimidine, and spermins (derived from bacterial decarboxylation of glutamic acid). Some of the important bacteria responsible for biogenic amines are *Morganella morganii*, *Klebsiella pneumoniae*, *S. putrefaciens*, *Escherichia coli*, *Vibrio* spp., *Aeromonas* spp., and halophilic organisms. The amine forming bacterial population varies with storage temperature. *P. phosphoreum* plays an important role in decarboxylation of histidine to histamine in fish stored at or below 10°C. Certain *Pseudomonas* spp. convert glutamic acid to putrescines. In the muscles of invertebrates (squid and cuttlefish), arginine is extremely abundant in the free state and is easily converted into agmatine. Its concentration is low in fresh samples and increases with storage time exceeding 30 mg% at the stage of initial decomposition. Hence agmatine could be considered as an index of freshness of these invertebrates. In scallop adductor muscle and kuruma prawn, putrescine and ornithine have been reported as freshness indices, while in crustaceans (shrimp and lobsters), putrescine can be used as an indicator of quality. Cadaverine seems to be a useful chemical indicator for tuna, salmon, rainbow trout, *surimi*, and analog products.[72,73]

Histamine toxicity is related with consumption of fresh, canned, vacuum packed, salt-cured, and smoked fish items, if they contain appreciable levels of the amines. Histamine has been detected in commercial samples of scombroid fish fillets in Taiwan at concentrations as high as 12 to 29 mg histamine per 100 g.[74] An index of quality in terms of biogenic amine contents (in terms of mg amine per kg meat) for canned tuna, rockfish fillets, lobster tails, and fresh salmon steaks has been suggested as:

$$\frac{\text{[histamine]} + \text{mg/kg [putrescine]} + \text{mg/kg [cadaverine]}}{1 + \text{[spermidine]} + \text{[spermine]}}$$

Another index, the Biogenic Amine Index (BAI), namely, total contents of histamine, cadaverine, tyramine, and putrescine, has been found to be more valuable for the grading of tuna. End of shelf life was defined as a BAI value of 50 mg/kg. Another quality index relying on biogenic amines and pH has been developed recently for vacuum packed cold-smoked salmon.[69] Chromatographic techniques including TLC, HPLC, and GLC are used for determination of biogenic amines in foods including fishery products. "Histamarine", a histamine analysis kit for fish based on the ELISA method has been developed recently.[69] The regulatory limits for histamine in fish and fishery products has been established in several countries, with a permissible level of 20 mg% of histamine stipulated by the U.S. FDA, EU, Canada, Germany, India, and Sweden.[72]

2.3.2.5 Rancidity

Rancidity in fishery products as a result of autooxidation is measured in terms of the content of malonaldehyde, the principal compound in oxidized lipids.[75–77] The 2-thiobarbituric acid (TBA) test is one of the most commonly used methods to determine rancidity in fishery products. The test relies upon the reaction between TBA and aldehydes giving rise to a colored alkanal whose absorbance can be measured spectrophotometrically at 450 nm. Despite its widespread use, there are a few drawbacks for the technique. These include uncertainty about the nature of the color generation reaction, lack of specificity of the TBA reaction, and its reactivity with only malonaldehyde, one of the components of lipid oxidation.[75] Another method is determination of peroxide value that involves titration of iodine released from the reaction between a lipid extract from the fish and potassium iodide solution.[65] Techniques based on fluorimetry have been developed to measure lipid oxidation, primarily because of potentially much higher sensitivity than is possible with TBA tests.[75] Gas chromatographic (GC) methods are becoming increasingly popular to measure oxidative rancidity in oils and foods. Volatile oxidation products can be measured directly by headspace GC methods. In cold-storage mackerel, the rates of hydroperoxide formation, an index of rancidity development, varied as follows: vacuum packed fillets < foil-wrapped fillets < ungutted fish < gutted fish.[78] Free fatty acids have been suggested as a criterion for assessing quality of seafood containing significant amounts of fat.[65]

2.3.2.6 Instrumental methods

Instrumental methods for fish quality evaluation involves correlating the data generated by these systems with the sensory data. Dielectric properties of fish muscle have been relied upon as indices of quality during the past 40 years. The Intelectron Fishtester VI (Intelectron International Electronics, Hamburg, Germany), the Torrymeter (Distell Industries Ltd., Fauldhouse, West Lothian, UK), and the RT-Freshtester (RT Rafagnataekni, Reykjavik, Iceland) use instruments with increasing degrees of sophistication. Readings from all the instruments reflect dielectric properties of fish, which almost linearly change with storage time. The main advantages of these instruments are their immediate response and potential in field use. Based on these rapid and nondestructive measurements, the RT-Freshtester allows automatic grading of 60 to 70 fish per minute. The electrical measurements can also be used to determine whether the fish has been previously frozen.[63] Nevertheless, electrical properties of fish may not be always directly related to sensory spoilage. Further, these instruments need calibration depending on the season and fish handling practices, and they are not suitable for grading frozen or thawed, superchilled fish, and fish fillets. These disadvantages coupled with the high cost of the instruments limit their practical use in the seafood industry.

Spectroscopic methods have gained acceptance in detecting whether a fish has been frozen and also to estimate the storage time of fish in ice. Near infrared (NIR)

reflectance is another technology where measurements are rapid and have the potential for on-line quality grading.[63] This technology is useful for the indirect measurement of oil, water, and WHC in different types of fish. L*a*b* is an international standard for color measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976. L* is the luminance or lightness component, which ranges from 0 to 100, and a* (from green to red) and, b* (from blue to yellow) are the two chromatic components which range from −120 to +120. The principles of food color measurement based on this system have been discussed.[79] Table 2.3 summarizes some of the conventional methods for quality evaluation of fishery products.

TABLE 2.3
Conventional Methods for Quality Evaluation of Fishery Products

Method	Remark
Sensory evaluation	Depends upon sight, smell, taste, touch, and hearing as judged by experienced panelists
Total volatile nitrogen	Good correlation with bacterial spoilage
Trimethylamine	Not generally useful for freshwater fishery products. Not very good correlation with total bacterial counts
Ammonia	Indicates advanced spoilage of finfish and shellfish
Volatile acids	Good correlation with bacterial spoilage
Nucleotide catabolites (K-value), Hx	Degradation products of ATP. Reliable quality indices for several fish/shellfish based on K-value
Indole in shrimp	Rapid test for shrimp quality
Biogenic amines	The amines are thermostable and hence cooked fish can also be analyzed. HPLC method is used
H_2S, CH_3SH, $(CH_3)_2S$	Indicates advanced degree of spoilage
Ethanol	Good quality index for several fish such as salmon, raw tuna, redfish, pollock, flounder, and cod
Rancidity	TBA value is a good index of oxidative rancidity. Reasonable correlation with sensory properties. Other methods include peroxide and carbonyl values
Instrumental methods	Electrical properties, pH, and vision properties of fish muscle. Presence of parasites and blood clots in fish fillets are determined by computer-aided vision techniques
Total microbial counts, SSOs	Indicative of microbial spoilage of fresh and processed fish
Pathogenic microorganisms and toxins	Indicative of hazards. Above threshold levels indicate unsafe for consumption

Source: Adapted from Venugopal, V., *Biosens. Bioelectr.*, 17, 147–157, 2002. With permission from Elsevier.

2.4 DETERMINATION OF SPOILAGE RATES AND SHELF LIFE

The ultimate aim of freshness evaluation techniques is to combine different standard methods that use rapid measurement techniques with a mathematical model to predict freshness, as well as the postharvest or remaining shelf life of an unknown fish sample. Spoilage of fish is linearly related to storage temperature, since autolytic reactions by muscle as well as microbial enzymes are directly related to temperature. In most cases, the spoilage rate can be determined by the slopes of the plots of the sensory, bacteriological, or chemical quality indices of fish against storage time at each temperature, since these plots are essentially linear. The QIM has also proved useful for obtaining a straight line relationship between quality scores and storage time.

If the shelf life of a fish at 0°C and another temperature, t°C are known, their ratio gives relative rate of spoilage (RRS) at t°C.[8]

$$\text{Relative rate of spoilage at } t°C = \frac{\text{shelf life at "0°C"}}{\text{shelf life at } t°C} \tag{2.1}$$

The relationship between shelf life and temperature has been studied in detail.[80] It was found that spoilage rate at varying temperatures followed the Spencer and Baines equation,[81] given below:

$$k = k_0(1 + Ct) \tag{2.2}$$

where k is the spoilage rate at temperature t°C, k_0 is the spoilage rate at 0°C, and C is the linear temperature response. The relative rate concept has made it possible to quantify and mathematically describe the effect of temperature on the rate of spoilage of various types of fish products. In the temperature range 0 to 8°C, the RRS of fish may be computed as:

$$k/k_0 = 0.24 \times t \tag{2.3}$$

where k and k_0 represent the rate of spoilage in spoilage units per day at temperature t and 0°C, respectively.

The spoilage rate of fish can also be determined by knowing the influence of temperature on growth of contaminant microorganisms. A two-parameter square root model for the effect of sub-optimal temperature on microbial growth is given as:

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) \tag{2.4}$$

where T is the absolute temperature (Kelvin) and $T_{\min}$ is a parameter expressing the theoretical minimum temperature of growth. The square roots of the microbial growth rates plotted against the temperature give a straight line from which $T_{\min}$ is determined. Based on the $T_{\min}$, a spoilage model has been developed on

the assumption that the relative growth rate of microorganisms would be similar to RRS.

$$\sqrt{\text{Relative spoilage rate}} = 0.1 \times t°C + 1 \qquad (2.5)$$

From this, the shelf life at any temperature, $T°C$, is given as:

$$\text{Shelf life at } T(°C) = \frac{\text{Shelf life at } 0°C}{[1 + 0.1 \times T(°C)]^2} \qquad (2.6)$$

While broad differences are observed in the shelf lives of various seafood products, the effect of temperature on RRS is almost similar for different fresh fish in general. At temperature of 5°C, the RRS of salmon and cod have been found to be 1.5 and 2.3, respectively, where as at 10°C, the corresponding values are 3.9 and 4.7.[7] The square root model (Equation 2.5) is the most popular to predict the effect of temperatures on the shelf life of different fresh seafoods from cold and temperate waters. This model has been successfully validated for products stored between −3 and +15°C.

Fresh tropical fish typically have a longer shelf life at 0°C than fresh fish from cold and temperate waters. The relative spoilage rates for tropical fish are more than twice as estimated for temperate fish species. For these fish, the logarithm of the relative spoilage rate has been found to be $0.12 \times T°C$.[7] An exponential tropical spoilage model has been developed for shelf life prediction,[69] based on Equation 2.4.

$$\text{Shelf life at } T(°C) = \frac{\text{Shelf life at } 0°C}{\exp[0.12 \times T(°C)]} \qquad (2.7)$$

Compared to fresh seafood, the effect of temperature on the shelf life of lightly preserved seafood is more variable and less well described. Temperature influences the RRS of cooked and brined MAP shrimp more than the fresh shrimp. However, in the case of hot smoked cod and mackerel, the influence of temperature on RRS is less than that of fresh fish. The reference temperature of 0°C that is applied for the calculation of RRS in fresh fish may be inappropriate for lightly preserved products, and a different reference temperature (T_{ref}), namely, 5°C can be used in the equation,[69] given below:

$$\text{Shelf life at } T(°C) = \frac{\text{Shelf life at } T_{\text{ref}}(°C)}{\exp[0.15 \times (T - T_{\text{ref}}(°C))]} \qquad (2.8)$$

The cumulative effect of time and temperature on storage and product quality allows spoilage models to be used for prediction of the effect of variable temperatures on product shelf life. Electronic time-temperature integrators (TTIs) have been developed based on these results. The shelf life of different types of fresh and

lightly preserved seafood varies substantially with temperature and processing treatments. Despite this, it is possible to develop simple and entirely empirical relative-rate-of-spoilage-models to predict the shelf life of different seafoods at various storage temperatures.[69]

2.5 HAZARDS ASSOCIATED WITH FISHERY PRODUCTS

Food-borne hazards are on the rise throughout the world. A hazard is defined as a biological, chemical, or physical agent in food, or a condition of food with the potential to cause harm. An estimate of the probability and severity of the hazard to populations caused by the consumption of foods is called risk. Diseases caused by consumption of food contaminated with pathogenic microorganisms are food *infections*, while those resulting from their toxins are referred to as food *intoxications*. The severity of the diseases depends upon the nature of contamination, and may range from mild diarrhea to death. The major reasons for such outbreaks are: the increase in population, tourism, industrialization, and the rise in international trade of processed food associated with lesser care during processing.[82–86] The various types of hazards associated with fishery products have been summarized recently.[84]

The World Health Organization (WHO) has observed that in Asia-Pacific region alone, more than 700,000 people die every year from consuming contaminated food.[85] An estimated 76 million cases of foodborne illness occur each year in the United States costing between $6.5 and $34.9 billion in medical care and loss of productivity.[83] In the late 1980s seafood attracted significant media attention as a carrier of environmental pollutants and other health hazards. Much processed seafood have been recognized as carriers of pathogenic bacteria, viruses, and parasites responsible for food-borne hazards worldwide. Contamination of wild as well as farmed fish can occur during harvesting, distribution, and storage, through biological and chemical hazards from both freshwater and coastal ecosystems.[84–88] The tendency of some fish to absorb and concentrate heavy metals such as mercury and other industrial pollutants and also hazards associated with aquacultured fish, such as presence of antibiotics at nonpermissible levels attracted the attention of regulatory authorities and consumers. The public health issues associated with seafood have been grouped as environment, process, distribution, or consumer-induced.[89] Some of the important hazards associated with fishery products are discussed below.

2.5.1 Bacterial Pathogens

A number of pathogenic bacteria have been implicated in seafood-borne diseases. These include *Salmonella* spp., *Shigella* spp., pathogenic *E. coli* and *Campylobacter* spp., *Vibrio cholerae*, *V. parahaemolyticus*, *Aeromonas* spp., *Plesiomonas* spp., *Yersinia enterocolitica*, *Clostridium botulinum*, and *Listeria monocytogenes*. The leading cause of food-borne illness during the last few

TABLE 2.4
Pathogenic Bacteria Indigenous to the Aquatic Environment and Naturally Present on Fish

Pathogen	Primary habitat	Carrier seafood	Quantitative level
Clostridium botulinum (nonproteolytic types B, E, F)	Temperate and arctic aquatic environment. Multiplication in aquatic carrion (type E)	Smoked fish, white fish, ready-to-eat (RTE) products	Mode of action, preformed toxin, generally low (<0.1) spore g^{-1}, but up to 5.3 spore g^{-1}
V. cholera	Ubiquitous in warm ($>15°C$) seawater environment	Shellfish, RTE products, shellfish, raw oysters, clams, crabs	Up to 10^2–10^3 cfu g^{-1}
V. parahaemolyticus			Up to 10^4–10^6 cfu g^{-1}
V. vulnificus			
Aeromonas spp.	Aquatic environment	Aquacultured fish/shellfish	Generally low, but up to 10^4 cfu in seawater
Salmonella typhi	Environmental	Mussels, scallops, oysters	—
Shigella spp.		Bivalves	—
Salmonella spp.	Tropical aquatic environment	Fish/shellfish/ Bivalves from warm waters, cultured fish	$<10^2$–10^6 cfu g^{-1}
Listeria monocytogenes		Cold smoked fish (trout, salmon), shrimp, RTE products	Not known

Source: Adapted from Lee, J.S. and Hilderbrand, S. Jr., Hazard analysis and critical control point applications to the seafood industry. Oregon Sea Grant, ORESU-H-92-001, Corvalis, OR.

years was salmonellosis followed by shigellosis, staphylococcal intoxication, and gastroenteritis.[87] *Salmonella* spp. including *S. paratyphi* and *S. enteritidis* have been detected in processed shrimp and bivalves.[90] Shellfish have been a common carrier of this pathogen. Others included fecal coliforms, *S. aureus* and *V. parahaemolyticus* in addition to Norwalk virus and Hepatitis A virus.[91] Many seafood-importing countries do not permit the presence of the organisms in imported consignments.[92] The major pathogens of concern are listed in Table 2.4.

Presence of *Salmonella* spp. in seafood indicates contamination with sewage. There is a higher prevalence of *Salmonella* in tropical than in temperate waters, although seasonal variations occur. *E. coli* strains that colonize the tract are generally harmless. However there are at least four types of pathogenic *E. coli* strains. These can be enteropathogenic, enterotoxigenic, enteroinvasive, and enterohemorrhagic strains. *E. coli* O157:H7 is both enterohemorrhagic and cytoxin producing strain and hence, hazardous. Where animal manure, particularly bovine

manure, is used as pond fertilizer in aquaculture, there is a risk that pathogenic strains of *E. coli* O157:H7 may be present in pond water. *Shigella* spp. have also been isolated in aquacultured systems. Poultry guts invariably harbor *Campylobacter* spp. and hence use of poultry-based manure may pose hazards in inland and coastal aquaculture. Nevertheless, the risk associated with consumption of cultured fish infested with this bacterium is low.

Currently, more than ten species of *Vibrio* are known to be involved in human infections acquired by consumption of contaminated food and water. These organisms being salt tolerant, occur in marine and estuarine waters, while *V. cholerae* and *V. mimicus* also occur in freshwater. All members of this group show an increase in abundance in warmer waters and an apparent reduction in numbers during cooler months. Some *Vibrio* spp. are both human and fish pathogens. The diseases associated with *Vibrio* spp. are characterized by gastroenteritic symptoms varying from mild diarrhea to cholera. The hazards are associated with consumption of raw fishery products, particularly farmed finfish and crustaceans harboring *V. cholerae* and *V. parahaemolyticus* and they have been major causes of gastroenteritis in Japan.[93] *Plesiomonas* spp. and *A. hydrophila*, which are common in estuarine waters, have also been isolated from fishery products. The former has been implicated in the outbreaks of gastroenteritis by fish consumption.

C. botulinum type E, naturally found in soil and aquatic sediments, is often isolated from fish. Human botulism is a serious but rare disease and is due to the intoxication caused by a toxin released by the bacterium. *P. shiglloides*, *L. monocytogenes*, and *A. hydrophila* have not so far been unequivocally incriminated in disease outbreaks involving fish products. However, *Listeria* spp. have been isolated from tropical fish, shrimp, crab, lobster tail, and *surimi*-based products.[94,95] Several of these pathogenic organisms including *L. monocytogenes*, *Y. enterocolitica*, and *A. hydrophila*, are capable of survival even at refrigerated temperatures, posing a threat to the safety of refrigerated products.[90,91] *L. monocytogenes* is frequently reported from fish items, particularly aquaculture products from temperate regions; however, it is rarely detected in tropical waters.

Fishery products that are not processed under strict hygienic conditions may harbor pathogens. Screening of 1264 samples of individually quick-frozen (IQF) peeled and deveined raw and 914 samples of cooked ready-to-eat shrimp commercially produced from farm raised black tiger (*Penaeus monodon*) were shown to be contaminated with *E. coli*, coagulase positive *Staphylococcus*, and *Salmonella* spp. The coliform count in raw products ranged from 1.0×10^1 to 2.50×10^3 cfu/g. Although the cooked shrimp samples were free of coagulase positive staphylococci, *E. coli*, and *Salmonella* spp., the frozen raw shrimp samples tested positive for these pathogens.[90]

There is recent evidence that viable but noncultivable (VBNC) state of microbes may be formed, in the stressing environment of food processing. This is a cause of concern because the microbial pathogens in such a state may retain the capacity to cause infection after consumption by the consumer, despite their inability to grow under conditions employed for determining their

presence in food. VBNC organisms can cause potentially dangerous public health problems.[96]

2.5.2 Viruses

The prominent food-borne viruses are Hepatitis-type A, Norwalk virus, and small round viruses (calicivirus, astrovirus, and parvovirus).[83,91] The symptoms of diseases caused by these viruses include paralysis, meningitis, respiratory illness, and myocarditis among others.[97] Consumption of raw molluskcan bivalves is a major cause of viral disease as a result of filtration of water by these shellfish.

2.5.3 Parasites

Parasites such as flatworms, roundworms, and protozoa infest the gills, viscera, and skin of marine, freshwater as well as farm raised fish and shellfish, and can pose health hazards to consumers.[91,98,99] The most common parasites associated with fish that can infect consumers include nematodiases, trematodiases, and cestodiases. Fish-borne nematodiases (roundworms) can be detected in humans as incidental infections, whose natural definitive hosts include marine mammals, birds, and pigs. The mode of infection is ingestion of fish containing the infective larvae. Anisakids (particularly *A. simplex*) are among the most common nematodes in marine fishes.[87] Others include *Ascaris lumbricoides*, *Trichuris trichura*, *T. spiralis*, *Capillaria philippinensis*, and *Pseudoterranova decipiens*.[100] Cod, whitefish, and salmonids can carry *T. spiralis*. Nematodes in fish can be killed by thoroughly cooking the fish, freezing, or curing with salt and acetic acid.[4]

Fish-borne trematodiases are major diseases in various parts of the world, causing morbidity and complications leading to death. Clonorchis and opsthorchis are the two major genera that are of great concern for human health. Clonorchiasis, caused by clonorchis, is endemic in some countries in East Asia. For liver fluke species such as *Clonorchis sinensis*, *Opisthorchis felineus*, and *O. viverrini*, the intermediate hosts are snails and freshwater fish, while dogs, cats, and humans are the final hosts where the fluke lives and develops in the bile ducts of the liver.[101] Other trematodes of importance are *Nanophetus salmincola*[94] and *Crytotyle lingua*,[4] the latter existing under the skin of gadoids such as herring and mackerel. Cestodiases (tapeworms), which mature in the small intestine, cause cestodes infection in humans. The disease is not very pathogenic. *Diphyllobothrium* spp. cause another major human cestodiasis and is transmitted by various species of freshwater, marine, and anadramous fish.

The smallest of the parasites are the protozoa, which are single-celled organisms. The three most important ones found in food are *Entameba histolytica*, *Giardia lamblia*, and *Toxoplasma gondii*. A well-known protozoan infection is caused by mixosporidian *Chloromyscium thyristes*. It occurs in a number of species of fish although its presence is difficult to detect in fresh fish.[4] During ice storage, the flesh of badly infected fish becomes softened by proteolytic enzymes produced by the parasite.[4] The abnormal color of shrimp may be due to infestation

of a protozoan that decomposes the meat, giving it a soft and white appearance. Strict regulations regarding the presence of parasites in the edible portions of fish are in force in countries such as Germany.[102]

Compared with capture fisheries, aquacultured fishery products are more exposed to hazards, both biological as well as chemical, due to possibilities of their contamination through agents from both freshwater and coastal ecosystems. In addition, the use of antibiotics such as chloramphenicol to control diseases has resulted in the accumulation of residues of these compounds in shrimp and other products. The high prevalence of bacterial pathogens in coastal and inland aquaculture environments than in open seas has been recognized. *Salmonella* spp. have been reported in fish ponds, which are usually scavenged by birds, and therefore, have been detected in the gut of cultured tilapia and carp.[93,101] Surveys have revealed that 21% of Japanese eel culture ponds, 5% of North American catfish ponds, and 22% of shrimp ponds in one of the major shrimp-exporting countries in Southeast Asia are contaminated with *Salmonella* spp.[103] Viral disease has been a major reason for the setback in shrimp aquaculture during the last few years. The disease spread mostly through unhealthy seeds. The safety issues associated with aquaculture products have been addressed by a Joint FAO/NACA/WHO Study Group.[101] The Study Group evaluated options for implementing risk management strategies to reduce or eliminate risks associated with products from aquaculture systems and recommended food safety measures to control these hazards (see also Chapter 12).

2.5.4 Insects

A number of insects such as flesh flies (Saracophagidae), beetles (*Dermestes*, *Cornestes*, and *Necrobia* spp.), and mites (*Lardoglyphus* and *Lyrophagus* spp.) infest fish, particularly during sun drying.[104] The most destructive pest is the hide beetle, *D. maculatus Deg*. A case study of the losses in traditionally cured fish showed the infestation of blowfly as the major cause of losses in dried sardine products.[105]

2.5.5 Biotoxins

A large proportion (approximately 80%) of seafood-borne disease outbreaks is caused by biotoxins and histamine.[87] The main types of fish poisoning include ciguatera, paralytic shellfish poisoning (PSP), diarrheic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and puffer fish poisoning.[94,106] PSP is associated with consumption of clams and mussels harvested during occurrence of "red tide," when these animals concentrate the dinoflagellates (usually species of *Ganyaulax*). Such poisons or toxins are not destroyed by heat or processing. Ciguatera poisoning, which causes gastrointestinal and neurological symptoms, is associated with a number of fish species, most notably reef fish such as groupers, sea basses, and snappers. Scombroid poisoning has been recognized as the greatest cause of seafood-associated disease linked to post-harvest contamination and

improper storage of fish.[94] Scombroid poisoning is associated with the consumption of fish of the scombroid family (mackerel, tuna) and is due to ingestion of appreciable amounts of histamine, and the characteristic symptoms are headache, dizziness, nausea, vomiting, and urticarial eruptions.[72,73] The maximum concentration of histamine permissible in 100 g fish flesh as per the E.U. and U.S. regulations, and as per the Codex Alimentarius Standards is 20 mg. Contamination of aquacultured fishery products with mycotoxins may also be possible. These are transmitted through agricultural components used in feed, which are contaminated with fungi such as *Aspergillus flavus*, *A. parasiticus*, *A. rubur*, and *Penicillium veridicatum*.

2.5.6 Chemical Hazards

Some species of fish may contain significant levels of methylmercury, polychlorinated biphenyls (PCBs), dioxins, and other environmental contaminants. These are present in low levels in freshwater bodies and those found in oceans. However, they are bio-concentrated in the aquatic food chain, so that their levels are generally high in older, larger, predatory fish and marine mammals. Large, predatory ocean fish such as swordfish, tuna, king mackerel, tilefish, and shark bio-accumulate methylmercury in the edible portions. Levels may be 1,000 to 10,000 times greater in these fish than in any other food.[82,84] However, because methylmercury is distributed throughout the muscle, skinning and trimming does not significantly reduce its contents in fillets. Many marine organisms have also high tissue levels of selenium.[82] Selenium and zinc as well as vitamin C and vitamin E have a profound protective effect against mercury and methylmercury toxicity.

Uses of chemicals in food production and processing have contributed to increase in chemical hazards. The WHO has identified 18 chemical contaminants in food, which include industrial chemicals, organo-chlorine pesticides, organophosphorus pesticides, and mycotoxins.[104,105] Incidences of chemical hazards are particularly widespread in aquacultured fish and shellfish, since some of the chemicals are also used to sterilize pond soils between production cycles.[99] When used as per good aquaculture practices, none of the water treatment compounds can be considered a hazard. Food additives and processing aides such as nitrosamines are carcinogenic.[107]

Presence of PCBs in farmed salmon has created some controversy recently. However, the reported content of the chemicals was below the tolerance level of 2000 ppb established by the U.S. FDA.[108] The WHO has fixed an upper limit of 4.5 ng of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in fish, other marine animals and their products and by-products.[108] Presence of antibiotic residues in imported aquacultured fishery products is a matter of major concern to regulatory agencies. High levels of chloramphenicol in aquacultured shrimp have adversely affected international trade in the commodity.[109]

The various hazards associated with seafood can be broadly grouped into three categories: (i) those that can cause illness in healthy adults (*C. botulinum*,

C. perfringens, *Salmonella*, *Shigella*, *S. aureus*, *V. cholerae*, *V. parahaemolyticus*, hepatitis A virus, Norwalk-like viruses, Diphyllobothrium, Anisakis, toxins, heavy metals, and PCBs, (ii) those *not* capable of causing illness in healthy adults, but are dangerous to susceptible people such as immuno-compromised individuals, children, elderly people, pregnant women, etc. (*L. monocytogenes, V. vulnificus*), and (iii) those having uncertain pathogenicity (*A. hydrophila* and *P. shigelloides*).[110] Sensitive populations such as the immuno-compromised, diabetics, and those with impaired liver functions are at an increased risk of infection if seafood contaminated with these hazards is consumed.

2.5.7 Some Practical Implications of the Hazards

Contaminated seafood is reported to be the leading known cause for food-borne illness outbreaks in the United States.[86,97,106] During 1990 to 1998 the U.S. FDA reported that 7.2% imported and 1.3% of the domestic samples from a total of 11,132 seafood products including crustaceans, shellfish, and other aquatic animals were contaminated with *Salmonella* spp.[91] During the period from 1973 to 1987, seafood items were found responsible for 753 incidents of food-borne diseases out of a total of 7,458 in the United States.[88] Of the 530 general food-borne outbreaks of food poisoning reported in England and Wales between 1992 and 1996, 54.9% cases were due to *Salmonella* spp. Seafood was responsible for 15.7% of the contaminations, next to red meat (18.7%) and poultry (15.5%).[111] In the Netherlands, seafood caused 23 outbreaks during 1983 to 1990; while in Canada 140 outbreaks were reported during 1982 for the same reason.[112] Improper handling of foods significantly contributed to these disease outbreaks. Major reasons of *Salmonella* infections were improper heating (39.8%), inadequate storage (32.1%), cross contamination (22.3%), and ingredients (21.9%).[111,112]

The risk of exposure to seafood related hazards is more common in several Asian countries where consumers often eat raw or partially cooked fishery items.[87,110] Biological and chemical hazards have been implicated in a majority of the cases. In a massive outbreak that occurred in Shanghai more than 16,000 people were estimated to have contracted hepatitis as a result of eating raw fish.[94] In June 1997 the European Union banned import of seafood from India due to its poor microbiological quality. The ban was lifted later as a result of corrective action such as upgradation and renovation of processing units. At present about 200 fish processing units have been given approval for export to European countries.[109] The presence of pathogenic microorganisms and antibiotics have occasionally affected the export of shrimp to Europe from Asian countries such as Vietnam and Indonesia also.[113] These incidences should serve as a pointer for the need of strict quality control measures in processed fishery products, whether intended for domestic use or international trade.

2.6 CONTROL OF HAZARDS

Seafood related health hazards have forced the global regulatory authorities to introduce stringent quality standards in traded fishery products. The food

TABLE 2.5
Seafood Hazard Categories in Order of Decreasing Risks

Category	Description	Example
1	Those consumed raw without any cooking	Mollusks, including fresh and frozen mussels, clam, oysters, and raw fish such as *sushi/sushimi*
2	Non-heat processed raw foods often consumed with additional cooking	Fresh/frozen fish and crustacea
3	Lightly preserved fish products (with <6% salt in water phase, pH > 5.0)	Salted, marinated, fermented, cold smoked fish
4	Semi-preserved fish (salt >6%) or pH < 5.0 with added preservatives	Salted, marinated fish, fermented fish, caviar
5	Mildly heat-processed (pasteurized, cooked, hot smoked) fish products	Pre-cooked, breaded fillets
6	Heat processed (sterilized, packed in sealed containers)	Canned, retort-pouch packaged items

Source: From Huss, H.H., Reilly, A., and Ben Embarek, P.K., *Food Control*, 11, 149–156, 2000. With permission from Elsevier.

regulations have been traditionally based on the principle that ideally all pathogenic microorganisms should be absent in food. However, the concept of "zero tolerance" is impracticable and hence, permitting a certain level of pathogens in some raw foods is explicitly acknowledged by the Codex Alimentarius Commission. According to the Codex guidelines, fish and fishery products should not contain microorganisms in any amount that may represent a hazard to public health.[114] Table 2.5 depicts seafood hazard categories in order of decreasing risks.

Biological hazards can be reduced by conventional food-processing treatments. Chemical hazards can be controlled by specifying quality of raw material and ingredients, and control of incidental contamination during processing and distribution. Removing the skin and fat from fish before cooking them can reduce the levels of PCBs. The Codex Alimentarius Commission, the U.S. FDA, and the European Union have laid down action plans to limit these chemical contaminants. The U.S. FDA has set action levels for maximum permitted concentrations of 5.0 mg DDT and its breakdown products, DDE and TDE, and lower limits for dieldrin and aldrin. It is likely that these limits may be further reduced to improve the quality of the processed products. The U.S. Institute of Food Technologists in a Scientific Status Summary has provided information on the foodborne hazards and advises on measures to control these hazards, which included the U.S. National Shellfish Sanitation Program Guidelines to limit *Vibrio* contamination and growth.[115] Table 2.6 shows measures that can be taken to control seafood pathogens. Experience has shown that end product inspection does not

TABLE 2.6
Control Measures Against Bacterial Pathogens and Parasites in Seafood

Pathogen	Control measures
Salmonella spp.	Proper sewage disposal, sanitary handling and processing, cooking just prior to eating, prohibit harvesting from polluted waters, maintenance of water quality in aquaculture
Vibrio spp.	Sanitary handling and processing, adequate refrigeration and freezing, cooking just prior to eating
C. botulinum	Correct processing, cooking just prior to eating food
Viral infections	Proper sewage disposal, adequate cooking, prohibit harvesting from polluted waters
S. aureus	Sanitary handling and processing, maintenance of proper hygiene during processing
L. monocytogenes	Check raw materials, sanitary handling and processing, adequate cooking, prevent cross contamination from raw to processed foods
C. botulinum	Correct processing, cooking just prior to eating food to inactivate toxin
Parasites	Check raw material, freezing/heating/curing

Source: Adapted from Lee, J.S. and Hilderbrand, S. Jr., Hazard analysis and critical control point applications to the seafood industry. Oregon Sea Grant, ORESU-H-92-001, Corvalis, OR.

safeguard production lines from exposure to various food-borne hazards. In contrast to end product analysis, a preventive strategy based on thorough analysis and control of the prevailing conditions can ensure better safety of the processed products. These include food surveillance in order to trace origin of contamination, creation of consumer awareness, improvement of infrastructure for food quality control systems, stipulation of standards, and upgradation of manufacturing standards. These can be affected by applying Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP), certification under the International Accepted Standard (ISO 9000 Series), Total Quality Management and a well designed Hazard Analysis Critical Control Point (HACCP) program.[111] Guidelines have been given with respect to fish consumption by regulatory agencies worldwide.[124] The U.S. FDA recommends that women who are pregnant or nursing and young children eliminate shark, swordfish, king mackerel, and tilefish (also referred to as golden bass or golden snapper) from their diets completely and limit their consumption of other fish to 12 ounces per week (3–4 servings per week) to minimize exposure to methylmercury. The FDA has stipulated that the concentration of mercury in edible portion should not exceed 0.5–1.0 ppm on wet weight basis.[115]

2.6.1 Hazard Analysis Critical Control Point

Hazard Analysis Critical Control Point is internationally recognized as the ideal method of assuring product safety by controlling food-borne safety hazards. HACCP helps to identify and monitor points where there is risk of contamination on a hazard. At present, it is the widely acknowledged, cost-effective method of controlling hazards in fishery products. Introduction of HACCP-based regulations for fish and fish products, particularly in the European Union and the United States, has triggered the need for production under the HACCP system in most fish-exporting countries.[84,114,116–119] The underlying principle of HACCP is to make seafood processors responsible for assuring safe seafood. The seven elements of the HACCP system are: (i) identification of potential hazards, (ii) determination of critical control points (CCPs) (A critical control point is defined as a point in the processing steps where the failure to effectively control a potential hazard may create an unacceptable risk), (iii) description of the critical control point, (iv) establishment of a monitoring system, (v) establishment of corrective action when CCP goes out of control, (vi) establishment of procedures for verification, and (vii) establishment of documentation and record keeping. HACCP, with its seven principles, form the framework for the rational consideration of actual hazards of seafood. Hazards may be caused by the fish species and its environment, or by the processing method. The HACCP processors can use their own scientific or technological principles to establish the hazard prevention system that works for a particular processing situation.

The U.S. FDA, as per notification dated December 18, 1995, (Federal Register, 21 CFR Parts 123 and 124) has made it mandatory for all seafood processors exporting to United States to adopt HACCP-based seafood quality assurance system for fish and fish products from December 18, 1997 onwards.[114] The regulations require that all seafood products must have been processed in accordance with the HACCP principles. These requirements apply to both imported as well as domestic products. From January 2006 onwards the European Union will require a safety monitoring system in place based on HACCP principles for all food businesses (http://www.foodsciencecentral/lm/fsc/ixid/13993) A detailed discussion on HACCP and its application to seafood processing are beyond the scope of this chapter and can be seen elsewhere.[100,112,114,116,118–120] The HACCP system has been harmonized with the General Principles of Food Hygiene of the Codex Alimentarius Commission and the official regulations of the European Union.[114,117,121] Practical experience generated during the last few years has shown that HACCP systems can control most of the hazards related to indigenous pathogens, as it is possible to identify Critical Control Points with respect to these pathogens.[112,116] Nevertheless, HACCP system cannot ensure complete safety of processed seafood. Some of the examples include the presence of pathogens in mollusks and fish that are consumed raw or steamed (undercooked) and growth of *L. monocytogenes* in some lightly preserved fish products (e.g., cold smoked fish). It is recommended that consumers should be warned about a possible risk in the case of the former and a limiting shelf life of the products should be recommended for the latter product.[116] In many cases, adoption of the recommendations by

TABLE 2.7
Key Areas for Quality Control for Food Processors

- Raw material: Monitor the quality and reliability of the raw material. It helps to set up a proper traceability of input ingredients and produce quality products
- Sanitation: Proper hygiene is a prerequisite with all standard operating procedures
- Good Manufacturing Practices
- Implementation of HACCP (Hazard Analysis Critical Control Points)
- Analytical testing — microbiological, chemical, sensory, and shelf life
- Environmental monitoring
- Standard Operating Procedures (SOP) and document trail through incoming, processing, finish product and outgoing products
- Process verification and validation studies to ensure safe product with extremes. The market storage conditions and instructions to be validated
- Proper pest control program to avoid any contamination
- Recall and crisis management programs, involving trained technical personnel to handle the issues of product safety.
- Employee training and education

Source: Vyas, H., *Indian Food Ind.*, 23, 16, 2004. With permission from Association of Food Scientists and Technologists (India), Mysore.[125]

processors needs much to be desired. This was shown by a recent survey of GMP in seafood processing plants that export a large amount of processed seafood. While the processors have adopted some recommendations such as sanitary conditions, maintenance of temperature of cold storage, personal hygiene of workers and glazing of the materials before freezing, recommendations regarding packaging material for IQF shrimp, and containers for transportation showed lower level of adoption.[122] Recently, the U.S. government enacted a new legislation intended to enhance the security of the U.S. food sources. The legislation requires all food facilities exporting to the United States to register with the U.S. FDA. The new law entitled Public Health Security and Bioterrorism Preparedness and Response Act of 2002, aims to track the origin and destination of food to evaluate credible threats through food chain.[123] These measures present good opportunity for producers to take the initiative to demonstrate competence and interest in assuring product safety. While developing novel products for trade, it is important that the protocols are strictly adhered to gain mileage in building up seafood trade. Table 2.7 gives key areas for quality control for food processors. In summary, the quality of seafood is influenced by a number of factors including the various environmental hazards that the commodity is exposed to while harvesting, processing, and marketing. Utmost care is required while developing seafood products for domestic or export markets.

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3 Bulk Handling and Chilling

3.1 Introduction

The prevailing situation of diminishing global fish stocks demands that harvesting of fishery products be integrated with optimal onboard handling techniques to ensure minimal wastage and transportation of the catch in prime quality to processing plants. The rough and primitive methods during fishing and postharvest handling still existing in several parts of the world are major causes of quality deterioration and wastage of the harvest. A number of factors such as nature of fishery, type and size of the vessel, and the final product intended from the harvest may decide the nature of handling onboard.[1–5]

3.2 Coastal Fishery

Coastal fishing generally is of short duration for a day or two. Because of this, the catch is invariably of good quality, unless it is handled roughly. The landings often comprise of several species, with exception when selective gear is used. The quantum of the catch comprising of a variety of species, is small and often in a state of rigor when landed. In these situations, gutting and icing may not be employed. Handling, if any, involves shielding the fish from the sun to keep it moist and ward off the flies. Since the catch is of mixed varieties, it is sorted by species and size wise to remove the undesirable species. The high-value species are then washed and stored in boxes under ice, since quality is better maintained in boxed than in bulk-stored fish. Exposure, even for a few hours, to high ambient temperatures can shorten the acceptable shelf life of fish. With increase in fishing duration, fish remain at ambient temperature for 12–18 h or more, which necessitates improved handling methods. In developed countries, icing in insulated containers is being practiced. Because of economic reasons, ice is not being used by developing countries; however, the situation is changing and fishermen have started giving more care to the catch.[5]

Coastal fishery also provides fish and shellfish in live state. Mollusks, for example, can survive out of water for extended periods. Hence it is possible to deliver it to the consumers in live condition. Reducing the temperature of the molluskan catch onboard can prolong their lives. Crustaceans are usually placed in suitable containers onboard after the harvest. The claws of lobsters are kept tied with rubber bands to prevent injury among the animals. Cooling the catch using chilled seawater (CSW) lowers the metabolic rate, which in turn reduces the oxygen demand and the aggressive nature of the animal.

```
        Pelagic fish          Demersal fish
                  ↘      ↙
                    Catch
                      ↓
                Transfer of catch
               from gear to vessel
                      ↓
             Holding before handling
                ↙              ↘
                          Sorting by species
                                ↓
                        Bleeding/washing/gutting
         Sorting/grading               ↓
                              Grading by size
                ↘              ↙
                Transport to hold
                      ↓
                    Chilling
                      ↓
                  Chill storage
                      ↓
                   Unloading
```
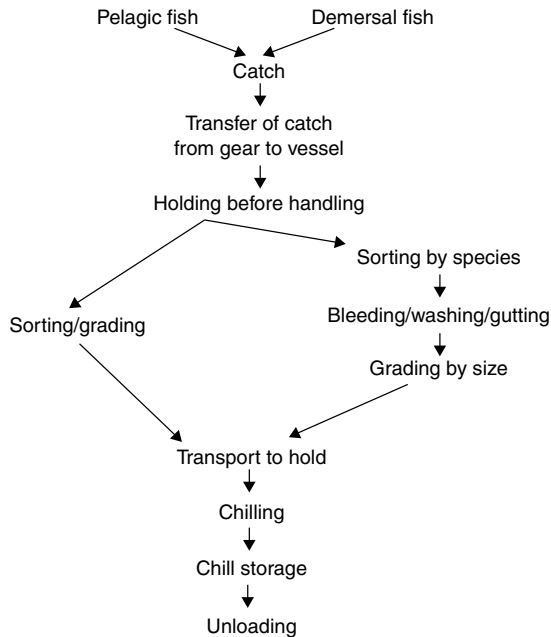
FIGURE 3.1 Unit operations in fish handling (*Source:* Venugopal, V., Doke, S.N., and Thomas, P., *Crit. Rev. Food Sci. Nutri.*, 39, 391, 1999. With permission)

3.3 DEEP-WATER FISHERY

Trawlers and purse seiners are the most common deep-sea vessels. Longline fishing is used for high-value species such as tuna. The fishing voyages by the vessels last up to a week or even more. Large catches of pelagic fish are generally caught by purse seiners, whereas, trawl catches include both pelagic and lean demersal species. The deep-sea vessels engaged in fishing voyages of longer duration have better facilities for processing such as chilling, freezing, recovery of meat, and even making *surimi*. Freezer capacity of the vessel is generally designed on the basis of an average day's catch. Batch type freezers with fixed time cycles are common onboard. Modern commercial fishing operations aim at harvesting only selected, important species, in quantities permitted by the regulatory authorities, reduction of by-catch and juveniles with the help of suitable devices, and rapid handling of the catch, including steps to improve the quality of the raw material.[4,6,7] The unit operations generally involved in harvesting and postharvest handling of seafood are shown in Figure 3.1.

3.4 COMPOSITION OF THE CATCH

The catch generally is a mixture of demersal and pelagic species, depending upon the method employed for fishing. Large catches of small pelagic fish including

species such as capelin (*Mallotus villosus*), anchoveta (*Engraulis* spp.), sprat (*Sprattus sprattus*), herring (*Clupea harengus*), sardines (*Sardinella* spp.), mackerel, and several other species are caught by purse seiners. These fish are susceptible to rancidity and loss of quality, if they have been fed heavily prior to harvest. Exposure of small pelagic fish to air during handling and storage may also bring about rancidity problems. When fishing trips exceed a couple of days, the fish stored at ambient summer temperatures deteriorates rapidly. Bulk storage of fish onboard may also induce anaerobic conditions with accumulation of ammonia, hydrogen sulfide, and other compounds. Such quality problems can adversely affect the ultimate use of the species for processing. While some small pelagics are sold as gutted or fillets, or used for curing and canning, a large portion of the catch is currently reduced to fishmeal and oil. The annual global catch of small pelagic fish exceeds 40 mt and comprises nearly half of the world's marine fish harvest, while 10 mt comprises of unidentified and miscellaneous species.[8]

Most pelagic species are of poor commercial value, and hence, they receive less attention and are often landed in poor condition. The main reasons for their poor acceptability include unappealing shape and taste, smaller size, presence of small bones, high oil content, and susceptibility to rancidity.[9] Negative consumer reaction to these fish species may not be actually correlated with their intrinsic characteristics.[6] Some of these species, while still remaining underutilized, may also be overexploited as they represent a major portion of the by-catch. For example, pelagic sharks constitute a major by-catch of sword fishing in the Atlantic and Gulf of Mexico regions and are overexploited.[7] Proximate compositions of several underutilized fish species suggest their potential role in providing high-quality protein as well as other nutrients.[10–16] Nevertheless, process development for underutilized species demands a thorough knowledge of each of these species with respect to their biology, proximate composition, catching technique, maximum sustainable yields, product development, marketing, and industry response.[17] Therefore, it is important that these fish also receive good handling care onboard.

The environmental hazards with respect to capture fisheries are overfishing and by-catch. Efforts to control overfishing include implementation of moratorium and quota systems on endangered species. It is a common practice that during fishing of commercially important species such as shrimp, a large amount of the fish is incidentally harvested as "by-catch." While the highly priced species is carefully tended to onboard vessels with ice, the by-catch on the other hand are often discarded into the sea or heaped on the deck of the vessel without any preservation, for eventual use as poultry and animal feed. It has been estimated that during shrimp trawling, five times the weight of the shellfish is caught as by-catch.[2] In some regions, the ratio could be as high as 20:1.[10] Wastage of fish as by-catch has been a subject of great international concern.[11] While efforts have been made to reduce by-catch using selective gear, many of these fish species have started attracting acceptability in some countries due to reasons such as population growth, increase in consumption, and shortage of preferred fish species.[2]

3.5 QUALITY CHANGES OF THE CATCH

The consumer acceptance of fish in the market is determined by their freshness, mainly the appearance and odor, while in the case of processed products, flavor and texture are the determining factors.[12] It has been recognized that fishing season as well as techniques of harvesting influence quality of fishery products and postharvest handling.

Catching techniques and season influence color, blood clot, injury, odor, texture, and overall grade of raw cod. Sensory analysis showed that the method of catching significantly affected color, browsing, and final overall grades. Muscle pH was significantly affected by method of catch and season. The season also influenced the texture of cooked muscle.[5] The temperature of seawater may affect the protein composition of the muscles. At water temperature around 0 to $-2°C$ the content of extractable protein in the muscle was about 50% higher than in fish caught at 10 to $12°C$[18]. Fish caught in warmer waters tend to keep better during chilled storage.

3.5.1 Influence of Season

The season in which the fish are caught can have a profound influence on their properties.[3] Normally, fish are at their optimum health during the period between the end of the main feeding season and the initial stages of gonad development, and at their poorest condition just after spawning.[13] The fat content in pelagic fish is influenced by season, which can go up to 30% in the case of some species. In spawning fish, much of the lipids and proteins in the muscle are used for either eggs or sperm. As a result of this, fillets tend to become softer and watery. In addition, once spawning is complete, fish need to replenish their depleted protein and energy stores. This, in turn, may lead to build-up of muscle glycogen and consequently lower muscle pH, adversely affecting the muscle quality. The ultimate pH of the fish tissue may have a direct impact on the texture of fresh and frozen fish. Lower pH in cod makes the fillet tougher and drier as a result of decreased negative charges on the myofibrillar lattice, associated expulsion of water, and contraction of the filaments. This results in poor texture and storage stability.[14,15] A higher pH in the fish, on the other hand, encourages development of an unacceptable flavor before textural toughening becomes a problem.[16] As salmons near their spawning grounds, their lipids are used to produce eggs and sperm, their protein content decreases, and moisture content increases. So, for the harvesting of salmons, the farther the spawning stream the better the quality.

3.5.2 Deck Temperature

Storage temperature exerts a profound influence on the shelf life of fresh fishery products. The temperature of the fish immediately after catch is that of the water, which slowly changes to that of the air onboard. Under tropical climates, this

rise in temperature is quite rapid, which adversely affects the fish quality. Lack of immediate onboard chilling and poor cold storage facilities enhance autolytic changes and spoilage of fresh fish. Belly bursting is a major adverse effect of autolytic changes in pelagic fish such as anchovies, which have significant proteolytic enzyme activities in the intestines.[19] while ideally the temperature of the catch should be immediately brought down to 0–2°C, it may not be the case in all instances. It has been generally observed that for every hour fish are held on deck at 14–18°C, there is a loss of one day's shelf life.[30]

3.5.3 Hygiene

Importance of hygienic conditions onboard the fishing vessels to ensure the high quality of the catch needs no emphasis. The possibility of contamination and the survival of psychrotropic pathogenic bacteria have a detrimental effect on the hygienic quality of the catch. The recommendations of the Codex Alimentarius and the Food and Agriculture Organization stress the necessity of strict observation of hygienic quality of the catch.[21,22] Rational design of fishing vessels, use of easily cleanable materials that are resistant to bacterial attack, strict sanitizing procedures as well as new systems of handling the catch are among the major steps required to improve hygienic standards.

One of the common ways of sanitation of fishing vessels and utensils is washing with aqueous chlorine (hypochlorite OCl^-; $HOCl$). It has been recognized that this may be objectionable because of health concerns related to trihalomethanes and other reaction products generated during the treatment with aqueous chlorine. An alternative disinfectant, particularly in Europe, is chlorine dioxide (ClO_2). The compound has bactercidal, viricidal, and fungicidal properties. Washing fish with water containing 100 to 200 ppm ClO_2 can significantly reduce its bacterial load. However, a lower concentration may be used in certain fish such as snapper and red grouper to avoid skin discoloration. The disinfectant can also find applications in depuration systems for bivalves.[23] Another compound is ozone, which is perhaps the strongest commercially available oxidant and disinfectant. It rapidly destroys gram-negative microorganisms including *Escherichia coli*, coliform, and also viruses and parasitic cysts. An ozone concentration of 0.3 g/m^3 is considered appropriate for sanitizing chill storage rooms. Although extensively used for water sanitation, its use in foods has recently emerged since it leaves no residue and taste in the treated food product.

3.5.4 Harvesting Conditions

Different types of gear and fishing methods have specific effects on the biochemical state of the fish muscles just after catch. Rigor mortis commences earlier and lasts longer in trawled fish than in fish of the same species caught by handline. Fish trapped in a gill net may be in an advanced state of postmortem changes, or else may be still alive when hauled on board, depending on the time elapsed between

capture and hauling. The same situation prevails on inshore fishing vessels using seining, droplining, longlining, and mesh netting. The quality of Atlantic krill is significantly affected by trawling and the size of the hauls. In large hauls of up to 15 t taken in about 15 min of trawling time, some krill are still alive when brought onboard. However, after several hours of trawling, even in very small hauls, the krill is to a large extent crushed and broken.[7] Atlantic salmon caught by seine and gill net are handled as much as 20 times before reaching the distributor, compared with trawler caught fish, which is handled 3 to 4 times only.[24] This can have a significant impact on the fish quality. Many times, the fish, especially aquacultured species, may not be harvested at the highest quality. Since management decisions are often made to preserve the stock, the processing plant receives fish with wide variations in quality.[25]

3.6 Onboard Handling of Catch

Mechanical graders are now available for grading the catch onboard. A typical example is the cylindrical grader. The fish pass through the length of the cylinder, while the smaller size industrial fish fall through the cylinder into a trough equipped with a continuous supply of small pieces of ice to chill the fish. The larger fish passing through the rotating grader are a mixture of many species and sizes.

3.6.1 Chilling Onboard

For prolonged shelf life, it is essential to chill the fish immediately after catch. Immediate chilling of fish affords good quality products that could be stored up to 8 days.[3,4] For every 10°C reduction in temperature, the rate of deterioration decreases by a factor of 2–3.[13] However, chilling of large catch of small-size fish species many not be possible sometimes. Physical damage such as belly burst occurs if icing is delayed. Delay in icing is of particular concern in the case of fish of the Scrombridae family because of the possibility of histamine poisoning, a chemical intoxication resulting from decarboxylation of histidine.[26,27] When large catches are handled, it is necessary to prechill the catch before holding in deck-pounds using ice, refrigerated seawater (RSW), or a mixture of ice and seawater. Prechilling systems are mostly used on pelagic trawlers, which size-grade their catches before storing in boxes or in portable RSW containers. If no sorting is done onboard, the fish is conveyed directly to the chilled storage in the hold. The effectiveness of temperature exchange depends on the thickness of the layers of fish and the distribution of ice. For example, an 80-mm layer of fish requires 2 h to lower its temperature from 17 to 10°C when exposed to double-sided cooling in ice. For transportation extending up to 24 h during the cold season, when the ambient temperature is less than 10°C, 1 kg of flake ice is sufficient to cool 8 kg of fish. When ambient temperature exceeds 10°C, 1 kg of flake ice suffices for only 4 kg of fish.

3.6.2 Dressing

Struggling of the fish, particularly commercially important species, in the catch results in depletion of high-energy phosphate and glycogen reserves, leading to rapid onset of rigor and quality loss after processing. The more a fish struggles during catch and killing, the faster the pH fall after death. A pH of about 6.0 encourages protein denaturation of muscle during frozen storage.[15] It is possible to minimize struggling in large-fish species to get a better product by instant killing, preferably by spiking through the brain. This technique has been shown to yield a superior product, commanding much higher prices, for example, in the case of New Zealand snapper.[33] Administration of anesthesia (AQUIS-S[TM]) and also conservative handling can minimize struggling to give rested fish, as observed in the case of salmon.[34] The technique has been recently extended to harvest yellow-eye mullet (*Aldrichetta forsteri*) and snapper (*Pagrus auratus*) in a "rested" state. Hyperbaric and hyperoxic storage extended the prerigor period in the rested white muscle by delaying postmortem changes. Prolongation of prerigor could be maximum in rested snapper muscle.[35]

Dressing operations of the catch including heading, bleeding, and gutting have to be carried as early as possible without significant bacterial contamination of the flesh. Shellfish, both mussel and crustacea, are not normally gutted on capture, and where possible, are kept alive until sold to the processors, because their muscle spoils very rapidly after death. The larger fish are sorted and gutted by hand, washed, and iced. Gutting of high-value fish can be done mechanically, provided the catch is of uniform size. Gutting helps to remove digestive enzymes responsible for early autolytic changes, foul-smelling compounds in the guts and also prevents entry of nematodes from the intestines into the muscle tissues. Furthermore, it prevents accumulation of bloodstains and controls hemoglobin-catalyzed lipid oxidation in the fillets.[28] On factory ships or large trawlers, small white fish may be filleted without gutting. In most North European countries, gutting of lean species is compulsory, since gutted fish have longer shelf life than their ungutted counterparts. Fatty fish are not gutted at sea prior to storage. The roes are either iced or frozen for further processing onshore.

Bleeding and gutting can help in extension of chilled-storage life. Effective bleeding onboard also preserves the desirable color of white fish flesh. Another advantage of bleeding is inhibition of hemoglobin-catalyzed degradation of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (HCHO) in gadoid fish, the blood of which contains high TMAO-reductase enzyme activity. On factory ships or large trawlers, small white fish may be filleted without gutting. Some trawlers keep certain species such as cod alive in intermediate storage, to supply a fresh fish market.[29] Bleeding and gutting extended the shelf life of dogfish.[24] Heading and evisceration of Atlantic croaker and gray trout helped retain their top quality during ice storage for 7 to 10 days longer than that of uneviscerated fish.[30] Fatty fish such as mackerel are not gutted at sea. Problems may arise with ungutted fish, such as herring, mackerel, sardine, and the like, during periods of heavy feeding due to belly burst. These fish therefore spoil quickly if not chilled immediately after harvest.[31] Belly bursting of pelagic fish could be controlled if

the pH of the medium is reduced to 5.0 by the addition of lactic or acetic acid.[32] Roe are either iced or frozen for further processing onshore.

The onset of rigor mortis has implications in processing because handling or filleting fish prerigor or inrigor can change the product properties and quality.[29] Commercial processing of fillets usually starts after the resolution of rigor mortis, which often delays production for 2–4 days. When filleting fish, the fillets need to be left untouched on a smooth surface and protected from surface drying, while rigor lasts. Fillets made from postrigor fish do not show shortening. On the other hand, prerigor filleting can lead to shortening of fillet length. Maximum contraction of salmon fillets reached after 12 h at 20°C, while it took up to 40 h at 0–10°C.[29] Most white fish are filleted and sold as such or in some form derived from fillets.[3]

3.6.3   Onboard storage

Traditionally, three methods of fish storage onboard are used, namely bulking, shelving, and boxing. Generally, fish are stowed in bulk on ice onboard the vessel. Bulk stowage, however, is rarely used for high-value fishes. Stowage should be in well-drained, shallow layers of fish and ice; deep layers cause loss of weight and some damage to the fish. For a day's fishing, with small boats on short trips delivering to a larger facility (mother ship or shore plant), ice may be the best way to chill the catch promptly. The larger facility's ice system supplies the smaller boats with enough ice. Fish stowed in plenty of ice are typically at a temperature of −0.5°C. Generally, fish and ice are mixed in a ratio of 1:2 in temperate regions, while in tropical climate, a ratio of 1:1 has been the practice. If the stacks are more than 5 ft deep, the fish near the bottom may be damaged. This can be prevented in shelving, where fish and ice are held between shelves of corrugated aluminum, usually 18–22 in. apart.[36] Shelving is primarily used for gutted larger species that are placed on the ice with the gut cavity facing down. For storage, separate holds are usually provided in each trawler.[37]

Boxing offers the best method for onboard storage of fish. Fish of the same size and species are usually placed in the same boxes. These boxes could be held one over another and are stacked without damaging the fish. Ice stored at ambient temperature contain 12–20% water on its surface depending upon the nature of the ice (flake, crushed ice, or chips), which has a negative cooling effect on fish. This water is useful only to improve the heat transfer and to keep the fish moist.[27] To compensate for water loss from melting and bad handling, it is advisable to add about 12–20% extra ice to the fish.[38] Even when fatty fish are boxed and well iced, they can be kept only for 5–6 days, in contrast to gutted haddock or cod, similarly stored will keep for 12–15 days.[3] Containerization in box lots of 50–250 kg capacities facilitates rapid and easy unloading of the catch from the vessel. Insulated containers have added advantages of better fish handling and less requirement of ice. On small boats in which the boxed-iced fish has to be stacked on the open deck, an insulated cover is desired to reduce melting of ice and hence there is demand for ice. Suggestions for ideal bulk stowage and boxing and designs

TABLE 3.1

Some Quality Problems in Bulk Handling of Large Catch of Small Fish

Problems onboard	Surface oxidation, rancidity, belly bursting, formation of excessive drip containing blood, oil, slime, etc. Quality loss depending upon delay in chilling and high fish temperature. Anaerobic conditions in bulk fish during storage. Formation of ammonia, etc. depends upon fish temperature
Chilling systems	*Ice:* Traditional method
	RSW: Seawater in a tank is chilled to 0°C and the fish is loaded into it up to 70% and remaining with seawater. The water is passed through heat exchangers for chilling
	Champagne system: Insulated tanks equipped with perforated air pipes on the bottom and connected to an air compressor. Ice is used for chilling seawater. The fish from the seine is loaded, up to 65% capacity; the air is blown through the pipes to mix ice and water for chilling to 0°C
Container storage	Galvanized plate iron containers equipped with fiberglass-coated polyurethane insulation and air pipes. Protect fish from time of loading to production. Usual capacity, 800 kg. Good for direct transportation
Unloading	*Scooping*: Scoops generally unload fish stored in RSW tanks.
	Dry suction pumping: Good for small fish such as sardines.
	Wet suction pumping: Needs pumping of water to keep the fish floating during unloading. Can cause spoilage and microbial contamination if chilled water is not used or the chilling is delayed

Source: Adapted from Hansen, P., *Infofish Mark. Dig.*, 2, 34, 1983; Jensen, J.G., *Infofish Mark. Dig.* 3, 38, 1983. With permission from *Infofish*.

of fish rooms have been provided.[27] Table 3.1 indicates some quality problems in bulk handling of large catch of small fish.

3.7 FREEZING AT SEA

Modern trawlers are equipped with facilities for freezing and frozen storage of fishery products. For freezing, the fish must be of high quality, properly headed, gutted, and washed. For best quality product, a freezing temperature of −25°C or lower is recommended. Blast freezers or plate freezers can be used for freezing. When the fish is frozen prerigor, thawing may be accompanied by thaw rigor, which is characterized by rapid and strong muscle contraction causing high drip loss, gaping, and a firm texture.[39] High-quality frozen cod fillets have been prepared on fishing trawlers, whose quality could be monitored by sensory assessment, measurements of salt-soluble protein, DMA and free fatty acid, and also water-holding capacity (WHC).[40]

3.8 UNLOADING THE CATCH

For unloading small pelagic fish, mid-water trawlers and purse seiners use pumping systems onboard. The fish are unloaded from the net by elevator or pressure/vacuum pumps. This considerably reduces bruising of the fish, which otherwise may induce an increased rate of rancidity development on subsequent frozen storage.[37] Unloading by using fish pumps is becoming more popular worldwide. These pumps may be elevators, dry suction pumps, or wet suction pumps, the latter two being widely used for handling fish for human consumption. Pumping is highly suitable for unloading small-size fish such as menhaden, sardine, and herring. Many of the wet suction pumps cause less damage to small fish than do the dry suction pumps. Most purse seiners in northern Europe, particularly in the Scandinavian countries, which land herring and mackerel for the food industry, employ scoops for unloading at a rate of about 30 to 50 t/h. Mechanization and use of fish pumps are effective to reduce handling delays. Automatic unloading pumps have been used for rapid transfer of RSW stored anchovies, sardine, and jack mackerel to the processing plants. Fish stored in ice-cold seawater in containers onboard, may be lifted by a crane and transported to the plant by truck. Bulk handling and chilling of a large catch of small fish has been discussed in detail.[27,36,41–44] General handling, processing, and preservation methods for cephalopods have also been provided.[45]

3.9 EFFECT OF HANDLING ON QUALITY

Methods of handling significantly influence on fish quality and the yield of fish fillets. The fillet yield, defect levels, thaw drip, cooked texture, and the bacteriological content of rock sole (*Lepidopsetta bilineata*) handled and processed under different conditions have been examined.[46] Boxing, unloading by hand, and hand filleting favor the highest quality fillets, while bulk-stowed, wet pump-unloaded fish give the lowest quality of fillets. The lowest bacterial counts were found in machine-filleted, wet-trimmed fish, which also has a firmer cooked texture. Prerigor filleting of fish gave the highest yields with least drip loss but underwent gaping and had the shortest shelf life. Lowest yield was obtained when fish were filleted after 3 days of ice storage. Shelf stability of fillets could be improved by a dip in carbonic acid before packaging. For example, cod fillets dipped in carbonic acid had a shelf life of 7 to 21 days.[47]

Atlantic salmon fillets from fish processed pre-, in-, and postrigor stages were compared for microbial growth, odor, water loss, texture, and gaping in two separate experiments. Salmon were chilled to a core temperature of $< 2.5°C$ prior to slaughter, and stored gutted at $0°C$ on ice. Fillets were made from prerigor (4 h after slaughtering), in-rigor (2 days), and postrigor (5 days) fish, and were stored vacuum packaged or exposed to air on ice at $0°C$ up to 14 days. The prerigor fillets had lower bacterial numbers (total viable counts, H_2S-producing bacteria and psychrotrophic bacteria) when stored in air and for most samples in vacuum. The

prerigor fillets were also characterized by higher flavor, lower gaping, and higher water loss compared to postrigor samples. The results show that prerigor filleting of Atlantic salmon is a preferable alternative to traditional postrigor filleting.[48] In Norway, the processing of prerigor saithe (*Pollachius virens*) is an established industry.[29]

Proper and speedy handling of tuna with efforts to minimize bruising is important for its market value for use as *sashimi* and *sushi*, in Japan. *Sashimi* is a Japanese word for raw seafood having natural appearance, texture, and flavor. *Sushi* means snacks made from rice that are seasoned with vinegar, salt, and sugar. The most common fish used for *sashimi* is red meat fish, especially tuna, bonito, and salmon, while *sushi* is raw seafood served as bite-sized pieces in soy sauce or in a small ball of rice. About 70% of tuna traded in Japan is consumed as *sashimi* and *sushi*. The tuna species preferred for *sashimi* and *sushi* are Northern hemisphere bluefin (*Thunnus thynnus*), southern bluefin (*T. maccoyi),* bigeye *(T. obesus)*, and yellowfin (*T. albacares*). Skipjack tuna (*Katsuwonus pelamis*) is rarely used for *sashimi*. For these products, tuna must be killed by driving a spike into the brain, within a few minutes of landing. The fish is bled, dressed immediately to remove gills, gut and fins, and chilled in ice slurry. Taste and color of the flesh deteriorate in yellowfin and bigeye after about 12 to 14 days at 0°C.[49]

Machines are available for faster processing of fish, which include gutting, washing, heading, dressing, splitting, filleting, skinning, and deboning. The advantages of mechanized fish processing are saving in labor costs, rapid handling of the catch, particularly in times of glut, and better quality of the products. The machines, however, should suit the wide variety of fish species, different in size, shape, and texture, and also have the desired capacity of production.

3.10 CHILLING OF FISHERY PRODUCTS

The common chilling media for fish are wet ice, RSW, CSW, solid and liquefied forms of carbon dioxide (CO_2), liquid nitrogen, and chill air. It has been recommended that fish after catch should be cooled to the temperature of melting ice (0°C) as quickly as possible.[44] For tropical fish species and for species that are naturally exposed to wide seasonal variations in temperature, chilling too slightly above 0 ± 1°C has been suggested. Low temperature slows down the rate of autolytic and oxidative spoilage mechanisms and retards the growth of contaminating microorganisms. Endogenous spoilage processes are delayed as well as growth of spoilage and food-poisoning bacteria are significantly retarded. Chilling only slows down the microbial activity, because psychrotrophic species are able to survive in chilled fish. A chill temperature of -1 to -2°C is ideal to control the psychrotrophic organisms. In chilled fish, the lag phase of psychrotrophic and mesophilic bacteria is prolonged, from hours to days, depending on the bacterial species as well as the temperature at which the fish is stored. However, once the lag phase is passed, psychrotrophic bacteria will grow at near maximal growth rate upon entering the logarithmic phase. The generation times of mesophilic bacteria

are significantly longer in chilled foods as compared to growth at optimal temperatures. Temperature abuse can lead to rapid spoilage due to autolytic reactions as well as bacterial action, both of which have economic consequences.[50] A reduction in the storage temperature from 5 to 0.6°C has been observed to double the shelf lives of several fish species. Reducing the temperature from 0 to −2 to −4°C extends the shelf life of lean fish from 2 to 4 weeks. By reducing the chill room temperature from 5 to 3°C, the requirement of ice can be reduced by about 40%, resulting in a considerable increase in fish storage capacity.[38]

3.10.1 Chilling by Ice

Cooling by ice is the most widely practiced chilling method. Slowly melting ice, through the washing action of the melt water, maintains the fish in a condition where quality changes are minimal. The extension of shelf life by chilling is essentially due to the reduction in the growth rate and metabolic activities of spoilage causing microorganism.[51] The fish catch prior to chilling may be washed to remove the contaminating microorganisms, slime, and blood. Spray washing may help rapid cleaning of fish. Although the ideal temperature of chilled fish is 0 to 2°C, even at this temperature some activities of endogenous enzymes as well as proteolytic enzymes of contaminant microorganisms persist spoiling the fish at these temperatures.

Ice is available in several forms such as blocks, plates, tubes, shells, soft, and flakes.[44,52] Of these, flake ice is the most popular for industrial use because of its advantages related to cooling efficiency and economics of production. It is also dry and hence, will not stick together or form blocks when stored for long periods of time at subzero temperatures. Cooling capacity of this type of ice is more due to a large surface area for heat exchange. Flake ice also reduces less damage when pressed into the flesh of surrounding seafood. Figure 3.2 shows cooling curve for cod in boxes containing different types of ice. Flake ice production consists in freezing a thin layer of water on the cooled surface of a cylindrical evaporator and then scraping off the ice with a knife. The ideal freshwater ice has a temperature of 0.5°C. It stays loose and workable and can be stored without refrigeration. Flake ice may be made from potable water or seawater.

Ice is stored in multiton bins, silos, or houses of several types, with mechanical or pneumatic means of delivery. Mechanical rakes, screws, and other conveyors involve safety and maintenance issues. Pneumatic systems are more flexible and perhaps safer, but they may require the air to be chilled, which adds another step of refrigeration. In industrial plants, fish kept in ice flakes are generally stored in a chill room at 5°C, where fish is retained for about 12 h.

3.10.1.1 Ice requirement

The quantity of ice required to chill a batch of fish can be calculated as a function of both the quantity as well as temperature of the lot, and is represented by
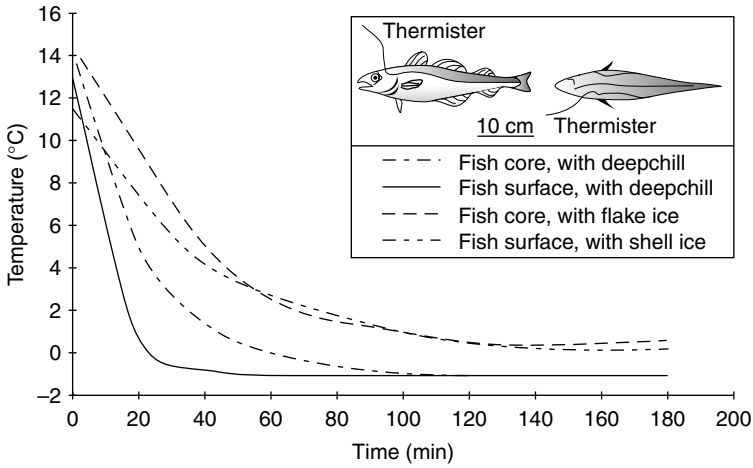
FIGURE 3.2 Cooling curves of cod in boxes with different types of ice (From Wang, M.-J., *Infofish Int.*, 2, 45, 2003. With permission from *Infofish*)

the equation,

$$C_{pf} = \frac{T_f \times M_t}{80} \qquad (3.1)$$

where, C_{pf} is specific heat of fish (kcal/kg), (approximately 0.80 for lean fish, 0.78 for medium fatty fish and 0.75 for fatty fish), T_f, fish temperature, usually taken as seawater temperature, M_t, mass of fish and 80 is the latent heat of fusion of ice (kcal/kg). Based on the equation, the amount of ice required to cool 1 kg of lean fish from 25 to 0°C would be approximately equal to 0.25 kg. While cooling fish to 0°C, some thermal loss of ice also takes place, which results in melting. Thermal loss of ice depends mainly on the external temperature and the type of container in which the iced fish is stored. Thermal loss of ice (L) can be expressed as,

$$L = k \times T_e \times t \qquad (3.2)$$

where, k is specific ice melting rate of the container, T_e, average external temperature, and t, time elapsed in hours since icing. The value of k can be determined experimentally for the insulated containers. The values of k (kg of ice per day) for plastic box (polyethylene, 40 kg capacity) and insulated container (Metabox 70 DK) have been found to be 0.22 and 0.108, respectively.[53,54] It has been observed that thickness of fish layer influences the cooling time. Thus, fish at the center of the layer with thickness of 10 cm takes 4 h to cool down from 10 to 2°C. The cooling times for thickness of 15, 20, and 60 cm are 9, 14, and 120 h, respectively.[27] The ice to fish ratio in tropical climate is almost 1:1, whereas 1.5 kg of ice is required for each kilogram of fish for chilling during collection from artisanal fishermen
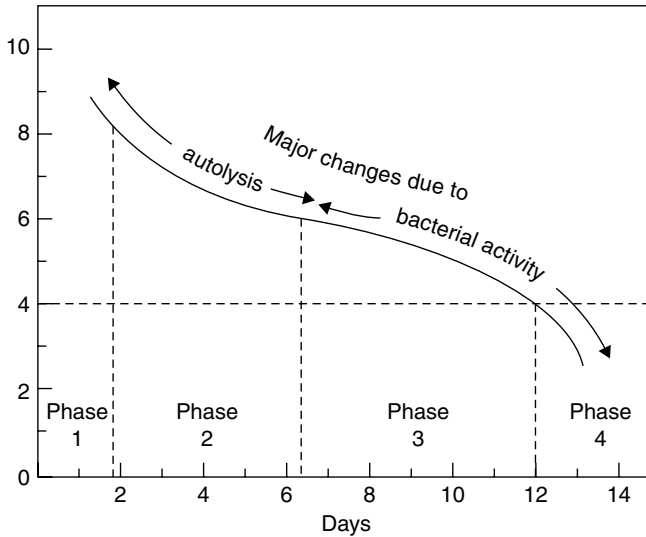
FIGURE 3.3 Changes in typical quality of iced fish with storage time (From FAO Fisheries Technical Paper 348 Huss, H.H. (ed.) With permission from Food and Agriculture Organization of the United Nations Rome.)

and at collection centers. At the processing center, an amount of 2 kg is required for chilling 1 kg of fish, while 4 kg of ice is required for chilling each kilogram of shrimp.[27]

3.10.1.2 Shelf life of iced fishery products

Freshly caught fish undergoes quality changes as a result of autolysis and bacterial activity, shown in Figure 3.3.[55] Extent of these changes with time determines shelf life of the product. Shelf life is defined as the maximal period of time during which the predetermined quality attributes of food are retained[56] (see Chapter 2). The chilled storage life of fish is determined essentially by sensory evaluation. Apart from temperature, chilled storage life depends on several factors such as composition, lipid content, microbial contamination, and type of microflora. The normal shelf life of cold-water fish chilled to 0°C immediately post mortem is 1–2 weeks, while fish from tropical water remains somewhat longer at this temperature. White-fleshed lean fish such as cod, haddock, and hake remain in ice for 11–13 days, whereas flat fish such as sole and plaice have a longer shelf life of 15–18 days in ice. Large fish, particularly those like halibut, seer, and tuna, which tend to have a low postrigor pH, keep for 21–22 days. Table 3.2 gives shelf lives of some commercially important marine finfish species in ice. Freshwater fish such as trout has a shelf life of 9–10 days in ice (see Chapter 12). Delay in icing can adversely affect the shelf life, as shown in Table 3.3. While optimum quantity of high-value products can be processed from fish stored in ice only for a few days, prolonged storage results in products that are of medium or low value. The "ice time," namely,

TABLE 3.2
Shelf Life of Some Commercial Fish Species in Ice

Fish species	Shelf life (days) in ice
Temperate, marine	
Cod (*Gadus morhua*)	12–15
Grenadier (*Coryphaenoides* spp.)	14–18
Haddock (*Melanogrammus aeglefinus*)	14–15
Hake (*Merluccius* spp.)	8–15
Halibut (*Hippoglossus hippoglossus*)	21
Herring (*C. harangus*)	5–7
Mackerel (*Scomber* spp.)	7–9
Plaice (*Pleuronectes* spp.)	14–18
Redfish (*Sebastes* spp.)	13–15
Skate (*Raja* spp.)	12
Tropical marine	
Emperor	15
Red snapper (*Lutjanus* spp.)	20
Grouper (*Plectropomus* spp.)	28
Spanish mackerel (*Scomberomorus* spp.)	11
Tuna	29
Sea bream (*Pagellus* spp.)	22
Bumper (*Chloroscombrus* spp.)	22
Catfish (*Netuma serratus*)	20
Threadfin bream (*Nemipterus* spp.)	21
Purple emperor (*Lethrinus* spp.)	25
Stripped mullet (*Mugil cephalus*)	21
Grunt (*Pomadasys* spp.)	27
Gray mullet (*Liza subviridis*)	29

Source: Adapted from Ninan, G., *Product Development and Seafood Safety*. Central Institute of Fisheries Technology, Cochin, India, 2003, pp. 44–57. With permission.

the days of preprocessing storage of raw fish in ice, and the remaining shelf life and further utility of the fish can be computed.[57] Figure 3.4 shows a general pattern of change in utility of raw fish with respect to preprocessing ice storage. Considering a maximum yield of 35–50% of finished product from the raw material, the utility of raw fish does not change within the first 5 days on ice, and yields high-value products up to 35%. After this period, the yield of high-value products decreases to give rise to more medium-value products. Beyond 9 days of ice storage, no high-value products could be processed. In fish stored up to its terminal shelf life in ice, only low-value products could be produced. Consequently, a change in utility will cause a change in the profit margin per kilogram of raw material.[57] The shelf life of fish can be profoundly affected by changes in storage temperature. If fish having a normal storage life of 15 days at 0°C is held initially at

TABLE 3.3
Effect of Delayed Icing on the Storage Life of Some Tropical Fish and Shellfish

Species	Delay (h)	Storage life in ice (days)
Mackerel (*Rastrelliger* spp.)	0	9
	3	7
	6	4
	9	1
Tilapia (*Oreochromis* spp.)	0	16
	4	13
	8	5
	12	<1
Milk fish (*Chanos chanos*)	0	14
	4	12
	8	6
	12	<1
Oil sardine (*S. longiceps*)	0	7
(Caught during November–December)	3	5
	6	1
Prawn (*Penaeus indicus*)	0	16
	3	14
	6	9
	9	4

Source: Adapted from Ninan, G., *Product Development and Seafood Safety*. Central Institute of Fisheries Technology, Cochin, India, 2003, p. 44. With permission.

10°C for one day and at 5°C for 2 days, before keeping at 0°C for the rest of the storage time, the likely shelf life can be calculated. This is by considering one day at 10°C is equivalent to 4 days at 0°C, and 2 days at 5°C is equivalent to 4.5 days at 0°C.[27] Cephalopods, being highly perishable due to autolysis and bacterial spoilage, should be chilled immediately after the harvest till it is consumed. Although icing is the economic method of preservation, the shelf life depends upon the quality of ice, method of icing, and the storage time. Being delicate in nature, flake ice is preferred for chilling squid, which will prevent the tissue from damage. However, in most commercial practices, dressed squid mantles are stored under crushed ice prior to freezing. Since squid contain a significant amount of water-soluble proteins the melt water from ice becomes turbid due to the leaching of these proteins together with nonprotein nitrogen (NPN) compounds. With the decrease in NPN compounds, there is a parallel increase in moisture content of the mantle for about 5%. When the total volatile basic nitrogen content of the muscle increases to 30 mg/100 g, as a result of bacterial action, the mantle becomes mushy. There is a need to keep cephalopods in ice for a minimum period prior to freezing.[58]
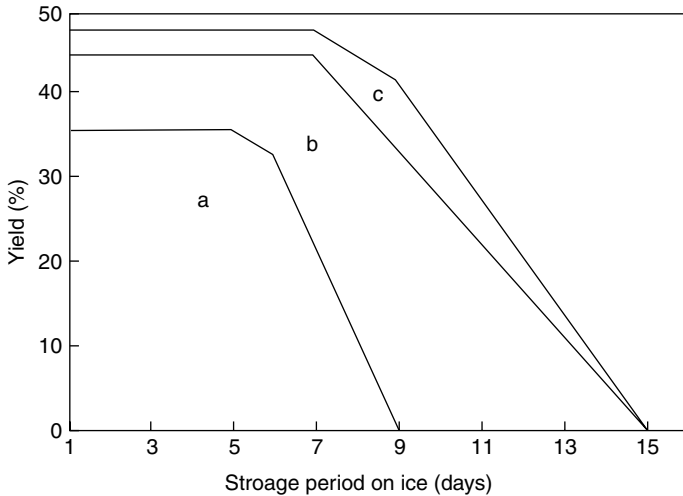
FIGURE 3.4 Influence of ice storage of fish on yield and quality of processed products: a, high value; b, medium value; c, low-value products (From Venugopal, V. and Shahidi, F., *Food Rev. Int.*, 14, 35, 1998. With permission from Marcel Dekker, New York)

Most small, cold, or temperate water shellfish like shrimp, scampi, abalone, scallop, and clam will remain edible for up to 6–10 days, and the corresponding warm water species for up to 8–12 days. Other shellfish such as oysters, cockles, mussels, crab, lobster, and rock lobster are not normally iced in the raw state. Prolonged ice storage results in defects in shrimp, as summarized in Table 3.4. These defects are important factors to be considered in determining the quality of shrimp for commercial processing. Table 3.5 gives suggested checklist for the information that should be provided when shelf life or extension in shelf life is measured or quantified.

3.10.1.3 Limitations in chilling by ice

Icing of fish has some disadvantages. These include bruising of the flesh with concurrent leaching of flavor compounds and water-soluble proteins and vitamins. Furthermore, ice may not be distributed uniformly and prolonged ice storage may lead to textural changes. Breaking strength and hardness of fillets may get reduced during ice storage. Elasticity of cod fillets was reduced, while cohesiveness of salmon increased during ice storage. Ice storage has been shown to adversely influence protein solubility, water-holding capacity (WHC), and activity of collagenolytic enzymes of salmon (*Salmo salar*) and cod (*Gadus morhua*) fillets.[59] Gelatinolytic activity associated with spoilage bacteria active during cold storage is responsible for the degradation of proteins including core protein of proteoglycans in halibut muscle, adversely altering its WHC of the fish muscle.[60] Chilling alone cannot completely arrest the activities of psychrotrophic organisms in fish, posing

TABLE 3.4
Some Common Defects in Shrimp

Defect	Description
Abnormal colors	Indicative of diseases, infestation of protozoa (color referred as milky spots) effect of mutation, pollution, etc.
Black spot	Darkened area present on either the shell or the flesh. The blackening starts as dark bands where shell segments overlap, giving the tail a banded zebra like appearance (can be prevented by dipping in dilute sodium bisulphite solution)
Decomposition	Detectable change from the normal freshly caught shrimp. Characterized by an odor varying from sweet to putrid
Dehydration	Desiccated white area on the flesh portion of the shrimp due to drying or chemical burn
Extraneous odors	Any typical odor other than that of fresh or decomposed material, such as oily, soapy, etc.
Flippers	Refers to tail fins, sometimes including the last shell segment, but containing no meat
Head, legs, and loose shell in peeled shrimp	Any one or all of head, shell and legs that may be present as either loose or attached to peeled shrimp
Balling and bare spots in breaded shrimp	Refers to adherence of lumps of the breading material to the surface of the product (referred as balling up) and lack of coating (bare spots or voids)
Blackening in canned shrimp	Caused by reaction between iron and a sulfur compound from the meat. The reaction can be prevented by inside-coating of the can, by creating high vacuum in the can or washing the meat before canning in a low acidity solution

challenges to refrigerated foods.[61] These observations point to a need for chilling of the products to subzero temperatures for enhancing shelf life.

3.10.2 Chilling Using RSW

Use of RSW provides a method to chill the product at a temperature lower than that obtained by ice (as shown in Table 3.6). The lower temperature is due to the salt content of seawater, which lowers the freezing point of water. The salt content is remarkably steady throughout the oceans at about 3.5%. However, this may occasionally vary depending upon such factors as dilution by river waters and concentration by high rates of evaporation, which in turn, influences the properties of RSW. RSW is generally used when a mechanical refrigeration unit is available to cool the water, while CSW is more often used where ice is added to seawater for cooling.[27] The process, also known as "superchilling" was originally introduced onboard trawlers in the 1960s. In RSW chilling systems, seawater is

TABLE 3.5
Check List for the Information that should be Provided when Shelf Life or Extension in Shelf Life of Fishery Products is Measured or Quantified

Definition of shelf life
General purpose of measurement
Fishing method (details on stress, catch, and postcatch processing)
Time when the fish is caught
Beginning of the shelf life considered
Time-temperature history to end of shelf life
End of shelf life considered
Destination of the product

Source: Adapted from Tolstoy, A., *Int. J. Food Microbiol.*, 13, 225–230, 1991. With permission.

TABLE 3.6
Properties of Salt Water

Salt content (%)	0	1	2	3
Freezing point (°C)	0	−0.53	−1.08	−1.64
Maximum density	1.000	1.008	1.016	−1.024
Temperature of maximum density (°C)	4.0	1.8	−0.5	−1.64
Solubility of carbon dioxide (ml/ml of water)	1.70	1.61	1.54	1.46

chilled to −1° to −2°C using a mechanical cooling unit. The fish dipped into the water, may undergo partial freezing, since freezing point of fish vary between −1 and −2.5°C.[62] The percentage of frozen water in superchilled fish is highly temperature-dependent. At −1°C about 19% of the water is frozen; 55% water is frozen at −2°C; 70% at −3°C; and 76% at −4°C.[63] In superchilled products, where up to about 50% of the water remains unfrozen, deteriorations, characteristic of frozen storage proceeds somewhat less rapidly. It may be however noted that water with a low salinity may give rise to difficulties with the cooling system, since there is an increased possibility of the build-up of ice on refrigerated surfaces, resulting in lower efficiency. When designing the layout of the RSW tank system for a fishing vessel, consideration has to be given to the stability of the vessel and the storage conditions within the tank at each stage of operation. The operation of the system should also ensure that adequate quantities of prechilled water are available for the fish and that movement of water and fish within the tank is minimal. Generally a three or six storage tank systems are used on small fishing vessels.[27] The details of onboard construction of the tanks have been provided.[37,42]

The advantages of using RSW include greater speed of cooling, reduced pressure and bruising on the fish, less exposure to oxygen, possibilities of lower holding temperature and quicker handling of large quantities of fish with little delay or labor involvement, and in many cases, an extended shelf life.[27,64,65] RSW can extend the prime quality or top-notch quality of fish from 4–5 to 7–8 days, and the normal shelf life from 14 to 18–20 days giving more time for distribution of the product.[25] RSW systems have been used for storing and transporting large quantities of sardine, salmon, halibut, menhaden, shrimp, mackerel, herring, blue whiting, and many other species.[27] This technique has been used industrially for species such as tuna and salmon.[4] The most successful commercial projects have been confined to bulk applications where the fish are to be used for canning or other industrial processes.[27] RSW systems are often used on freezer and factory trawlers, where delays can occur between catching and processing. Purse seine fishing vessels use these systems for chilling catches mainly of pelagic fish, which are pumped from the net directly into RSW tanks. RSW systems also allow longer fishing trips, improve handling, fast bulk chilling of the catch, and reduction of losses. Japanese studies have indicated a potential for successful application of the technology for seabass, carp, rainbow trout, and mackerel.[4]

Superchilling by RSW can be combined with other processing techniques. Bulk-packaged, superchilled, whole, or gutted salmon combined with a high CO_2 atmosphere maintained a high microbiological and sensory quality for more than 3 weeks (see also Chapter 6). Atlantic salmon fillets under CO_2 and N_2 in the ratio 60:40 and superchilled at $-2°C$ maintained a good quality with negligible microbial growth for more than 24 days as assessed by both sensory and microbial analyses. Superchilled mackerel and salmon in air had a 21-day sensory shelf life, whereas modified atmosphere and air-stored fillets at chilled conditions spoiled after 10 and 7 days, respectively.[64–66] The practice of injection of CO_2 into RSW system has been shown to delay spoilage by lowering the pH and bacterial activity and provides additional shelf life in the case of some fish such as rockfish and salmon.[4]

Refrigerated seawater systems, nevertheless, does have some disadvantages. In the circulating water, the spoilage-causing organisms may spread very rapidly and accelerate deterioration of the fish. Other disadvantages include excessive uptake of salt, uptake of water by species with low fat content, loss of protein, and problems with anaerobic spoilage bacteria.[37] RSW storage may impart a salty taste to several fish species due to uptake of salt from the seawater. For example, cod when held in RSW for 5, 9, and 15 days gave fillets that contained 0.3, 0.5, and 1.0% of salt, respectively.[39] Other detrimental effects on fish quality are discoloration of flesh, drip formation, hydrolysis of phospholipids, denaturation of proteins, loss of texture, and appearance on fish such as cod.[3,5,67] RSW systems may turn the eye color of some freshwater fish to opaque.[5] Cod stored at $-2°C$ for 10 days had an appearance and texture inferior to fish stored at $0°C$ in ice. The drip of the superchilled fish was increased and at $-3°C$ the texture of whole cod became unsuitable for filleting. Apart from the quality problems, RSW systems are complicated and require sophisticated equipment. RSW has found use as a cooling
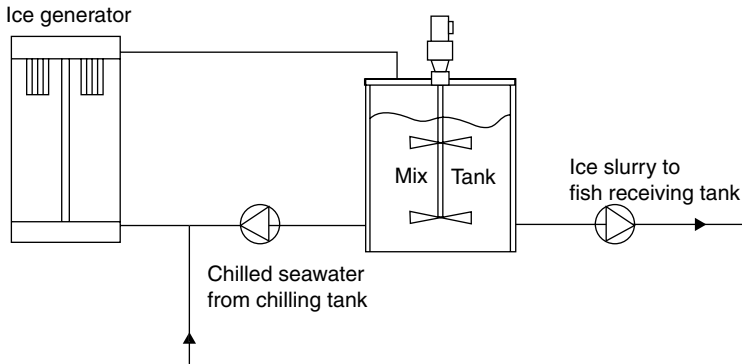
FIGURE 3.5 Schematic representation of making of chilled seawater slurry

medium only in some fisheries, particularly in situations where the disadvantages of salt uptake are not important. The cost and practical difficulty of arranging the conditions render the method unattractive for some fisheries.[3]

3.10.3 Champagne System

The problems met with RSW systems have been reduced in the less expensive "champagne system." In this system, plain water in insulated tanks is cooled by ice and mixed with fish by blowing air through pipes. For this, the tanks in champagne system are provided with perforated pipes in the bottom for air percolation and connected to an air compressor placed on deck. Air bubbling is stopped when the temperature reaches 0°C. CSW system has also been used for fish storage. The containers may be made from galvanized iron plates and insulated with polyurethane foam coated with fiber glass.[68]

3.10.4 Use of Ice Slurry

Chilling by ice slurry is a modification of the RSW system. Figure 3.5 schematically represents preparation of chilled seawater slurry. The ice slurry system developed by Sunwell, Ontario, Canada, comprises millions of microscopic spherical (usually 0.25–0.5 mm) ice crystals of pure water suspended at 20–40% levels in seawater or brine.[69] The slurry, having 20–25% ice can be easily pumped by standard centrifugal pump. The temperature of the ice slurry is a function of the salinity in the mixture and is typically −2°C. The superior cooling achieved by ice slurry is attributed mainly to the 30–40 times higher contact of the fish with the slurry as compared with flake ice. In addition, removal of air pockets in the bulk fish and movement of the slurry results in rapid reduction of the core temperature of fish. A bulk chilling of herring to −1.8°C could be achieved within 30 min, while capelin could be chilled from 11°C to 0°C in 6–8 min. The superior

cooling efficiency of the slurry has been found more efficient than that of conventional RSW systems for cooling fish such as anchovy, salmon, tuna, pollock, hoki, pilchard, and cod. The slurry having 25% ice could be pumped into fish boxes having drainage facility to remove melt water. After cooling, the fish is firmly packed in ice for the rest of the trip. In long liners fishing for high-value species like tuna, fish chilled, and stored in crystal ice are preferred for freshness. The slurry production systems can be installed onboard trawlers, long liners, purse seiners, shrimp boats, and also in onshore processing facilities. The advantages of cooling by the slurry system are: protection of the fish from skin bruising and body damage, easy handling, possibility of its preparation from seawater to have different salinity and consistencies, flexibility in placing the compact ice generation and storage equipment, and operational savings in energy and labor.[68,69]

3.11 COMBINATION OF CHILLING WITH FOOD PRESERVATIVES

Chilling can be combined with other treatments for further enhancement of shelf life. Some of the common chemical preservatives are sodium benzoate, sodium and calcium propionate, sodium bicarbonate, sorbic acid, and sulfur dioxide. Some chemicals act by preventing the growth of microorganisms while others prevent oxidation or enzymatic deterioration.[47,70,71] Dipping cod fillets in ammonium or sodium bicarbonate solution (8%) markedly reduced microbial growth after 8 days at 4°C. The treated fish had improved texture and moisture retention revealed by both objective and subjective testing, but had significantly lower aroma and overall acceptability scores.

A dip in 1 to 3% aqueous solution of potassium sorbate can extend the shelf life of fish fillets by several days.[5] The chemical is generally recognized as safe (GRAS) food preservative, by the U.S. Food and Drug Administration (FDA), which has proven effective in inhibiting spoilage causing bacteria, yeast, and moulds in chilled fish. Several polyphosphates including sodium tripolyphosphate, disodium orthophosphate, tetrasodium pyrophosphate, and sodium hexametaphosphate, are known to enhance the quality of fresh as well as frozen fishery products. The advantages of using these compounds are reduction of fluid loss, maintenance of muscle integrity, tenderness, meat particle adhesion and color, improvement of texture and flavor, promotion of protein gelation, prevention of microbial spoilage and lipid oxidation, and enhancement of cooking yields in fresh and frozen muscle. These compounds preserve fishery products by acting as metal ion chelators, altering pH, interacting with proteins to promote hydration, and WHC, preventing lipid oxidation and inhibiting microbial enzyme secretion and growth.[67,70]

The preservative action of organic acids such as lactic and acetic acids and their salts has been well documented. Sodium lactate is used as humectant and flavor enhancer in meat and poultry products, which has also antimicrobial properties. Potassium and calcium lactates are equally effective in controlling the growth of aerobes and anaerobes in meats. Antibotulinal and antilisterial activities of the lactate anion have also been established.[71] Catfish fillets dipped in chilled dilute solutions of lactic acid followed by packaging in sterile plastic bags could enhance their refrigerated shelf life, as assessed by microbiological and sensory

methods.[72] Treatment of Chilean mackerel with dilute acetic acid extended shelf life associated with a decrease in the formation of total basic volatiles.[73] Monolaurin (monoglyceryl ester of lauric acid) in combination with phosphates and sorbates has potential to preserve fish and meat. A usual formulation consists of one part of monolaurin, 20 parts of a mixture of pyro- and polyphosphate in equal amounts and 40 parts of sorbic acid. The preservative mixture is added to the product to a final concentration having 0.3% sorbic acid.[74]

A number of antioxidants, both natural and synthetic, may be used to control rancidity in food products including seafood. The natural antioxidants include extracts of plants such as cocoa shells, tea, olive, garlic, red onion skins, apple cuticle, nutmeg, mustard seed, peanut seed coat, rice hull, and extracts of spices. The synthetic antioxidants include butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and *tert*-butylhydro-quinone (TBHQ). Addition levels of these antioxidants are generally limited to 100 to 200 ppm.[75] The antibacterial properties of several essential oils derived from spices have been examined against a number of bacterial strains. The gram-positive bacteria including *Clostridium botulinum* and *C. perfringens* appeared to be more sensitive than gram-negative microorganisms. Oregano, savory, and thyme showed broad antibacterial activity by distinctly inhibiting growth of all the organisms tested.[76] Fig extracts which contain 4-substituted resorcinols have been shown to have great potential in the food industry as novel antibrowning agents. These compounds are water soluble, stable, nontoxic, and enjoy GRAS status.[67] The enzyme glucose oxidase can be used as a preservative, through its action giving hydrogen peroxide and gluconic acid from glucose, which have antibacterial properties against microorganisms such as Pseudomonas.[77] Ascorbic acid and its isomer erythorbic acid have also been used in the food industry as antioxidants.[67] The antimicrobial potential of chitosan, a deacetylated product of chitin, has been reported.[78] The beneficial effects of chemical additives in combination with chilling and irradiation in extending the shelf life of fishery products have been detailed (see Chapter 10). Heat pasteurization in combination with chilling is a popular method of shelf life extension of shrimp, crab, and other crustacean products.[79] Such products have a limited shelf life due to their sensitivity to microbial growth including pathogens.

3.11.1 Antifreeze Proteins

Another combination method is the use of antifreeze proteins (AFPs) with chilling, which help to reduce the chill temperature, without freezing of the muscle tissue. Several marine organisms that dwell in extremely cold habitats survive due to the presence of AFPs that are responsible for depression of the freezing point of their blood. These compounds are generally glycopeptides, which have molecular weights in the range of 2.4–36 KD and remain in the colloidal fraction of the blood. On a molar basis, their effect in depression of freezing point is 200–300 times more than that of sodium chloride. There are mainly three types of AFPs, depending upon their amino acid contents and structures, all of them functioning in a similar way. The antifreeze glycoprotein producing fishes include Atlantic cod and Greenland cod and winter flounder, Atlantic wolf fish and sculpins. Certain

cold-tolerant microorganisms also produce AFPs, although in a limited quantity. Due to their ability to cause depression of freezing point, they have potential for use in frozen foods as normal ice modulators.[80,81]

3.12 PREFERRED HANDLING PRACTICES

Traditional methods practiced in onboard handling, stowage, offloading, and transport result in substantial loss in quality, because of the use of insufficient ice, poor handling methods, and inadequate hygienic conditions onboard the vessels. In addition, the traditional method of selling fish at the wharf and also in the markets, particularly in warm countries, leads to considerable deterioration in the quality of the fish. Duration of fishing trips should be in accordance with the practical storage life of the fish species sought. Generally it should not exceed 2–4 days for lean fish and one day for fatty fish. The practical storage life considered must, of course, encompass the entire period comprising the fishing trip and time in port (off-loading, sale, preparation for dispatch, etc.) as well as transportation time, which may run from 2 to 6 days. Fish may also be subjected to handling such as filleting, packaging, etc. Time required for these operations must also be minimized. If chilling/freezing is not carried out expeditiously and properly, the catch could be spoiled and cannot be marketed. Even when fatty fish are boxed and well iced, they can be kept only for 5–6 days. In contrast, gutted haddock or cod, when similarly stored will keep for 12–15 days.

Fish chilled at sea should be handled onshore in such a way that its temperature is maintained, as far as possible, throughout the distribution chains in order to prevent loss of quality. Shellfish or pelagic fish caught during short voyages, and that may not have been iced onboard, should be iced immediately after landing. The shellfish may be covered so that they are not exposed to direct sunlight. It is also important to keep the shellfish moist. Ungutted fish may require gutting as a first step onshore. Further dressing, such as heading, filleting, or splitting will depend on market requirements. Fish warm considerably during handling and processing. The temperature of prepackaged fish products can rise to the prevailing ambient temperature during processing. When they are finally packed in cartons and stacked during chilled storage, the recooling time could be prolonged resulting in a loss of potential shelf life. For example, fish fillets with an initial temperature of 19°C require more than 8 min to reach 2°C when immersed in ice water.[27] Even in temperate climates, although the fish has entered the premises close to 0°C, delays in filleting may result in a rise in temperature up to 10°C or more by the time they are processed and packed. Such temperature fluctuations adversely affect the shelf life.

3.13 PACKAGING OF FRESH FISHERY PRODUCTS

A characteristic of fresh fish is their moist and slippery surface, which makes handling difficult. Packaging of fishery products has a great influence in facilitating handling and also control of spoilage. The fresh fish packages are required to

TABLE 3.7

Requirements of Packaging Material for Chilled Package of Fish

Ability to hold the product
Compatibility with the food
Non-toxic nature
Appropriate gas permeability
Control of moisture loss or gain
Protection against light
Should have antifog properties
Resistance towards insect and rodent infestation
Compatibility with time/temperature indicators
Smooth filling of the product
Ability to withstand the packaging and distribution stress
Ability to prevent accidental physical damage
Prevention of dirt, chemical, and microbiological contamination
Protection from odors and taints
Tolerance to operational temperatures
Easiness to open
Ability to show evidence of tampering
Ability to communicate product information
Consumer appeal
Cost effectiveness

facilitate easy disposability of drip, handling convenience, and hygienic protection of the contents.[82] A suitable fresh fish package must also reduce fat oxidation, reduce dehydration, control bacterial and chemical spoilage, and prevent odor permeation. The use of oxygen impermeable packages and cold storage drastically reduces lipid oxidation. The requirements for packaging material for chilled food are given in Table 3.7. Vacuum packaging of fatty fish such as herring, salmon, and trout, however, has not shown any additional extension in shelf life at chilled temperatures.[3] When fresh fish is packaged, some drip gets accumulated in the package during extended storage, resulting in unattractive appearance of the product. An absorbent pad may be placed in the package to soak up the drip. Treatment with polyphosphates has been shown to reduce drip formation in fresh fish.[67,70] A good odor-barrier packaging can also help dissipation of odor-bearing compounds from the chill stored fish to the environment. Packaging operation may result in rise in temperature of the chilled fish. It is therefore important that fish packed in cartons are recooled to attain the required chilled temperature. While the cooling efficiency depends upon the type of chilling employed, the packaging material has a profound influence on cooling time, as shown in Table 3.8.

A shallow tray and transparent film overwrap is the most popular retail package for fresh refrigerated fish. Tray packs are wrapped with semimoisture-proof

TABLE 3.8

Influence of Packaging on Cooling Time of Fish Exposed to Air at Different Temperature and Speed

Product	Chilling conditions	Initial Temperature (°C)	Final temperature (°C)	Cooling time (min)
Unwrapped fillets	Air at −1°C, 0 min/sec	19	2	38
Unwrapped fillets	Air at −1°C, 1 min/sec	19	2	20
Wrapped fillets	Air at −35°C, 3 min/sec	21	2	3.5
Wrapped fillets	Air at −35°C, 3 min/sec	20	2	15
Wrapped fillets	Air at −35°C, 8 min/sec	20	2	5.8

The fish fillets were packaged in polystyrene trays with an overwrap before cooling by different methods.

Source: Adapted from Graham, J., Johnston, W.A., and Nicholson, F.J., Ice in fisheries. Fisheries Technical Paper 331, Food and Agriculture Organization of the United Nations, 1993. With permission.

cellophane, polystyrene, oriented polypropylene, or polyvinyl chloride. A significant disadvantage of such tray packs is that they tend to deteriorate if too much moisture is absorbed. Foam and polystyrene require the use of an absorbent paper. In order to obtain improved rigidity for direct film-wrapped packages, a plastic or wax-coated paperboard insert is used. Sometimes, a vacuum pack is used to improve shelf life. This requires higher barrier films such as polyvinylidene chloride (PVDC), polyethylenes, polypropylenes, or polyesters.[83] The U.S. FDA considers any hermetically sealed package containing fresh seafood as a reduced oxygen package (ROP) if the oxygen transmission rate of the package is less than 10,000 cm^3/m^2/day. The FDA has banned the use of ROP for fresh seafood in the United States unless adequate temperature control and thermal history monitoring are in use. Table 3.9 summarizes packaging options for processed fishery products.

3.13.1 Active Packaging

The environment within a sealed food package can be modified using scavengers, which cause some chemical reactions inside the package that favors enhanced quality of the product. One potential is the use of scavenging system to remove oxygen from inside of a sealed package. For example, a compound, *ageless*, (introduced in Japan in 1977 by the Mitsubishi Gas Chemical Company) which is basically an iron product, gets oxidized to ferric state inside a package thereby reducing oxygen in the package. The water activity of the food has to be high enough so that the iron will get oxidized. The iron is separated from the food by keeping it in a small, highly oxygen permeable, labeled sachet or pouch. Similar systems are available for controlling other parameters in the pouch such as high humidity, microorganisms, and other hazards[67] (see Chapter 6).

TABLE 3.9
Packaging Options and Materials for Processed Seafood

Packaging options and materials	Advantages
Single ply films (polyethylene, 50–100 μm), co-polymers (e.g., polyvinylidene chloride and vinyl alcohol). PVDC, commonly known as saran, has negligible water vapor, oxygen, and CO_2 permeability	Commonly used for packaging fishery products. Good rigidity and protection to frozen items
Laminates packs (e.g., polyester/polyethylene or nylon-polyethylene laminates)	Better protection. Reduced vapor and oxygen permeability
Trays and form-fill packs made of rigid plastics (polystyrene, expanded polystyrene, and polyvinyl chloride, PVC)	Permeable to oxygen, may need extra protection by overwrapping by PVC, cling film or skin packaging. Not thermostable
Shrink wrapping (shrinkable polyethylene), cellophane	Flexible films covering the trays carrying the food, for protection
Stretch wrapping using elastic films	Tight draping around the product by machine for better protection. Ideal for heat-sensitive products
Vacuum packaging (polyester, PVDC)	Control of rancidity development
Active packaging	Use of certain compounds as scavengers in packages to enhance better environment such as reduced oxygen, relative humidity, etc., for example, use of *ageless* to control oxygen
Retortable pouches (Polyester/ aluminum/adhesive/polypropylene)	For heat-processing foods products to provide long shelf life at ambient temperatures

Source: Adapted from Ashie, I.N.A., Smith, J.P., and Simpson, B.K., Crit. Rev. Food Sci., Nutr., 36, 87, 1996; Mendiza, T.F., et al., J. Food Sci., 69, FM90, 2004; Srinivas Gopal, T.K. and Ravi Shankar, C.N., in Seafood Safety, Surendram, P.K. et al., Eds. Society of Fisheries Technologists, Cochin, India, 2003, p. 122.

3.13.2 Monitoring of Temperature

Time-Temperature Indicators (TTI) have been proposed as one potential method to satisfy the thermal monitoring requirement for packaged seafood. Evaluation and selection of appropriate TTIs remain a difficult process for seafood manufacturers. A few TTIs are commercially available to predict the safety of fresh seafood in ROP.[84] A new tag available to measure freshness in seafood is called *FreshTag*, which is an adhesive tag having color indicators that can detect the production of gases such as volatile amines. The odor-causing chemicals react with the non-toxic dye-based indicator inside the tag to give a colored product, which indicates the degree of freshness of seafood. A rapid detection kit using this tag is now

available in the market and is useful in seafood processing including purchasing and inspection of raw material.

3.14 TRANSPORTATION OF CHILLED FISH

The transportation of seafood, fresh or processed, is increasing globally. It is important that the fish be chilled before it is packed for transport. This allows just enough ice to be used to offset the heat inputs that occur during transportation in insulated isothermal vehicles or to keep the fish wet in a refrigerated vehicle. Land transportation of chilled fish should be carried out in insulated, refrigerated, or mechanically refrigerated vehicles. The conventional standard emphasizes that refrigerated vehicles should have a minimum inside temperature of 7°C. Boxes used for carrying fish in land transportation are made of wood, aluminum, high-density polyethylene, or similar material. There is a tendency to use boxes made of expanded polystyrene or polyurethane. Boxes should be light yet strong enough to withstand the weight of the fish and the ice, and stacking should also have insulating properties as well. Boxes should provide for drainage of liquids and unless disposable, should be easy to clean. Boxes in which the melted ice water is collected in a double bottom are sometimes used to keep liquids from draining into the transport vehicle itself. For example, an expanded polystyrene box 25 mm thick measuring $60 \times 40 \times 48$ cm^3 has a capacity for 52 l of fish and ice, with a double bottom that can hold 12.5 l of melt water.[54,85] These measurements are also suitable for stacking on standard 120×80 or 120×100 cm^2 pallets. Containers for transportation of chilled fish by air should be completely watertight.

A process for maintenance of freshness and transportation of hake, commonly known as "whiting" in North America, has been developed.[86] The fish immediately after catch, is kept in special holds of the boats filled with ice and seawater, creating ice slush. Air is circulated through the hold, keeping the fish uniformly cold and fresh. The fish is transported under fresh super-chilled ice slurry to processing plant, where it is cleaned, inspected, and filleted. The procedure helps in preparation of fillets having good texture and acceptability.[87]

Air shipment is becoming popular as a result of increasing demand for fresh fish in interior consumption centers. A lightweight and protective container is essential for this type of transportation. Formerly, wooden boxes with parchment liners were used for bulk packaging of fresh fish. In recent years, plastic containers, plastic corrugated cartons, and metallic containers have been commonly used for fresh fish packaging. Modern insulated containers are made of high-density polypropylene or polypropylene with polyurethane insulation sandwiched between the inner and outer walls of the double-walled container. They are durable and in normal cases have a life span of over 5 years. Fresh fish are often packed with ice in these containers.[82,83,88–90]

For chilling the air cargo, several alternatives to conventional ice are employed. One such material used is the compartmentalized pad of nonwoven fabric encapsulating a unique synthetic absorbent powder. Once soaked in water and deep frozen, the pads could be used to cool the products. The pads could also be used as

absorbent for any drip or fluid in the containers. In the case of large fish, the frozen pad could be stuffed into the belly cavity of the fish to give additional protection from heat. Special thermal barrier films are often used in combination with the pads to protect the fish containers from heat. Some of these barrier films used with bubble rap material to cushion the fish are reported to cut off up to 97% of radiant heat, a common cause of temperature rise in fish containers in transit.[88] Most airlines specify compulsory standards for boxes for chilled fish. In conclusion, proper handling of raw material is essential for development of safe and wholesome products acceptable to consumers worldwide.

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4 Quick Freezing and Individually Quick Frozen Products

4.1 INTRODUCTION

Freezing is considered, all over the world, as one of the best methods for long-term preservation of perishable foods. Freezing reduces chemical reactions as well as microbial activity in the tissue, thus extending the shelf life of the product. Next to fresh foods, frozen items attract high consumer acceptance in terms of sensory parameters including appearance, texture, and flavor, as well as nutritional value, as presented in Table 4.1.[1] In developed countries, the proportion of fish that is frozen has been constantly increasing, which has reached a share of 40% of fish production.[2] Global production of frozen seafood including fillets and shellfish has increased from 24 mt in 1988 to 27 mt in 2001.[2] The growing world markets for frozen food products, attract innovations in freezing techniques, shifting from bulk freezing of commodities to consumer friendly and convenient, individually quick frozen (IQF), and value-added products. This chapter is intended to discuss the potential of individual quick-freezing technology for the value addition of seafood. At the outset, general aspects on freezing of fishery products are discussed including shelf life of frozen items, quality changes during freezing and frozen storage, and techniques to control such changes. This is followed by discussion on technology for quick freezing, IQF fishery products, advantages of the IQF process, and its potential applications.

4.2 FUNDAMENTALS OF FREEZING OF MUSCLE FOODS

Freezing involves cooling down of the materials from a refrigerated temperature (0 to 2°C) to a temperature below the freezing point (at least −15°C). There are three stages in freezing. During the initial stage, the temperature fairly falls rapidly to just below 0°C. In the second stage (also known as thermal arrest period), the temperature remains fairly constant at about −1°C as the bulk of the water in the product begins to freeze. This is the most critical period and to produce a good-quality frozen product, fish should pass through the thermal arrest period as quickly as possible.[3] More than 50% of the energy is expended for passing the fish through the second stage. In the third stage, nearly 75% of the water in the muscle turns into ice, leading to a further drop in the temperature. Bound water, the water

TABLE 4.1

Comparative Merits of Foods Preserved by Different Preservation Methods, Based on Sensory Evaluation

Quality attributes	Frozen foods	Canned foods	Dried foods
Freshness	71	14	5
Taste	56	30	7
Appearance	62	28	3
Nutritive value	51	20	11
Value for money	47	29	10
Ease of preparation	40	52	3

The values indicate % score.

Source: Adapted from Pruthi, J.S., *Quick Freezing Preservation of Foods — Principles, Practices, R&D Needs. Foods of Animal Origin*, Vol. 1. Allied Publishers, Mumbai, India, 1982. With permission.

that is the integral part of the chemical structure of the tissue, may be frozen only at extremely low temperature of $-55°C$.[4]

Freezing points for fishery products vary from about -1 to $-2.5°C$.[5] As temperature reaches the freezing point, a continuous freezing front forms on the surface, which proceeds from the exterior to the interior. Extracellular water freezes more readily than intracellular water because of its lower ionic and solute concentrations. As freezing is initiated, concentration of solutes in the aqueous phase increases with the formation of a subsaturated state or glassy domain (see also Section 4.3.1.2). Further fall in temperature results in the formation of ice crystals. Before crystallization process can occur at the initial freezing point, a significant energy barrier must be surpassed. This energy barrier is demonstrated by the withdrawal of sensible heat below $0°C$ without a phase change. The process, called under- or supercooling, results in a thermodynamic unstable state that initiates the formation of submicroscopic water aggregates leading to interface (seed) necessary for a liquid–solid transformation. In the absence of a stable seed, phase separation is not possible since the liquid does not easily align in the configuration of a solid. The process of ice nucleation is complex. In general, it involves creating a stable interface via a foreign particle (heterogeneous nucleation) or through a process by which crystals form spontaneously during intrinsic fluctuation (homogenous nucleation).[6] Heterogeneous nucleation is more important in the freezing process. This type of nucleation occurs when water aggregates assemble on a nucleating agent with some insoluble material such as meat particles. Water during freezing will nucleate at a higher temperature than that in a homogenous nucleation.[7] Ice crystallization can cause extensive microstructural changes to tissue foods during freezing.[8] However, unlike plant cells, which are more rigid, animal cells are less affected, essentially due to the flexible nature of the collagen fibers in the meat.

As the extracellular water freezes, there is a gradual movement of water out of the muscle cells. The outward movement of water brings about accumulation of additional amounts of extracellular water and concentration of solutes in the intracellular region. As a result of the concentration of solutes, muscle properties including pH, viscosity, oxidation–reduction potential, and surface tension change, leading to a loss of overall quality. In the case of proteinaceous materials, large increase in ionic strength during freezing affects stability of the proteins.[6,9] Proteins sensitive to ionic strength become irreversibly denatured and subsequently aggregate and precipitate. Moreover, at high-protein concentration, there is a formation of protein–protein cross-links. With meat systems, these processes can lead to curdling and drip following thawing.[8] In addition to composition and size of the product and kinetic (mass transfer) properties, mentioned earlier, several thermodynamic factors are also involved in food freezing. Thermodynamic factors include heat transfer capacity of the refrigerant, mass transfer properties of the food material, and other process variables. In recent years, considerable attention has been given to kinetic rather than thermodynamic processes that occur during freezing of food systems.[10]

4.3 FREEZING OF FISHERY PRODUCTS

Freezing is a very important method for trade in fishery products. Freezing and subsequent cold storage are particularly useful in making seasonal species of fish available all year round. In addition, freezing is beneficial for different value-added fishery products such as breaded and battered fish sticks, fillets, steaks, or nuggets. Table 4.2 shows some major fish species used in different frozen product forms.

4.3.1 Quality Changes Due to Freezing and Frozen Storage

4.3.1.1 Reasons for quality changes

Properly frozen fish under proper storage conditions will never spoil due to bacterial action since microorganisms cannot grow at commercial freezer temperatures. Nevertheless, the product will suffer other kinds of degradative processes. The changes have been explained as arising from biochemical reactions that occur in the muscle during frozen storage. The freezing process, prolonged frozen storage, and thawing conditions have adverse influence on the quality of the product.[11,12] The extent of these influences essentially depends upon the species and the initial quality. Considerable attention has been given to chemical and microbiological quality indicators to predict the shelf life of frozen fish. In most cases, the fish is processed after it has passed through rigor mortis. But in some inshore fisheries and during freezing at sea, fish may be filleted while they are in the prerigor state. Freezing fish prerigor can result in up to 40% shrinkage, leading to gaping of the fillets on thawing.[13] Fillet gaping is the term used to describe the phenomenon in

TABLE 4.2
Some Major Fish Species Used for Freezing into Different Product Forms

Fish species	Product forms
Atlantic cod	Standard, boneless and interleaved fillets in blocks, IQF fillets
Haddock	Boneless and interleaved fillets in blocks, IQF fillets, headed and gutted fish, whole round
Salmon	Fillets, smoked, breaded
Saithe	Fillets, breaded forms, nuggets
Atlantic pollock	Fillets, breaded forms, nuggets
Hake	Fillets, breaded forms, nuggets
Argentinian hake	Fillets, breaded forms
Atlantic red fish	Standard and interleaved fillets in blocks, skin-on, scale off, with interleaved blocks, headed and gutted fish in blocks
Catfish	Fillets, breaded forms
Turbot	Fillets
Atlantic plaice	Fillets
Atlantic mackerel	Fillets, blocks, whole fish, headed and gutted in blocks
Atlantic herring	Fillets, butterfly fillets, blocks, headed and gutted, whole herring, round

which the connective tissue of fish fails to hold the muscle blocks together. Mechanical skinning of fillets that have undergone gaping is difficult because of the slits appearing on the surface of the fillets. Gaping has often been associated with low final pH of the muscle and changes in the connective tissues during freezing.[14–16] To avoid shrinkage in prerigor frozen fish, the fish should be kept chilled and handled expeditiously and frozen in order to prevent it going into rigor. Although, fish that are frozen prerigor will probably have completed the rigor process during frozen storage and defrosting, the thawed fish may still require a further holding period in ice to complete the rigor.

The quality changes during freezing and cold storage of fishery products have been discussed in detail.[10,17–21] There are three accepted theories to explain denaturation of structural proteins during freezing and frozen storage, namely, (i) an increase in solute concentration, (ii) dehydration of the cell, and (iii) autooxidative changes that alter the balance of protein–protein and protein–water interactions. As freezing progresses, proteins are exposed to increased ionic strength in the nonfrozen aqueous phase that leads to extensive modification of the native structure of proteins.[13,22,23] In a dehydrated state, protein–water interactions in the tissue are disrupted, and protein molecules are exposed to an environment that is less polar than water. These changes result in an increased exposure of hydrophobic side chains, and therefore, changes occur in protein conformation.[24] In addition, it has been demonstrated that autooxidation of lipids is also involved in denaturation and deterioration of muscle protein functionality by causing cross-linking between

protein and lipid oxidation products.[25] Another major reason for protein denaturation is formaldehyde-mediated cross-linking of the muscle proteins. Formaldehyde is produced by the action of the enzyme trimethylamine oxide (TMAO) reductase on TMAO, which is present in significant amounts as an osmo-regulator in gadoid fish.[15] TMAO reductase and many other enzyme activities are accelerated during freezing (and also thawing) of cellular systems, due to disruption of cells[10,15] (see Section 4.3.5.1).

An understanding of the composition of the fish, rate of freezing, temperature, and duration of storage will be helpful to assess the freezing-induced quality changes occurring in the processed fish.[9] Freezing rate influenced the degradation of adenosine tripolyphosphate in cod. Freezing at -20 and $-45°C$ resulted in degradation of adenosine triphosphate (ATP) accompanied by corresponding increase in inosine monophosphate content. Glycolysis occurred during freezing of the fish as indicated by an increase in the content of lactic acid. These changes were more at a slower rate of freezing, due to longer thermal arrest time at $-0.8°C$.[26] Fish proteins are particularly sensitive to denaturation during frozen storage. The denatured proteins are incapable of absorbing the exudate drip water, resulting in a tough-texture characteristic of the frozen fish. However, cephalopod myofibrillar proteins have been shown to be resistant to freeze-induced denaturation.[27] Paramyosin, which forms about 14% of squid myofibrillar proteins, is involved in decreasing the rate of protein denaturation in the frozen-stored cephalopod. On the other hand, proteolytic changes during chilled storage before freezing can influence the functionality of proteins. The myosin heavy chain was the primary myofibrillar protein followed by paramyosin that was hydrolyzed during autolysis by endogenous proteases of octopus arm muscle. The proteolytic activity should be controlled before freezing of octopus to retain functionality of the muscle.[28]

Physicochemical changes during frozen storage of some tropical fish species have been reported recently. ATPase activities changed during storage of croaker, lizardfish, threadfin bream, and bigeye snapper during storage at $-18°C$. Lizardfish was the most susceptible to change in the enzyme activity. Croaker and lizardfish contained higher disulfide bonds as storage time increased. Surface hydrophobicity increased in all species, except in lizardfish, in which it sharply decreased after 12 weeks of storage. For all the species α-glucosidase and β-N-acetyl glucosaminidase activities increased with increase in drip formation.[29]

4.3.1.2 Glass transition

A phenomenon, known as "glass transition" has been observed in foods during freezing, which occurs at varying temperatures depending upon the food. The glass transition has been related to product stability during frozen storage.[8,30] A glass is an amorphous solid that has a liquid structure with a viscosity greater than 10^{14} Pa.s. As the temperature falls during freezing, the liquid viscosity increases and the transition movement of the molecules becomes slower. When molecular transition cannot occur within the experimental time scale, the variations of enthalpy and volume with temperature will resemble those of a solid, even though the molecules

are not ordered, as in a crystalline structure. The transition point from liquid- to solid-like behavior denotes the glass transition temperature, T_g. The general view is that the temperature of frozen fish should be below T_g, however, this is not necessarily true in the case of some frozen fish. T_g values vary significantly from fish to fish. Differential scanning calorimetry measurements have revealed thermal transitions at -11, -15, and $-21°C$ in the case of cod, and an additional transition at $-72°C$, in the case of tuna.[31]

4.3.1.3 Textural changes

As a consequence of frozen storage and associated denaturation of proteins, the texture of fish gradually changes from the usually soft, springy, moist succulent of fresh or recently frozen fish to unacceptably firm, hard, fibrous, woody, spongy, or dry ones. The problem is more acute for the white flaky fillets of fish such as cod, haddock, and hake. Flat fish such as sole and plaice show less sensitivity to freezing-induced textural changes.[32] It has been proposed that fiber diameter is a key determinant of firmness when comparing fish species; smaller fiber diameters correlate with increased textural firmness.[20] However, whole fish when thawed, exhibits less textural changes than fillets, basically as a result of the presence of the backbone, which serves as structural support for the flesh. The extent of textural changes also depends upon the fish species. Changes in texture are correlated with decrease in water-holding capacity of the myofibrils, affecting sensory and functional attributes of the muscle. Changes in firmness, juiciness, and fibrous nature are the significant textural changes in trout during frozen storage.[33] Mechanical tests to monitor changes in the firmness of fish fillets have been proposed. However, few models exist to relate mechanical properties of whole fillets to sensory attributes. Nevertheless, changes in sensory properties have been correlated with instrumental analysis such as Kramer Shear cell and puncture penetration tests.[34]

4.3.1.4 Changes in flavor

Generally, fresh fish has a fresh seaweedy odor, which can be retained even after freezing and frozen storage. During frozen storage, off-odors and off-flavors gradually develop, which become noticeable as the storage period advances. Characteristic off-odors and flavors of frozen lean fish and shellfish are variously described as acid, bitter, turnipy, cardboardy, musty, or singled; those of fatty fish are typically rancid, oxidized, painty, or linseed oil-like. White lean fish and shellfish become opaque and yellowish, fatty fish develop a "rusty" appearance. These changes result in the characteristic "cold-store" odor and flavor.[35] Lipid oxidation and generation of a variety of compounds are the major reasons for flavor changes during frozen storage, particularly of fatty fish such as herring and mackerel. White hake (*Urophycis tenuis*) initially gives weak off-odors of sweet, boiled milk, but gives out weak off-odors followed by a sour milk odor when frozen storage is extended. In the case of low fat white fish such as cod, less oxidative changes are observed during frozen storage.[35,36]

Oxidation reactions in frozen fish depend on the accessibility to oxygen, storage temperature, and nature of the tissue.[14,37,38] Lipid oxidation in fish may be initiated and promoted by a number of mechanisms. These include the production of singlet oxygen; enzymatic and nonenzymatic generation of partially reduced or free radical oxygen species (hydrogen peroxide and hydroxy radicals); active oxygen iron complexes; and thermal or iron-mediated cleavages of hydroperoxides.[32] In frozen mackerel stored at −15, −30, or −40°C, lipid oxidation was eight times faster in subcutaneous fat than in deep layers.[38] The volatile products of decomposition of fatty acid hydroperoxides (formed due to autooxidation), especially *cis*-4-heptenal in frozen cod and 2,4,7-decatrienal in oxidized mackerel contribute to cold-store flavor of fishery products.[9,35] The lipid oxidation products can also affect the nutritional value of the fish.

Recently H.O. Hultin's group at the University of Massachusetts, Gloucester, Massachusetts found a need for washing to remove blood to enhance retaining the quality of fish. A 1 min delay in washing the seafood blood did not affect the shelf life of refrigerated mackerel; however, it did reduce the shelf life and quality of the fish stored frozen. An antioxidant system consisting of a chelator and lipid and water-soluble free radical scavengers was developed to further stabilize the fish muscle. Using the system even fatty fish such as mackerel can be stored at −20°C for a year without the development of rancidity.

4.3.1.5   Changes in appearance

The pigments of fish and shellfish tend to fade and become duller or change in hue during frozen storage. Prolonged frozen storage of rainbow trout fillets resulted in increased lightness, redness, and yellowness and decreased hue values.[33] The carotenoid pigments of salmonids are sensitive to light, heat, and oxygen, but are stable during frozen storage.[39] Carotenoids content in the flesh of rainbow trout after six months of storage at −20°C was affected. Similar frozen storage reduced the content of astaxanthine in salmon fillets.[16] If freezing is carried out after smoke treatment, fewer changes in quality occurred, as compared with fillets subjected to smoking prior to freezing.[16] Some fish, like tuna, develop discoloration during frozen storage, reportedly due to oxidation of myoglobin to metmyoglobin in the blood.[40] Other freezing-induced changes are green discoloration of swordfish and shark, and blue or black discoloration of crab and lobster.[36] As compared with whole fish, more losses in the color and appearance are noticeable in fish steaks and fillets.

In the case of whole shrimp, black pigmentation, known as "melanosis" can occur within 2 to 12 h of harvest (see Chapter 2). Although melanosis does not make shrimp unfit for human consumption, it can significantly affect its market value.[41] To prevent melanosis, efficacy of some compounds has been examined. The most widely used ones are sulfites, particularly sodium bisulfite. These compounds interfere with the polymerization of the quinones forming colorless compounds. Other compounds that are effective against melanosis include ascorbic acid, citric acid, sodium benzoate, kojic acid, and 4-hexyl resorcinol.[42] While the oxidation

reactions leading to the formation of these black pigments can occur at 0°C, at −18°C, no visible spots were detected in brackish water prawns up to 3 months of storage.[43] Melanosis can be induced by the thawing conditions employed.

4.3.1.6   Microbial changes

As the temperature is reduced to cause a large portion of water to freeze, the growth of most microorganisms stops except for some psychrophilic bacteria, yeasts, and molds. At −20°C most cells will have lethal or sub-lethal injury. The rate of freezing and the lowest temperature of freezing dictate the extent of microbial damage from ice crystals. Damage and death are more extensive at slow freezing than at quick-freezing. Bacteria at the exponential state that undergo abrupt temperature fall from 37 to 0°C lose viability. During storage, death and sublethal injury are very high during initial period (about 7 days) and decrease during further storage. Fluctuation of temperature during frozen storage of food has great influence on growth, sublethal injury and death of microorganisms. The effect is more pronounced with gram-negative organisms such as *E. coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. The lethal effect of freezing on microorganisms in fishery products varies depending on duration of storage, rate of cooling and thawing, and storage temperatures.[44] Incidence of pathogens, including *Salmonella*, *Vibrio*, *Listeria* spp. among others, in frozen fishery products has been of great concern in the international trade of the commodities. Organisms that are very sensitive to lethal effects of freezing include parasitic protozoa, cestodes, and nematodes. The cysts of the tapeworms *Taenia* spp. are destroyed when stored for 10 days at −10°C.[45]

4.3.1.7   Dehydration

Dehydration and weight losses are the major problems in frozen fish and shrimp trade. "Freezer burn" is a defect that develops on the surface of exposed frozen fish, particularly unwrapped or unglazed steaks and fillets after a few weeks of frozen storage. It is usually in the form of patches of light-colored tissue, developed due to vapor pressure gradients within the product and between the product and external environments during temperature fluctuations.[45] Removal of water from the surface results in exposure of the muscle to air, contributing to autoxidation of lipids and associated flavor changes. Even a small patch of freezer burn on frozen fillets may cause loss of sensory quality, and hence it is rejected by the consumers. By using a tight water-vapor impermeable film over the product can reduce "freeze-burn." However, if there is a space between the material and the package, moisture will move into the space and condense inside the package or on the surface of the material, when its surface has a lower temperature than the environment. The weight losses of the product could be as high as 20%.[45] Maintenance of low, constant temperature is necessary to minimize moisture loss. In a good cold storage, the weight loss will be about 1 g/m^2 surface per day. However, in commercial situations of frozen storage, temperature may fluctuate. Reasons

for higher losses in weight include higher temperature of the material during loading, frequent opening of the cold storage door, excessive defrosting, and high air velocities (above 0.1 m/sec). A survey of 11 cold stores all operating between -25 and $-30°C$ and ranging in capacity from 240 to 24,000 m^3 showed an average weight loss of 0.3 to 13.5 g/m^2 surface area per day.[3]

4.3.2 Measurement of Quality of Frozen Fish

There are no commonly accepted simple and reliable tests for quality assessment of frozen fish. Biochemical indicators can be divided into three categories: (i) structural changes in proteins measured in terms of extractability in salt solution, hydrophobicity, viscosity, and electrophoresis pattern, (ii) decrease in activities of certain enzymes, and (iii) changes in concentrations of some metabolites. Enzyme tests as indicators of frozen fish quality include myofibrillar ATP, aldolase, malic enzyme, α-glycerophosphate dehydrogenase, acid phosphatase, 5′-nucleotidase, and phospholipase.[10] Metabolites include TMAO, free fatty acid level, peroxide value, and thiobarbituric acid reacting substances particularly malonaldehyde. Physical measurements include water-holding capacity and cooking losses. Textural measurements determine hardness, springiness, cohesiveness, gumminess, chewiness, resilience, and adhesiveness.[46] A few spectroscopic techniques have also been developed recently. Sensory methods are popularly used on as commercial practices.[18,19] Table 4.3 summarizes reasons for quality loss in frozen fishery products during storage, and Table 4.4 indicates spoilage characteristics of some frozen fish in the U.S. markets.

TABLE 4.3
Reasons for Quality Loss in Frozen Fishery Products During Storage

Parameter	Effect
Protein cross-linking, denaturation	Tough and spongy texture
Breakdown of TMAO into dimethylamine (DMA) and formaldehyde (HCHO)	Tough and spongy texture, loss of juiciness and succulence
Oxidation of lipids	Flavor changes
Loss of calcium-dependent ATPase activity	Dry and cottony on first bite
Loss of flavor-causing compounds	Bland or neutral flavor
Development of off-flavor compounds	Loss of natural flavor
Concentration of minerals	Lipid oxidation, protein denaturation, changes in pH and ionic strength
Ice crystal formation	Rupture of cells, release of cell constituents
Sensory changes	Loss of consumer value
Nutritional changes	Leaching of nutrients in drip. Loss of nutritional quality
Functional properties	Water-holding capacity, emulsification capacity

TABLE 4.4

Spoilage Characteristics of Some Frozen Fish in the U.S. Markets

Fish	Thawed-state characteristics
Herring, whole	Skin and meat show rusting
Mackerel, whole	Surface dehydrated, skin and meat showing rusting, rancid smell in skin and meat
Chinese pomfret, whole	Dehydration at lower part of belly and fin, head and belly parts yellowish discolored, sponge-like meat
White pomfret, whole	Skin and meat show rusting, sponge-like meat
Jew fish, whole	Slight rancid smell in skin
Lemon sole, whole	Surface dehydrated, spoiled and rancid smell, sponge-like meat
Haddock fillet	No smell, sponge-like meat, cracks
Cod fillet	No smell, sponge-like meat
Flounder fillet	No smell

Source: From Santos-Yap, En E.M., in *Freezing Effects on Food Quality*, Jeremiah, L.E., Ed. Marcel Dekker, New York, 1995, p. 109. With permission.

4.3.3 Control of Quality Changes

While it is difficult to completely control quality changes during prolonged frozen storage, the intensity of such changes could be reduced by several means. These include measures to be taken: (i) at the product level, (ii) during the freezing operation, and (iii) at the storage level. The control measures at the product level include use of fresh fish, incorporation of certain additives known as cryoprotectants, glazing, coatings, and packaging of the products. All packaging should be tightly fitted to the product to avoid free air space, which may cause "in-pack desiccation." Control at the level of freezing is mainly a quick-freezing process employing a technique to suit the product. Prevention of weight loss can be achieved by ensuring a lower product temperature during freezing and maintenance of ideal storage temperature.

4.3.3.1 Cryoprotectants

Cryoprotectants are compounds that are added to the meat system to protect the proteins and prevent loss of their functionality during frozen storage. Cryoprotectants improve the quality and extend shelf life of frozen foods by preventing deleterious changes in the myofibrillar proteins caused by freezing, frozen storage, and thawing.[9,47] Some of these additives are sucrose, sorbitol, sodium lactate, and phosphates, all of which have been found useful to protect the functional quality of frozen muscle foods. A few of them may display a synergistic effect. A sucrose/sorbitol mixture is commercially used in minced fish products.

Cryoprotectants having reduced sweetness and sensitivity to undergo Maillard type browning reactions are preferred in fishery products.

Cryoprotectants such as 8% of either sucrose or sorbitol or 1% sodium lactate with or without 0.5% polyphosphate containing 0.05% magnesium chloride were beneficial to enhance the protein functionality of trout fillets stored at $-20°C$.[48] The protein functionality could be measured in terms of hardness and cohesiveness of the fillets, surface hydrophobicity, total and free sulfhydryl contents, and susceptibility of myosin to thermal denaturation.[49] In addition to the above cryoprotectants, recently, antifreeze proteins have been found to protect fish muscle during frozen storage. Some of these compounds are naturally present in cold-water fishes.[32,50]

4.3.3.2 Glazing

Glazing is an effective and economic means of protecting frozen fish and shell-fish during storage and transport. Glazing forms a protective film or coating over the frozen product, which retards moisture loss and prevents oxidative rancidity. Glazing is done by spraying cold water over the product or sometimes by dipping in water, which freezes almost instantly, with slight increase in temperature of the product. The product takes up water to the extent of 5–10% of its weight, or sometimes more, providing a coating of ice of 0.5–2 mm thick. A rather better system is to freeze the fish in a small amount of water contained in an open metallic box with a plastic lining. For small, cooked and peeled shrimp the rise in temperature after glazing may well be as high as 4–8°C. Glazed Indian mackerel (*Rastrelliger kanagurta*) had a storage life of 6 months at $-20°C$ as compared with unglazed samples, which lasted only for about 3–4 months.[51,52] Whole fish such as herring can be frozen and stored successfully by placing the fresh fish in a polyethylene-lined paper bag in a vertical plate freezer and topping up the bag with water to fill the voids before freezing.[14]

Apart from the conventional chilled water, chilled water containing certain compounds may be used as glaze. These glazes include water containing inorganic salts, disodium acidphosphate, sodium carbonate and calcium lactate, alginate solution, antioxidants such as ascorbic and citric acids, glutamic acid, monosodium glutamate, and other edible coatings such as corn syrup solids.[51] Thaw exudates and cooking loss can be effectively reduced by dipping in polyphosphate solution prior to freezing.[52] Polyphosphates help improve solubilization, swelling, and water-holding capacity of myofibrillar proteins. These compounds are also known to reduce oxidative rancidity by chelating metal ions.[53] Glazing by ascorbic acid dips extended the shelf life of frozen chub and white bass fillets.[54,55] A Codex method and also another one based on enthalpy measurement are available to determine the glaze uptake in prawns.[56]

4.3.3.3 Edible coatings

In recent years, there has been interest in development of edible coatings from polysaccharides, proteins, and also lipids to extend shelf life of foods. Such coatings

can retain quality of fresh, frozen, and processed muscle foods including fish by retarding moisture loss, reducing lipid oxidation and discoloration, enhancing product appearance in retail packages, and also as carriers of food additives such as antimicrobials as well as antioxidant agents.[57,58] Edible films based on fish myofibrillar proteins have been developed.[59,60] A protein glaze for a fish fillet or mince can be prepared from a portion of the same fish meat, making use of gel-forming properties of washed fish muscle in presence of dilute organic acids such as acetic acid.[61] This gives a natural protection to the fillet or mince. Glazing of mince muscle blocks of Indian mackerel (*R. kanagurta*) using a dispersion prepared from the fish myofibrillar proteins significantly reduced lipid oxidation and moisture loss in the product during storage at $-17°C$.[61]

4.3.3.4 Packaging

In addition to glazing, packaging is essential for obtaining optimal frozen shelf life at a specific temperature. Packaging performs essentially four functions, namely, control of the local environmental conditions to enhance storage life, presentation of the product in an attractive manner to the potential buyer, provision for printing the nature of the contents and other details on an outer layer, and protection of the product during transit. In the case of seafood, packaging performs the following functions:

1. Prevention of moisture loss
2. Prevention of microbial contamination
3. Reduction of oxidative processes
4. Retention of volatile flavors
5. Flexibility to fit the contour of the food
6. Ease of filling
7. Resistance to puncture
8. Protection against deterioration at low temperatures

In addition to the above, packaging can also prevent the tissue from coming into contact with the secondary refrigerant.[62] Vacuum packaging is well established as a method to provide an oxygen-free environment to minimize the problems. Packaging may not be essential during cryogenic freezing, as commonly used cryogens are acceptable for contact with the products to be frozen.[36] The packaging materials and style of packaging depend upon the product and vary from flexible plastic polybag to waxed paper or polyethylene-lined board for industrial handling of fish fillets. Economic considerations suggest that ideally all the functions be met with a single layer of packaging, but sometimes several layers may be required.[63]

Frozen fish is generally wrapped in polyethylene or cellophane. These are packed in paraffin-coated paperboard cartons.[3] Paperboard (duplex board) carton, lined inside with 100 to 125-gauge (0.03 mm) low-density polyethylene, is the most important primary package used in the export of frozen seafood. Block-frozen shrimp, which are normally in units of 2 kg per block, are shipped in the above cartons. The specifications for packaging films have been recommended

by the Central Institute of Fisheries Technology, Cochin, India. According to these specifications, water-vapor transmission rate of the packaging film should be less than 18 g in 24 h/m^2 area at 90% relative humidity and 37°C. Low density polyethylene (LDPE) and high density polyethylene (HDPE) have water vapor transmission rates of 6 and 19 g of respectively, under these conditions. Its tensile strength should be not less than 120 kg/cm^2 in machine direction, and 85 kg/cm^2 in cross direction. The specification for elongation at break is 100% in machine direction, and 350% in cross direction. The cartons (usually duplex board) have to be given a wax coating to protect the contents from loss of moisture. Ten such cartons are in turn packed in a 5-ply corrugated fiberboard box, which serve as a shipping container. The boxes have to be strapped with 12 mm-wide high-density polyethylene or polypropylene, or rayon straps.[62]

4.3.4 Shelf Life of Frozen Fishery Products

The practical storage or shelf life of a product is defined as "the period of frozen storage after freezing during which the product retains its characteristic properties and remains suitable for consumption or the intended purpose."[44] One of the most common assumptions is that "quality" loss can be predicted from a simple relationship between storage time and temperature. However, many frozen foods systems are not in a stable equilibrium, and their storage stability may depend upon complex kinetics and diffusion processes depending upon multiple factors.[32] These are influenced by the nature of the species, their composition, prefreezing handling conditions such as evisceration and gutting, as well as quality, freezing rate, packaging, temperature of frozen storage, and thawing conditions. These factors can be grouped into three, namely *product*, *processing*, and *packaging*, referred as PPP factors.

At −30°C, when over 90% of the water in the fish has been frozen, the change in quality is slow. Fresh white fish will keep in good condition up to 8–9 months at −30°C and will become inedible due to bad texture and off-flavor only after a few years. However, maintenance of products at −30°C may not always be practicable. Therefore, a temperature of −20 ± 2°C has been suggested for handling, storage, and displaying of frozen fishery products for commercial trade. Whole or filleted blocks of lean fish can have a storage life of 4–8 months at −18°C, although at −30°C, the storage life can be as long as 24 months. At −18°C, the temperature of most display cabinets, fish, which has been frozen fresh, will remain in excellent condition only for 2–4 months. As compared with whole fish, IQF fillets of white fish have a shorter shelf life of 3–6 months at −18°C and 6–18 months at −30°C.[64,65]

In fatty fish, rancidity proceeds rather faster and quality remains good for only about 6 months at −30°C. Lowering frozen storage temperature may reduce rancidity development in fatty fish, but this may not be commercially viable. Fatty fish, such as mackerel and herring, have got less storage life than lean fish-like cod. Fat content of fish, which is dependent on seasonal changes, feed, and its maturity, has an inverse relationship with the storage life of the frozen fish. For example, the

storage life at $-18°C$ of frozen sardine (*Sardinella longiceps*) having a fat content of 42 and 10% are 20 and 40 weeks, respectively.[66] Frozen storage life of skipjack tuna at -4, -17, and $-25°C$ ranged from 1–2, 6, and 12 months, respectively.[9] Discoloration due to formation of metmyoglobin is a common problem in the fish during storage.[9] Similarly, chub mackerel (*R. brachysoma*) remains in good condition at $-10°C$ for 3 months, while at $-30°C$, the storage life is at least 12 months. Seer (*Scomberomorus commersoni*) has a storage life of 16–20 weeks at $-10°C$, which could be increased to 28–32 weeks at $-23°C$.[66] Practical storage life of cod has been suggested as 8 months at $-18°C$, 18 months at $-24°C$, and 24 months at $-30°C$.[67] Indian mackerel (*R. kanagurta*) with an average fat content of 3.8% has a shelf life of 16–20 and 40 weeks at -20 and $-30°C$, respectively.[62] Minced blocks exhibits reduced quality and accelerated deterioration during storage when compared with fillets. This is essentially due to the tissue damage and exposure of minced tissues to air, which cause rapid deterioration of the proteins present in the mince. The minced blocks of Pacific hake stored in moisture-proof, vapor-proof packaging films exhibited superior quality over glazed fillets.[64] Coated fish has a longer shelf life than noncoated samples. Catfish fillets stored at $-20°C$ for 11 months did not reveal significant differences in the sensory attributes when compared with fresh fillets vacuum packaged or packaged in oxygen permeable films.[68]

4.3.5 Freezing at Sea

The 1960s saw the introduction of freezer trawlers, which brought about significant improvement in the quality of fish, compared with that landed by the distant water fleets, which could be at sea for up to 21 days and used to land 5- to 14-days-old iced fish. Modern freezer trawlers are equipped to carry out varied operations such as filleting, block freezing, and even retail catering fish packs. They are in fact floating factories with the same hygienic designs and operating standards as land-based operations.

Fish for onboard freezing must be of high quality, properly headed, gutted, and washed. For the best quality product, a temperature for freezing of $-30°C$ or below is recommended.[69] Fish may be frozen prerigor to provide better quality. Horizontal plate freezers are used to freeze fillet packs, while vertical plate freezers are used for round-gutted fish. The temperature of the fish is brought from 0 to $-5°C$ within 2 h and further to $-30°C$. Weight of fish blocks produced in vertical plate freezer is usually about 25 or 50 kg.[69] The design of the cold store for the subsequent maintaining of the frozen fish is important. It must be capable of maintaining the temperature of the fish at $-30°C$ in the bulk cold stores, although intermediate cold stores used in the distribution chain and retail cabinets are often operated at $-20°C$. Temperature monitors have been developed that will monitor the functioning of the store and then, through the use of microprocessors, changes can be initiated to maintain the store at maximum efficiency. Cod fillets frozen at sea before the onset of rigor and stored for 17 months had lower microbial content and higher sensory score on thawing, as compared with cod fillets, which

are frozen postrigor and stored for the same period.[70] Sea-frozen fish command better price than unfrozen fillets, because of the superior freshness of the former.

4.3.5.1 Thawing

Thawing is the application of heat to the frozen fish until all the ice present is reverted to water. Thawing takes longer time because of poor conductivity of the frozen products.[13] Frozen products are thawed either in air, water, or in a microwave oven. Thawing in water is virtually the standard practice. Conventional water-immersion thawing has disadvantages of long thawing times, potential of cross-contamination, prolonged exposure of the outer regions to warm temperatures, use of large amounts of water, and generation of the possible large amounts of wastewater. Water thawing of fillets is not recommended because of water logging and loss of flavor. For air thawing, frozen fish is exposed to ambient temperatures until the frost that forms on the surface starts to thaw. The partially thawed fish is transferred to a chill room and held overnight to complete the thawing. Alternately, a spray of water at 5 to 8°C can be employed to thaw the whole fish. Use of systems with large temperature difference for rapid thawing is not advisable since it can result in changes in the texture of the product.

Formation of exudates, known as drip, is a general phenomenon associated with thawing of frozen foods. Some amounts of soluble proteins are lost in the drip. For a quick-frozen product, drip loss may vary from 3 to 8%, while for a slow-frozen product this may be as high as 20%. The drip formation is due to freezing-induced structural changes in the myofibrils and denaturation of proteins, which results in inability of the proteins to fully hold the initial amount of water. Large volume of drip is an indication of prolonged ice storage prior to freezing, frozen storage at inappropriate temperatures, or improper thawing of the fish.[36] Evaluation of structural changes in myofibrils of cod (*Gadus morhua*) during frozen storage showed decrease in the dimensions of the thick filament lattice coupled with disturbances to the regular hexagonal lattice spacing. It was also noted that the myofibrils within a frozen and thawed fiber were pushed closer together, with commensurate disappearance of the sarcoplasmic reticulum vesicles lying between the myofibrils.[32] In addition, the inability of the myofibrils to swell back to its original state once the fish is thawed, leading to loss of water-holding capacity, results in release of excess water as drip.

Thawing also results in loss of some flavor. There is exposure of the meat surface to temperatures above that of freezing, leaving it susceptible to bacterial growth and other deteriorative changes. Thawed meat is more susceptible to microbial spoilage than fresh meat. Slow thawing results in longer holding periods at higher subfreezing temperatures, where enzymatic activity occurs at significant rates. Slow thawing of trout in air at 5°C resulted in larger volume of centrifuged tissue fluid and higher activity of the enzyme, *β-N*-acetyl glucosaminidase in the fluid, than in samples that are fast thawed at 25°C in water. The enzyme activity was also affected by frozen storage temperature.[33] Rapid thawing is more conducive to microbial survival than slow thawing. Regulatory authorities generally

advocate that commercial thawing be undertaken at a low temperature ($\leq 10°C$) to ensure that the hygienic status of the meat is not compromised by the growth of mesophilic pathogens.

Quick thawing is necessary for maintaining the quality. Apart from the use of air, several other techniques for thawing, such as vacuum, dielectric, electrical resistance, microwave, and infrared thawing have been developed. The air in machines for blast-thawing blocks of frozen fish on an industrial scale is humidified to avoid drying of the product.[13] Air speed at 300 m per min at less than 20°C will thaw a 10 cm thick fillet block in about 4 h. Vacuum thawing helps achieve a large heat transfer coefficient. The surface of the product is heated using condensing system, but the whole system is maintained under vacuum. A commercially successful vacuum-thawing equipment has been developed to thaw blocks of medium and small whole fish. The blocks rest on bars in the equipment and as the thawing progresses, the blocks break up and fall between the bars, thus allowing the heat to penetrate throughout the fish.[14] Infrared thawing favors a high rate of surface heat transfer using the mechanism of radiation to supply heat. However, care must be exercised to ensure that the surface is not cooked before the center is thawed. Ohmic, dielectric, and microwave thawing techniques are methods of transferring electromagnetic radiation through a piece of meat, where it is transferred into heat energy. The methods differ largely in frequency of electrical energy used to heat the material. Resistive heating is applied at lower frequencies (50 Hz) in dielectric thawing, at moderate frequencies (0.01–0.1 GHz) in microwave thawing, and at high frequencies (0.1–2.5 Hz) in infrared thawing. The time taken for a 10 cm thick fillet block is less than 1 h. Incomplete thawing, where ice crystals are still present in the deep tissue, is liable to produce gaping and structural damage on filleting. It is not absolutely necessary to use packaging to keep the meat separate from the thawing medium. Fish that was frozen prerigor may still require some holding period in ice to complete the rigor process. Shrimp may also need to be held in ice for up to 24 h after thawing to enable the membrane between shell and tail to break down to aid peeling.[10] Microwave tunnels at 915 MHz are commonly used to temper fish fillet blocks to about $-10°C$ for automated slicing into fish sticks. Ohmic thawing has been suggested to thaw blocks of shrimp. Proper control of the operation eliminates local overheating. Moisture content of large shrimp was not significantly different when ohmically or conventionally thawed, although, small shrimp can have higher moisture content when conventionally thawed. Total aerobic microbial plate counts were not significantly different in shrimp that were thawed by both methods. Furthermore, sensory tests showed that there was no significant difference between conventionally and ohmically thawed shrimp.[71]

Thawed fish and shellfish are required to be immediately chilled for subsequent use by placing in boxes in ice, which are held preferably in a refrigerated storage at 2°C. Any frozen product, once thawed, should be treated as chilled fish and handled accordingly. However, it should not be marketed as *fresh* fish, because the term "fresh" indicates that the fish is not, or has never been, frozen, cooked, cured, or otherwise preserved. Furthermore, the shelf life of frozen fish, after thawing will be considerably less than that of the untreated fresh fish.

Techniques for differentiation between fresh and frozen-thawed fish or fish fillets have been examined. These include texture measurement using Torrymeter, physiological examination, and assays of enzymes such as α-glucosidase, β-N-acetyl glucosaminidase, and β-hydroxyacyl-CoA-dehydrogenase. These methods were evaluated for detection of freezing and thawing of plaice, whiting, and mackerel. It was observed that for whole fish, the Torrymeter measurement was a reliable method for detection, while for fillets, α-glucosidase assay together with sensory evaluation was recommended.[72]

4.4 QUICK FREEZING

Quick freezing is an industrially important method of increasing the shelf life of perishable foods including fishery products by subjecting them to conditions of temperature low enough to inhibit the oxidative, enzymatic, and microbial changes that are responsible for the changes in flavor and color of the foods.[73,74] For quick freezing, the temperature of the product should be brought down to $-20°C$ preferably within a period of 2 h (which gives a short duration of thermal arrest period), whereas in slow freezing, the required time could be anywhere between 3 and 72 h. The speed at which the thermal arrest period is passed through determines the quality of frozen foods (Figure 4.1). If the product is frozen very slowly (e.g., with a freezing time above 6 h), there is sufficient time for the moisture to migrate from the interior portion to the outside, which develops into large crystals. The coarse ice crystals thus formed rupture or lacerate the cells adversely affecting the basic texture of the food.[36] This results in a dark, translucent, vitreous fish fillet, whereas, the rapidly frozen fillets are densely white and opaque.[14]

In slow freezing, the movement of the freezing front is generally at a rate of 0.1–0.2 cm/h and it may take as long as 20 h for the product to be completely frozen. On the other hand, in the case of quick freezing, the freezing front travels at a rate as fast as 5 cm/h. The translocation of water from the interior to the exterior
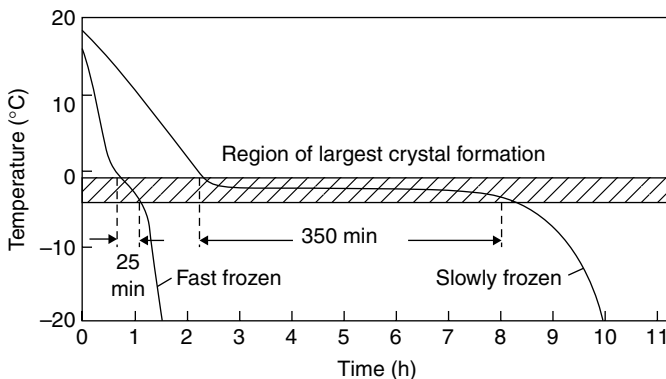

FIGURE 4.1 Slow and quick freezing of fish fillet (Adapted from Belitz, H.D. and Grosch, W., *Food Chemistry*, 2nd ed. Springer-Verlag, Germany, 1999)

TABLE 4.5
Freezing times for selected fishery products

Product	Freezing method	Initial product temperature (°C)	Operating temperature (°C)	Freezing time (h/min)
Whole cod, 100 mm thick	Vertical plate freezer	5	−40	3/20
Whole salmon, 125 mm thick	Air blast, 5 m/sec	5	−35	5/00
Whole herring, 100 mm thick	Vertical plate	5	−35	3/20
Cod fillets, laminated block of 50 mm thick, in waxed cartons	Horizontal plate	6	−40	1/20
Haddock fillets, 50 mm thick on metal tray	Air blast, 4 m/sec	5	−35	2/05
Haddock fillets, laminated block 37 mm thick in waxed cartons	Horizontal plate	5	−35	2/05
Whole lobster, 500 g	Horizontal plate	8	−40	3/00
Whole lobster	Liquid nitrogen	8	−80	0/12
Scampi, 18 mm thick	Air blast	5	−35	0/26

Source: Adapted from Sykes, S.J., *Refrig. Sci. Technol.*, 3, 41, 1988.

region of the cell is less during quick freezing, resulting in minimal changes to the native structure of the myofibrils. In addition, the fine crystals formed during quick freezing cause only negligible cellular damage. Therefore, in terms of quality, quick freezing gives a product that is juicier, having better texture, which exhibits less drip loss when thawed. Generally for quick freezing, the material is exposed to a temperature of about −40°C and the freezing is completed within 1–2 h.[13] In the case of IQF materials, the time could be as short as 3–20 min depending upon the equipment and temperature. However, it may be noted that extremely short freezing times are likely to cause structural damage to the fillets of some delicate varieties of fish.

Freezing time depends on the freezer type, freezer operating temperature, air speed and temperature in a blast freezer, thickness, and shape. Freezing times for several foods have been calculated.[73] Table 4.5 gives freezing time for some fishery products under various conditions of freezing. Software has also been developed for the determination of freezing time.[73] Packaging has also an important role in determining the freezing time.[74] In bulk freezing of fish packaged in a carton, a number of factors influence the heat transfer resistance between the geometric center of the carton and cooling air flowing over the carton on the top and bottom. These factors include convection boundary layer external to carton, carton wall, trapped air between carton and product, and the product itself. Based on a thermal
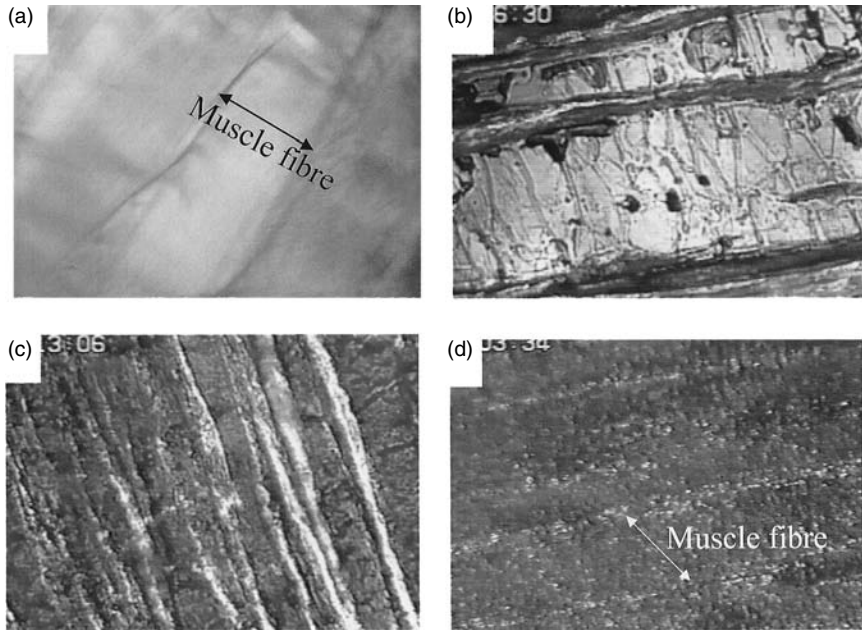
FIGURE 4.2 Electron micrographs of cod muscle. (a) Unfrozen muscle fiber, (b) fiber frozen in a domestic freezer (large ice crystals), (c) sample frozen in a blast freezer (ice crystals are smaller and more evenly distributed), and (d) cryogenically frozen sample (ice crystals are located within the muscle fibers) (From Hedges, N., *New Food* 3, 37, 2003, Russell Publishing, Brasted, Kent, UK. With permission)

conductivity of -1.5 W/m^2 K for frozen fish, these factors together contribute a resistance of 0.17 m^2 K/W in a block freezing 10 kg of fish fillets in a corrugated-wall carton. The heat transfer resistance attributable to packaging system may be as high as 59%.[63]

In the case of quick-frozen fish, the surface reflects more light than the surface of slow-frozen tissue. Consequently, the cut surface of fillets and steaks has better appearance. Another advantage of quick freezing is the efficient control of contaminant bacteria. Most bacteria in food are mesophiles and consequently their growth can be suppressed by lowering the temperature quickly. Quick freezing also results in killing of any parasites meat and has been recommended as a method for their elimination in fishery products. The process also minimizes oxidative and enzymatic changes, which are responsible for the changes in flavor and color of the foods. As compared with slow freezing, connective tissue and muscle fiber are not detrimentally affected by slow freezing. Electron microscopic studies have established the advantages of quick freezing with respect to the ultrastructure nature of fish muscle.[32] Figure 4.2 shows electron microscopic pictures of fresh cod muscle and the influence of freezing by different methods on the tissue. In unfrozen cod fillet, the muscle fibers are clearly visible. Freezing the fish in a domestic freezer caused relatively slow rate of heat removal resulting in large ice crystals. These

large crystals displaced the fish fibers leading to their structural damage. When the fish was subjected to air-blast freezing at $-30°C$, the ice crystals were smaller in size and evenly distributed, causing only minimum displacement of the muscle cells. It was interesting to note that there was no collapse of the muscle fibers when the fish were subjected to cryogenic freezing by dipping in liquid nitrogen.[32]

The influence of freezing rates on weight loss during freezing, thawing and cooking, water binding capacity, sensory properties, solubility of myofibrillar proteins, and other physicochemical properties have been investigated.[75] Maximum weight loss occurred at slow freezing temperature (freezing rate of 0.22 and 0.29 cm/h) resulting in lower water-binding capacity (higher exudates), decreased protein solubility, and tougher cooked meat. Quick-frozen meat (4.92 and 5.66 cm/h) had a higher rating than the slower frozen samples. Freezing rates of 3.33 and 3.95 cm/h exerted the least influence on physicochemical characteristics. Optimal quality of frozen meat was reached at an average freezing rate of 2 to 5 cm/h, which was achieved with a temperature below the eutectic points of meat (-40 to $-60°C$).[75]

4.4.1 Freezing Techniques

Three types of freezers are in general use, namely, (i) surface-contact freezers, (ii) direct-contact freezers, and (iii) immersion freezers. For freezing, the fish for freezing may be in direct contact with the refrigerant such as nitrogen, carbon dioxide, or "freon" substitutes, or in surface-contact freezers, by contact with refrigerated surfaces such as plates, belts, drums, or shelves. In these freezers, the material is frozen by a secondary medium such as chilled air or brine, which is recirculated and recooled by a refrigeration coil. Plate freezers, probably, are the most common contact freezers used in the fish industry. They may be vertical, horizontal, or rotary and may either be manual or automatic. Their main advantage is in the production of regular frozen blocks of uniform thickness.[76]

4.4.1.1 Surface-Contact freezing

In contact freezing, fish products are kept in contact with a metal surface cooled by refrigerants. Double-contact plate freezers are commonly used for freezing blocks of fish and shrimp. The equipment consists of a stack of horizontal cold plates with intervening spaces to accommodate single layers of the packaged product. The filled unit appears like a multilayered sandwich containing cold plates and products in alternate layers. When closed, the plates make firm contact with the two broad surfaces of the package, thereby facilitating quick heat transfer. A cold surface containing circulating refrigerant at $-40°C$, provides heat transfer as the product is conveyed over the plate surface. Due to the excellent contact between the product and refrigerated surface the crust-freeze time is short. Contact-plate freezing minimizes problem of product dehydration, defrosting of equipment, and package bulging. The packaged product must be of uniform thickness. A 3–4 cm thick packaged product can be frozen in 1–1.5 h, when cooled by plates at $-35°C$. Contact freezers can be used for initial freezing of wet, sticky products such as raw-peeled

shrimp, scallops, mussels, and delicate fish fillets, prior to being transferred into a spiral freezer for final freezing. The technology can be classified as "crust" or "surface" freezing and generally replaces cryogenics as a lower-cost method of freezing. Recently, plate freezers with carbon dioxide as the natural refrigerant in place of conventional ammonia has been developed. The advantages are better product quality, safety, less space requirements, and no negative impact on environment.[77]

4.4.1.2 Blast freezing

Blast freezing, available in many variations, is the common and versatile method for freezing seafood through direct contact with the coolant. Air-blast freezing is accomplished by placing the product on a mesh belt and passing it slowly through an insulated tunnel, where chilled air at -18 to $-40°C$ is circulated by fans around the product. The air moves in a direction opposite to the product, at speeds ranging from 1 to 20 m/sec. Air at $-30°C$ and at a speed of 10–12 m/sec is often satisfactory. Fans are used to increase the airflow, thus increasing the cooling rate. Since the chill air comes into direct contact with the surface of the meat, it improves the heat transfer and hence the freezing rate. In a typical batch process, fish are placed on trays of size usually 100×200 cm^2. A number of such trays are stacked about 200 cm high on a cart or other framework. Several of the carts are loaded into a chamber or cell that has one or more refrigeration coils, fans, and baffles to direct airflow evenly over the product. Capacities of the equipment vary from 200 to 500 kg/h. Air-blast freezing is economical and is capable of accommodating products of different sizes and shapes. Packaging is recommended to prevent excessive dehydration and weight loss in the product during freezing.

The conventional tunnel-blast freezers have been modified into spiral freezers, having continuous belts for carrying the product. The design consists of a self-staking and self-enclosing spiral belt for compactness and better control of airflow. The number of tiers in the belt can be varied to accommodate different capacities and line layouts. The products are placed on the belt outside the freezer, which are then taken inside the freezer cabinet. Both horizontal and vertical airflow can be used, although vertical airflow is more efficient. While straight-line belt freezer is generally used for particulate products, which include certain forms of shrimp, spiral freezer is used for products that require longer retention time such as fillets and various prepared foods. In both the systems, cold air is circulated through a mesh belt that conveys particulate products through the freezer. Some mechanically refrigerated systems have the ability to operate at temperatures as low as $-51°C$, providing excellent product quality. Linear belts of one or more layers can convey fish continuously through a tunnel. With perforated or mesh belts or pans, air can pass upward through the product as well as across it. Vibrating conveyors can be arranged to form a fluidized-bed freezer, but caution may be needed in the case of fragile products.[78,79]

Spiral freezers have the advantage of providing greater freezing capacity in a limited floor space. In order to cut down the frequency of defrosting, an air-defrost system has been developed to inhibit build-up of frost and ice on the evaporators. This facilitates longer production runs, and also improves the economics of the

freezing process. The more advanced spiral freezers are equipped with improved conveyor belt and auto-washing systems and drive technology that requires less maintenance, and thus suitable for installation in remote areas. More efficient airflow provides faster freezing without large dehydration losses and hence, yields higher products.[78,79]

In order to eliminate the problems of fillets sticking to the conveyor belt, the product may be initially pulled across a polyethylene film conveyor over a −40°C or colder freezer plate, which freezes the bottom surface of the fish fillet to a thickness of approximately 1 mm in 1 min. The fillet comes out with glass-smooth appearance instead of having wire mesh marks from the conveyor belt. This is then transferred to the spiral freezer[78,79].

4.4.1.3 Fluidized bed freezing

The advantages of fluidized-bed freezing as compared to air-blast freezing are more efficient heat transfer, faster freezing rates, and lower product dehydration. Small products such as shrimp can be fluidized on a mesh belt through which chilled air is pumped upward at a rate sufficient to partially lift or suspend the product (see Section 4.4.3). An air velocity of at least 2 m/sec is necessary to fluidize the product and air temperature of −35°C is commonly employed. The bed depth depends on the ease of fluidization and this in turn depends on size, shape, and uniformity of the product. A bed depth of slightly more than 3 cm is suitable for small prawns, whereas a depth of 20–25 cm can be used for products such as fish fillets. For fish fillets of 3 cm thickness, the freezing time could be of 30–35 min to bring down the temperature from 30 to −18°C. Some fluidized bed freezers employ a two-stage freezing technique, wherein the first stage consists of an ordinary air-blast freezing to set the surface of the product and the second stage consists of fluidized-bed freezing.

Modern design features incorporate fluidization airflow for better-frozen product separation. A precooling section at the entrance to the freezer can enhance product quality and also minimize clumping of soft, sticky products like raw-peeled shrimp. To ensure better hygienic quality of the product, stainless-steel contact parts with easily accessible coils are used in the construction of the machinery. Positioning the coils beside the conveyor belt instead of positioning beneath it, avoids falling of products on the coil and the area beneath the belt is completely open for cleanup. Sequential defrost systems allow continuous freezer operation as the evaporator coils can be defrosted and cleaned one section at a time.

4.4.1.4 Liquid immersion freezing

The problem of handling and freezing wet, soft and sticky products such as raw shrimp, mussels, and the like, which tend to form clumps or stick to the equipment was earlier solved by immersion freezing. Freezants used for liquid-immersion freezing should be nontoxic, inexpensive, stable, and should have low viscosity, low-vapor pressure, freezing point, and reasonably high-thermal conductivity.

Further, they should not have any undesirable effects on the product. One of the most efficient methods was to immerse the material in purified chlorofluorocarbon (CFC). However, after phasing out of CFCs due to environmental problems, aqueous solutions of propylene glycol, glycerol, sodium chloride, calcium chloride, and mixtures of sugars and salts are now used freezants. Water/glycerol/alcohol mixtures are also in use. The coolant passes through the baskets of product in one direction and returns through the refrigeration coil in the other direction, moved by propellers or high-volume, low-pressure pumps. For onboard applications, such tanks must be proportionately shorter and deeper or compartmentalized, to avoid spillage of brine due to rolling of the vessel. Dipping the seafood product in freshwater before loading into the brine freezer will diminish salt uptake by the product. Depletion of salt in the brine needs to be checked with continual addition of salt.[79]

4.4.1.5 Cryogenic freezing

Cryogenic freezers employ supercold gases such as liquid nitrogen or carbon dioxide as the freezing medium. Liquid nitrogen at $-196°C$ is sprayed into the freezer and circulated by small fans.[80,81] The freezant partially evaporates immediately on leaving the spray nozzles and on contact with the products. Heat removal from the product is by evaporative cooling of the cryogens. The rate of freezing obtained with cryogenic methods is much more rapid as compared with conventional air-blast freezing or plate freezing, although only moderately greater than that obtained with fluidized-bed or liquid-immersion freezing. Shrimp freeze in about 9 min in a commercial liquid-nitrogen freezer, whereas it takes about 12 min for freezing in a fluidized-bed freezer. Fillets may take slightly more time depending upon their thickness. For a 3 cm-cubic size shrimp wrapped in polyethylene, theoretical freezing times using air-blast and liquid-nitrogen tunnel freezer were 151.3 and 14.6 min, respectively[82,83] (see Section 4.4.3). The reason for the rapid cryogenic freezing (time required is almost ten times less than that for blast freezing) is due to the enormous temperature-driving force available for heat transfer. Apart from the lower time requirement, other advantages are high throughputs (low floor space), flexibility (adaptable to different products), low dehydration loss ($<1\%$), absence of contact with oxygen and associated oxidative changes, and hence improved quality in terms of flavor and appearance, minimal drip, and simplicity in the equipment. In addition, lower handling losses and possibility of freezing unpacked or thinly packaged products, flexibility, high throughput, reduced maintenance cost, minimum manpower requirement, and rapid installation and compactness are other benefits.[78,79] The rapid lowering of temperature in cryogenic freezing also helps inhibition of microorganisms, retention of quality through a greatly reduced enzyme action, and minimum destruction of the intact cells.[80–82] The capital cost of setting up a liquid nitrogen-based freezing unit would be about one third of a mechanical refrigeration unit, since drive motors, compressors, chilling units, etc. are not involved.[82]

Consumption of liquid nitrogen in cryogenic freezing depends on many factors other than freezer efficiency, such as product quality, its thermo-physical

TABLE 4.6
Comparison of Different Quick-Freezing Techniques for Fishery Products

Contact plate freezing	Air blast freezing	Cryogenic freezing
Low capital investment	Economic to construct and operate	High capital investment costs
Low operating cost	Higher operating cost	Higher operating cost
Controlled heat transfer	Efficient heat transfer	Efficient heat transfer
Generally bulk freezing	Flexible product line	Flexible product line
Large floor space	Large floor space	Less floor space
Refrigeration plant required	Refrigeration plant required	No refrigeration plant required
Low maintenance cost	Low maintenance cost	Minimum maintenance cost
Higher dehydration loss	Higher dehydration loss	Minimum dehydration loss
Reasonably good product quality	Good product quality	Superior product quality

TABLE 4.7
Advantages of Quick Freezing

The ice crystals formed are much smaller, and therefore, cause much less damage to the cell structure or texture of the food

The freezing period being much shorter, less time is allowed for the diffusion of salts and the separation of water in the form of ice

The product is quickly cooled below the temperature, at which the bacterial, mould, and yeast growth occur, thus preventing decomposition during freezing

The fourth and very practical reason in favor of over-slow freezing is the inherent speed and greater output and hence larger capacity for commercial freezing plant

properties, initial temperature, and temperature of the frozen product. Under an almost ideal condition, where heat load comes from the product only, the consumption of liquid nitrogen for freezing may be about 1 kg/kg. But it has been found experimentally that the consumption level varies between 1.5 and 2.2 kg/kg of product depending upon the freezer and the product.[80,81] High cost of liquid nitrogen is the major drawback for cryogenic freezing using the liquefied gas.

Another cryogen is carbon dioxide, which usually involves tumbling the product with powdered or liquid carbon dioxide. This method provides most of the advantages of liquid nitrogen freezing. Carbon dioxide, however, is absorbed or entrained by the product, which needs to be removed before it is packaged. The greenhouse effect and other disadvantages of using carbon dioxide pose limitation in using this technique. Table 4.6 compares different quick-freezing techniques for production of IQF fishery products, The general advantages of quick freezing are given in Table 4.7.

4.4.2 Major Seafood for Quick Freezing

Increasing international trade in seafood has necessitated quick freezing of popular seafood items.[73] The seafoods that are raw materials for quick-freezing are finfish,

shellfish, and mollusk. Cod, haddock, hake, saithe, and Alaskan pollock dominate the flat-ground fish groups, while the pelagic fish group is dominated by herring and mackerel. White fleshed fish such as cod, haddock, and plaice are preferred by maritime nations, whereas, inland countries often prefer dark-fleshed species with firmer texture such as saithe and red fish (ocean perch).[10] Crustacea including different species of shrimp and lobsters and cephalopods consisting of squid and cuttlefish make bulk of the frozen products. Scampi (*Nephrops norvegicus*) caught in Scotland, Iceland, and Norway; blue crab (*Callinectes sapidus*) caught in the United States and Canada; the popular shrimp in the western hemisphere; North Atlantic prawn (*Pandalus borealis*), and black tiger shrimp (*Penaeus monodon*), are all mostly frozen. Annual international trade in fresh and frozen shrimp is in the range of US$8.5 to 9 billion, nearly 50% of this being generated from seven Asian markets, namely, Japan, Malaysia, Singapore, China, Hong Kong, Taiwan, and Thailand. Molluskan shellfish such as mussels, cockles, abalone, oysters, and scallops are raw materials for freezing for localized industries. Most of the aquacultured shellfish are also candidates for freezing. Freezing technology has given impetus to growth in aquaculture production throughout the world, particularly in Asian countries.

4.4.3 Individual Quick Freezing

Consumer demand for easy-to-prepare convenient seafood is shifting focus from traditional bulk freezing to IQF and value-added fishery products. The IQF process allows the processor to supply customer with seafood in small, ready-to-cook quantities instead of large solid blocks, which have to be cut or thawed prior to packaging or use. High-value products like shrimp and certain seafood such as fish fillets are the favorite raw material for this technology.[84] The global demand for quality seafood in a competitive market is the driving force behind the demand of IQF products.[85] Freezing of major seafood products, with particular reference to IQF is discussed in the following section.

4.4.3.1 Shrimp

4.4.3.1.1 Preprocessing steps

The various preprocessing steps prior to freezing of the shellfish include cleaning, beheading, peeling, cooking, icing, and dip treatment in preservative solutions such as bisulphite.[86,87] Shrimp for freezing may be prepared as whole-head on, headless-shell-on (HL), peeled and undeveined (PUD), peeled and deveined (PD), fan-tail/butterfly, center-peeled, cooked, or peeled, deveined and cooked (PDC). In the case of center-peeled samples, peeling of whole shrimp is done keeping the head and two segments near the tail intact, and then deveined. Butterfly shrimp is made by giving a dorsal cut, which opens up the muscle block giving a fan-shaped product.

In order to make butterfly-shaped product, shrimp is washed in chilled water containing 5 ppm chlorine, beheaded, deveined, and again washed in chilled water. A small bamboo stick is then pierced from the head portion to tail between the

shell and the meat and then cooked in 1% brine for 2 min at 100°C. The cooked shrimp is then cooled in chilled water, bamboo stick removed, and then peeled completely, including the tail fans. The vertical side is then gently cut down completely lengthwise, using a sharp scalpel. The cut surface is gently opened up to form the butterfly shape. Instead of the dorsal cut, a ventral cut extending toward the vein gives a more triangular product, the latter being used in preparing *sushi* shrimp in Japan. For skewered shrimp, the process is similar as mentioned for butterfly shrimp, but piercing of shrimp is carried out in such a way that 4–5 shrimps are arranged in a skewer in an inverted "U" shape. There is also a high demand for very small prawns and pieces for value-added products such as pastes, cheeses, and pizzas. For making stretched (*Nobashi*) product, two or three light transverse cuts on the belly side of the peeled and tail-on shrimp are made, which are then pressed uniformly with a metal or plastic die. The treatment stretches the shrimp up to 20 to 30% in length. These products are also used for breading (Chapter 9).

The shellfish are graded into various sizes designated by the number of individuals in 450 g (1 lb). These size grades include, 0–10, 10–15, 16–20, 21–30, 31–50, 51–70, 71–90, 91–110, and 110-up pieces per pound. Speed in handling and processing of shrimp is of utmost importance in determining the end-product quality as well as storage life. Method of processing and storage temperature are two major criteria in this respect.[77] During the last few years, automations in shrimp and other shellfish processing operations including peeling, deveining, and grading have made rapid advances.[78,88–90] The peeling equipment is capable of handling shrimp varying in size from 10/15 to 71/90 per pound to prepare products such as tail-on round and butterfly cuts. The advantages are faster processing, higher productivity with less manual labor, flour space and operational costs, and improved hygiene. Figure 4.3 shows the various steps involved in shrimp processing and preservation.[86,87]

For traditional block freezing, the shrimp is washed three times with ice-cold water, drained, and packed in trays in 2-kg lots. After glazing with water (ice-cold water chlorinated at 5 ppm level) the material is frozen ideally at −40°C. Since glazing increases the product temperature, as mentioned in Section 4.3.3.2, it is advisable to rapidly lower the temperature by freezing. Studies have shown that if the product after glazing is not refrozen and kept in a cold storage maintained at a temperature of −25°C, there would be a 7% drip loss after thawing the product, with a yield of the only 90% of the original weight. On the other hand, if the shrimp is refrozen at −20°C immediately after glazing, before holding in a cold storage at −25°C, the drip loss was only 1%. Due to the positive quality characteristics of rapidly refrozen products, many processors in countries such as Greenland, Denmark, and France have installed equipment for refreezing shrimp.[78]

4.4.3.1.2   The IQF process

There are two major freezing methods for IQF shrimp. For smaller shrimp, fluidized-bed freezers are normally used. The through fluidization guarantees a full IQF product with negligible loss in weight. Normal handling capacities in the shrimp industry are 250 to 1000 kg/h. For larger shrimps and prawns, spiral-belt
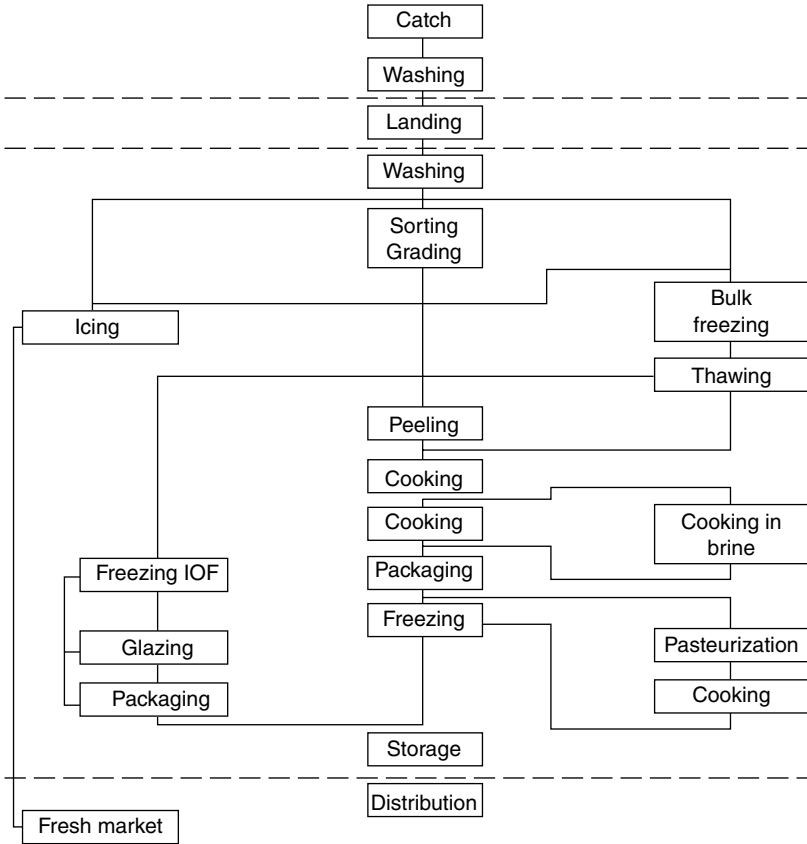
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                          │  Catch   │
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                          │ Washing  │
- - - - - - - - - - - - - └──────────┘ - - - - - - - - - - -
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   ┌──────────┐           └──────────┘
   │ Glazing  │           ┌──────────┐        ┌──────────────┐
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   │Packaging │                               ┌──────────┐
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  ┌──────────────┐        │Distribution│
  │ Fresh market │        └────────────┘
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FIGURE 4.3 Shrimp processing line (Reprinted from Chandrasekaran, M., *J. Food Sci. Technol. (Mysore)*, 31, 441, 1994. With permission from Association of Food Scientists and Technologists (India), Mysore)

freezers are commonly used. As the belt speed is variable, optimum-holding times can be set for each product. The controlled counter-flow air-distribution system keeps weight loss down to a minimum. Shrimp frozen in a standard IQF-tunnel freezer will take the natural curved shape of the animal. Due to the short time taken to freeze, the quality of a product frozen using a belt freezer would be better than a product which has been subject to longer-freezing process. In contrast, in conventional freezing, shrimp frozen in a plate freezer will freeze the way the product is laid on the plate. The stationary product in a plate freezer will also gather water/ice unevenly; often the surface touching the surface of the plate will be encrusted with a thicker layer of ice. Further, glazing on conventional products too would not be normally uniform. The area of the shrimp touching the plate will have a thicker glaze than the rest of the body. Butterfly and skewered shrimp are usually packed in thermoform trays under vacuum and frozen at −40°C. The storage life of IQF-frozen shrimp is about a few months when held at −18°C. Vacuum

TABLE 4.8
Comparison of Bulk and IQF Shrimp

Parameter	Bulk-frozen product	IQF product
Freezing machinery	Conventional contact plate freezer	Cryogenic/belt freezer
Product size	Usually 2 kg	Individual shrimp
Glazing	Amount of glaze employed varies. Chemicals such as polyphosphates may be used in the glaze	Uniform coating of the product by the glaze. Usually water without any additive is used as glaze
Freezing time	3–6 h	15–30 min
Price	Determined by drained weight. Hence, glaze with additives such as polyphosphates are used to increase drained weight	Price not determined by drained weight
Sensitivity to temperature fluctuation	Low	High
Consumer acceptability	Poor due to difficulties in handling	High. Easy to handle

packing along with 8% water glazing can extend the shelf life up to 10 months at this temperature. Table 4.8 presents comparison of bulk and IQF shrimp.

Innovations have been made recently to further improve the product quality. A cryogenic freezer used for shrimp, mussels, etc. is CRUSTOFREEZE®, an equipment utilizing liquid nitrogen, designed for installation before a traditional freezer. The product is fed into the freezer from a vibrating conveyor entering a stainless steel IQF pan together with a horizontal flow of liquid nitrogen. The nitrogen cushions the products fall and separates the pieces, freezing the product surface instantly. After the first stage the products are dropped onto a belt where the crust freezing is completed in a combined nitrogen dip and spray. A shiny ice crust forms around each shrimp ensuring the best texture and flavor of the shrimp. The product is then transferred to an out feed belt within the equipment in order to minimize the consumption of liquid nitrogen, and fed into a traditional freezing equipment for complete freezing.[78] The texture is not affected by the method employed for quick freezing. In prawns frozen with liquid nitrogen and stored for one month at −20°C, the intermuscle fiber spacing and the degree of cell integrity were similar to those observed in prawns frozen with air blast at −30°C and stored at −20°C.[9] Table 4.9 shows comparison of IQF shrimp prepared by different freezing processes.

The IQF process also helps development of novel menus as per consumer requirements. A process has been described for IQF cooked-shell-off North Atlantic shrimp, *P. borealis*.[10] For retailing, the thawed shrimp from frozen blocks are size-graded, steam-blanched for 50–120 sec in order to reach a temperature of 76°C. The cooked shrimp is peeled using center-rotating rollers, dewatered, and the remaining shell on the meat is separated by air blowing. The cleaned shrimp

TABLE 4.9
Comparison of IQF Shrimp Prepared by Different Freezing Processes

Parameters	Contact/plate freezing	Belt/cryogenic freezing
Freezer used	Plate/contact freezer using generally ammonia as coolant	Belt or cryogenic freezer using liquid nitrogen or carbon dioxide as coolant
Freezing time	1–3 h depending upon product size and ambient as well as cooling temperature	15–30 min depending upon product size, belt speed and ambient temperature
Appearance	Varying size	Uniform size
Packaging	Loosely packed in retail packs followed by bulk packaging	Loosely packed in retail packs followed by bulk packaging
Retention of water from glazing	Uneven gathering of water	Uniform
Consumer acceptability	Low	High

TABLE 4.10
Storage Life of Shrimp (*P. borealis*) Quick Frozen Under Various Conditions

Product	Storage life, months, at −18°C
Blockfrozen, vacuum packed	6–7
IQF, polybag	3–4
IQF vacuum packed	6–7
IQF vacuum packed, 4% glazed	8–9
IQF vacuum packed, 8% glazed	>10

Source: From Londahl, G., *Infofish Int.*, 3, 49–53, 1997. With permission from *Infofish*.

are sprayed with brine in order to impart 1 to 2% salt content. The brined shrimp is IQF, water glazed to about 10% level, and packed to bulk cold storage or directly packed into retail and catering packs of 8 oz, 1, or 2 lb sizes, usually in polyethylene bags. Size grade will vary from 80 to 120 per lb to small, with a count of 400–600 per lb. Storage lives of quick-frozen shrimp (*P. borealis*) under various conditions are given in Table 4.10.

4.4.3.2 Lobster tails

For processing lobster tails, the raw material should be fresh, having its characteristic color, appearance, and odor. The tail should be free from eggs, saw dust, sand, dirt, and any other extraneous matter. The first preprocessing step for lobsters is thorough washing with potable water to remove all the adhering sand,

dust, loose eggs, etc. The lobster tails are then removed, washed well with potable water containing 5 ppm chlorine, and transported in chilled water or ice to the processing plant. After washing with potable water containing 5 ppm chlorine at the plant, the tails are wrapped individually in polyethylene film. The material is then quick-frozen at or below −40°C in minimum possible time in a blast or cryogenic freezer. It is a common practice to subject lobster tails to a heat treatment by dipping in boiling water for 1–3 min. This helps cooking the meat only next to the external shell. The treatment facilitates meat removal when required. In the case of spiny lobsters, the tail is about one third of the total lobster weight and contains about two third of the entire meat. The tails are washed and graded into four sizes (170–450 g) for freezing. The raw tails are frozen individually (or in blocks), protected with an ice glazing (to prevent dehydration) and packed in waxed cartons. The frozen lobster tails store well at −23°C or lower.[10]

4.4.3.3 Scampi

Whole, large scampi with intact claws and appendages is a valuable commodity. Before freezing, they are normally given a dip in sodium metabisulphite to retain the bright pink color of the shell or carapace and to prevent darkening or melanosis. The concentration of the chemical should not exceed 100 ppm in the whole scampi or 30 ppm in edible meat. The scampi tail meat is IQF, glazed and packed in 1 or 2 lb bags for use in cooked dishes.

4.4.3.4 Bivalves

Some of the popular IQF-bivalves products include vacuum-packed half shell oysters, clams, cockles, and scallops, which are also available as coated products or as ingredients in complete ready-made meals. While slow freezing of oysters causes darkening of the muscle and drip formation, the problem is reduced by quick freezing. Molluskan shellfish such as oysters, clams, and mussels are filter feeders and accumulate microorganisms and other particulate matter in the gut from the surrounding water. The important preprocessing step for bivalves is depuration. In depuration, the shellfish are stored in tanks of potable water usually for 36–48 h and allowed to decontaminate naturally. Specially-made depuration plants with attached recirculating seawater systems are available for the purpose. In a process for IQF mussel, the animals are declumped, precleaned, and transferred to a rewatering station for 8–12 h for depuration. The samples are then cooked at 95°C for about 30 sec. The cooking process opens the shells and the meat is removed by shucking over vibrating graders. Cooking also ensures adequate inactivation of viruses such as small round viruses, which are associated with coastal sewage pollution and food poisoning. The "beard," a tough, inedible anchor muscle is removed from the meat after shucking. The cooked meat is weighed and filled in a bag, in many cases containing some sauce. The bags are vacuum-sealed, cooked in a suitable cooker, cooled and frozen at −18°C, and stored at this temperature. The product has a shelf life of 2 years.[90] A process for retaining taste and also for reducing bacteria in frozen oyster has been patented.[91] In the process, the top shell of the oyster is initially removed, followed by rapidly lowering the temperature of

Raw material

↓

Washing, declumping, and grading

↓

Conditioning

↓

Depuration

↓

Infrared heating

↓

Cooling

↓

Shucking

↓

Individual quick freezing

↓

Packing

↓

Cold storage
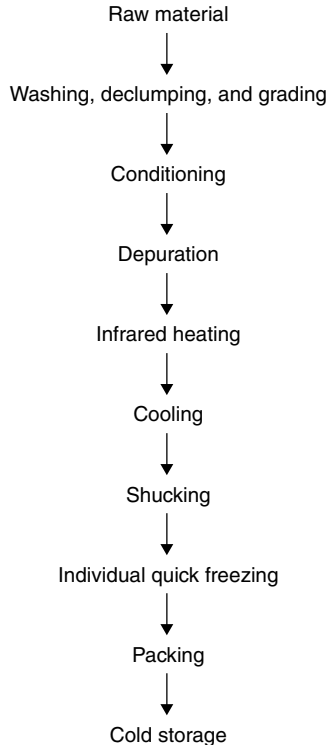
FIGURE 4.4   Process flow chart for half shell mussel (Cited from Gorski, Z., *Infofish Int.*, 6, 44, 1999. With permission from *Infofish*)

the shucked oyster to below −32°C. The frozen oyster is glazed by spraying water to form a thin layer of ice on the surface. The oyster is then placed in a cold storage at a temperature not higher than −32°C for at least 10 days. The process is also useful for cockles. Figure 4.4 depicts process flow diagram for half shell IQF mussel.

Automatic machinery is available for processing of fishery products including mollusks.[89,90] Machinery for shucking of mollusks has been developed. For mussel, the incoming material is first washed, declumped, and graded. Graded mussels are conditioned at constant temperature. From the conditioner, the mussels go to a debyssing machine for depuration and further to the half-shell processor. The main characteristic of the half-shell processor is the heating chamber where each mussel is ensured separate heating by high-frequency infrared lamps to loosen the meat and opening the shell on one side. The opened upper shells are water-cooled, the shells are removed manually, and then subjected to individual quick freezing. Optionally, an automatic garlic–butter or other sauce-dosing unit can be positioned between the inspection belt and freezer. The end product, mussels on a half shell, is usually eaten as an appetizer and is most popular in France, the United States, Spain, and Japan.[90] Similar operations are used for oysters too. The oysters are washed often with water under high pressure to remove mud. It is then steamed in special retorts causing the shell to gap open. They are then shucked and the meat is

used for freezing or canning.[90] Frozen shelf life of clam is limited to 4 to 6 months at −18°C due to development of rancidity and toughening. Cryogenic freezing prevents these deteriorative changes. Scallops are marketed either as frozen in blocks with plate freezers or as IQF with or without breading.

4.4.3.5 Cephalopods

In the case of squid, the term fillet is applied to the mantle, which is opened lengthwise along the dorsal side and wherein the viscera, ink sac, skin, and head with the tentacles are removed. Squid rings are cut from cleaned squid tubes. Fillets and rings are raw materials for IQF products.[92] It is ideal that these products prepared from freshly harvested squid are frozen immediately. Prolonged Chilled storage of mantle before freezing results in significant loss in protein functionality, particularly thermal gelation profiles. Further, keeping the mantle more than 4 days in ice prior to freezing results in yellowing of the frozen product. The functional properties are not affected during frozen storage, although appreciable protein hydrolysis takes place.[93] However, Canadian squid stored at −30°C can remain in acceptable condition for 18 months.[92] Similarly, prolonged chilled storage affects the quality of cuttlefish. A frozen shelf life of 2 months has been observed for freshly harvested cuttlefish mantle. Yellowing of the product in some species could be controlled by a dip treatment in 2% brine containing 0.2 to 1% of acetic, citric, or ascorbic acid, before the freezing process.[94]

4.4.3.6 Fish fillets

A fillet is a piece of meat consisting of the dorsal and abdominal muscles of fish. Fillets may be cut suitably to remove undesired portions. For example, the nape may be completely removed to produce a "J" cut fillet. In some flat fish fillets, the black membrane covering the nape area is removed by surface trimming. Large, flat fish fillets may be cut into half, longitudinally, to produce cross- or quarter-cut fillets. For fillets to be used in boneless fish blocks, it is necessary to remove the small pin bones in the thick nape end. A popular equipment for this purpose is Baader 184, a white fish-filleting machine. Removal of pin bones is achieved by "V" cutting, which involves cutting out a wedge of fillets. Meat from the "V" cut fish material can be recovered by mechanical deboning.

Fish fillets as skin-on or skinless are suitable for retail and catering. Presence of skin in skinless fillets, bones, and occasional presence of nematode parasites in the meat are quality problems. The parasites could be checked visually by inspecting skin-on fillets under a strong light. Skinless fillets are inspected over candling tables consisting of a back-lit translucent plate, in order to detect and remove visible nematodes, particularly from the nape region. Fillets of fish displaying soft texture (e.g., due to seasonal circumstances such as spawning and feeding) gaping and fillets that have to be severely trimmed to remove nematodes or blood spots are selected for second-grade fish blocks. Automation systems for fillet inspection are available.

4.4.3.6.1 IQF process for fillets

Fillets are blast frozen on trays in continuous flat-belt freezers. To protect them from dehydration, the fillets are dipped in a water bath or passed through a spray to impart a glaze 0.5 to 2 mm thick. The frozen material is bulk packaged in protective waxed cartons. Frozen fillets are marketed as IQF or shatter packs for catering and further processing. Shatter pack consists of fillets individually placed on polyethylene sheets, which are layered within a frame and frozen in a contact freezer. The individual packaging prevents moisture and oxygen permeability. On leaving the freezer, the product should be minimally exposed to humidity and warmer temperatures and moved to a cold warehouse as quickly as possible and should have enough dwell time for temperature equilibration. Packaging outer cases for frozen products should be of good quality to prevent contamination, ensure the integrity of the product during storage, and prevent dehydration. Pack-up coding should be adequate for all effective identification. Outer-case coding is useful to enable proper stock rotation of individual cases. Each warehouse should have adequate capacity and equipped with suitable mechanical refrigeration to maintain a temperature of at least −18°C or lower. The end user separates the fillets by knocking them apart and peels off the film without thawing. IQF process for farmed-fish species such as catfish has commercial applications. The fish are harvested and kept alive till they are processed (see Chapter 12). The fish are slaughtered, cleaned, processed, and IQF to temperature −40°C. The entire processing is completed in 30 min. There are 20 certified catfish processing plants in the United States with a total capacity of 10 million pounds.[95]

4.4.3.6.2 Fillet block manufacture and other processes

The fillet block from boneless and skinless fillets of whitefish such as Alaska pollock, South African hake, Pacific cod, and Atlantic cod was developed over 50 years ago and has since then remained the most important raw material for further processing of fish products in North America and Western Europe. It was originally developed to respond to the need for a standard product of fixed dimensions that could be used with maximum yield by sawing into small, size-controlled, rectangular portions for further processing into breaded fish sticks and portions.[3] The tails and nape ends of the fillets are also used in block manufacture. Addition of edible adhesive materials could be used for better binding. The 16.5 lb (6.5 kg) block of dimension of $48.0 \times 25.3 \times 6.2$ cm is the most commonly internationally traded fish blocks. In 2001, close to 300,000 t of single-frozen fillet blocks were produced from the major whitefish stocks. A further 300,000 t were double-frozen blocks, that is, originally produced as frozen-headed and gutted fish, for later processing to frozen fillets and blocks, most often in China.[96]

For composite fillets, smaller fillets are placed in a large fillet-shaped mold, and compressed in presence of some binders, with a low-pressure ram. After release of the mold, a fillet piece is obtained, which after freezing (also battering and frying) is almost indistinguishable from fillets obtained from large fish.[3] A process for value addition of hake, commonly known as "whiting" in North America, has been developed.[97] The fish, immediately after catch, is kept in special holds of the

boats filled with ice and seawater, creating an ice slush. Air is circulated through the hold, keeping the fish uniformly cold and fresh. The fish is transported under fresh super-chilled ice slurry to the processing plant, where it is cleaned, inspected, and filleted. These fillets are subjected to individual quick freezing, which are then glazed with water. Interleaved fillets with or without skin in consumer packs are also a product of commerce.

Tuna and salmon fillets are some of the other fish items that are popular when processed by techniques such as smoking, in combination with individual quick freezing.[16,98] Freezing before smoking resulted in an increased product yield and water content, but with softer texture in the case of salmon.[16] The quality of fillets have been improved by injecting additives such as polyphosphate into the material.[99] Several commercial products developed based on individual quick freezing and related technologies are available. Some of the items include "fish strips" cuts from whole fillets of whitefish, cooked IQF lobster (prepared by a patented "sea lock" process), and blast-frozen Maine lobster tails.[98]

Individual quick freezing may have applications to supply fresh seafood for *sashimi* (raw seafood) products in Japan. The most common fish used for *sashimi* is red meat fish, in particular, tuna, marlin, sailfish, mackerel, tilapia, flounder bonito, and salmon, (and also non-fish items including squid, prawn and abalone) which are usually served uncooked and chilled with soy sauce. *Sashimi* freshness is indicated by the color of the raw meat, its texture and flavor. Prolonged exposure to high-ambient temperature may result in loss of the natural color of fish, as a result of oxidation of oxymyoglobin in the muscle and hence the value of the fish for *sashimi*. While the problem could be solved by treatment with carbon monoxide, this is not a permitted method. Quick freezing, particularly, individual quick freezing, which takes 1 to 3 h, can supply good quality fish for distant *sashimi* markets.[9]

4.4.4 Merits of Individual Quick Freezing

As compared with bulk freezing, individual quick freezing has several advantages. These include lower freezing time, better quality, and consumer acceptability as well as higher price for the product. The lower freezing time is essentially due to the increased surface area of the latter, which favors enhanced heat transfer. In addition, since generally the material is frozen under nonpackaged conditions, the resistance of the packaging material to heat transfer is not involved in determining the freezing time of IQF products. Rapid lowering of the temperature favors retention of the biological condition of the fresh food at a point at which it is frozen and to give a thawed product that has high-sensory qualities. The process is suitable for small and thin products having varied or irregular shapes.

IQF products will have a higher freight component as the products are normally loosely packed in retail packs of bulk packs. The IQF products generally enjoy a slightly higher market price than block-frozen products. However, it is difficult to compare the prices of products prepared by block freezing and IQF processes. The price of block-frozen products is quoted in terms of drained weight whereas the price of IQF products is quoted in the glazed form. Processors looking to install,

TABLE 4.11
Advantages and Disadvantages of Individual Quick Freezing

Advantages	Best freshness close to natural freshness
	Better taste
	Better flavor/aroma
	Better color and appearance
	Higher nutritive value
	Less dehydration loss during freezing
	Minimal drip formation during thawing
	Requires less time for cooking
	Greater convenience in handling
	Less fuel required
	"Ready-to-heat and serve" frozen meals possible
	High hygiene quality
	Less chances of adulteration of the product
	"See-through" pouches/containers help the purchaser/consumer in prompt evaluation/selection purchases
	Provides a method to make different varieties of seasonal foods "all the year round" in almost fresh condition and at a reasonable cost
Disadvantages	Requirement of larger storage space
	Possibility of thawing and refreezing in case of wide temperature fluctuations during storage

upgrade, or expand the IQF operation need to carefully consider the comparative data to determine the technology that will serve their particular requirements better. The benefits of quick freezing to ultrastructure of myofibrils of cod and hake have been noted[32,103]

Some disadvantages of individual quick freezing have also been noted. IQF products require higher storage space. Further, the products are more sensitive to dehydration because of large exposed surface. There is also a possibility of thawing and refreezing of the product into large lump in case of wide fluctuations in storage temperatures. Due to the insulation properties and poor heat conductivity of air in packed products, proper temperature regulation of IQF products during transportation is of utmost importance. Table 4.11 shows the merits of individual quick freezing.

4.4.5 Recent Innovations in Individual Quick Freezing

As discussed, seafood can be IQF employing several types of freezers. In the recent past, sophisticated machinery has been developed for preparation of high-quality hygienic IQF products. Impingement freezing is one of the methods used to increase the speed of a mechanical system. Food-processing system using air impingement consists of several jets of high-velocity chilled air (10–100 m/sec) exiting from nozzles and impinging on a food product at the sides, resulting in freezing at a rate faster than cryogenic freezing. Figure 4.5 depicts schematic presentation of the impingement process. The factors affecting impingement
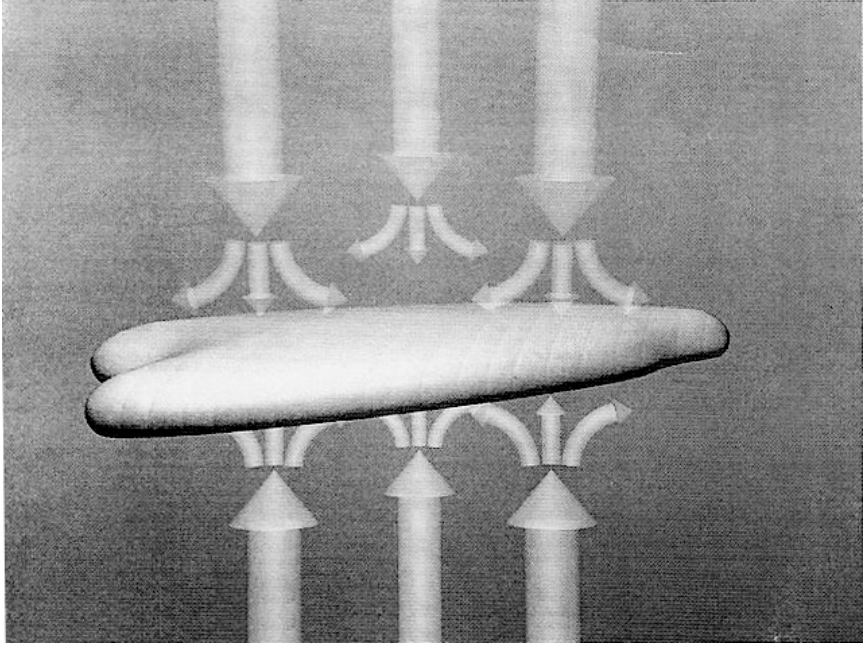
FIGURE 4.5   Schematic presentation of the impingement process (Courtesy, *Frigoscandia*, Equipment, Sweden)

systems include nozzle exit velocity, nozzle design, boundary layer, and characteristics of the product surface and design of the equipment. This technique helps to reduce the freezing time to less than 2 min depending upon the size of the product. Using this technology, shrimp and fish fillets could be frozen within 200–300 sec, with minimum dehydration losses. Generally, products having 2.5–4 cm thickness are ideal for impingement freezing, since in thicker products heat transfer from the center to the surface takes longer time and hence affects the freezing time. In the case of white fish fillets, dehydration during impingement freezing is only 0.6–1%, as compared with 5–6%, 4–5%, and 1.5–2.0% in cold storage, air-blast, and conventional in-line spiral freezers, respectively. Further, the products when thawed suffer only negligible drip loss. Operating costs are similar to those of traditional mechanical equipment. These systems have the potential to provide high-yield, high-quality seafood products such as frozen-peeled shrimp and fish fillets.[85,100] The freezers are provided with stainless steel components equipped with automated cleaning system to ensure product safety. The equipment can also be held cold continuously for periods up to a week.[100] A specialized impingement freezer has also been developed for onboard freezing of fish fillet.[90] Figure 4.6 shows operation of an impingement freezer.

Another novel method of freezing, namely, pressure-shift freezing is under investigation. The technique produces smaller and more uniform ice crystals than
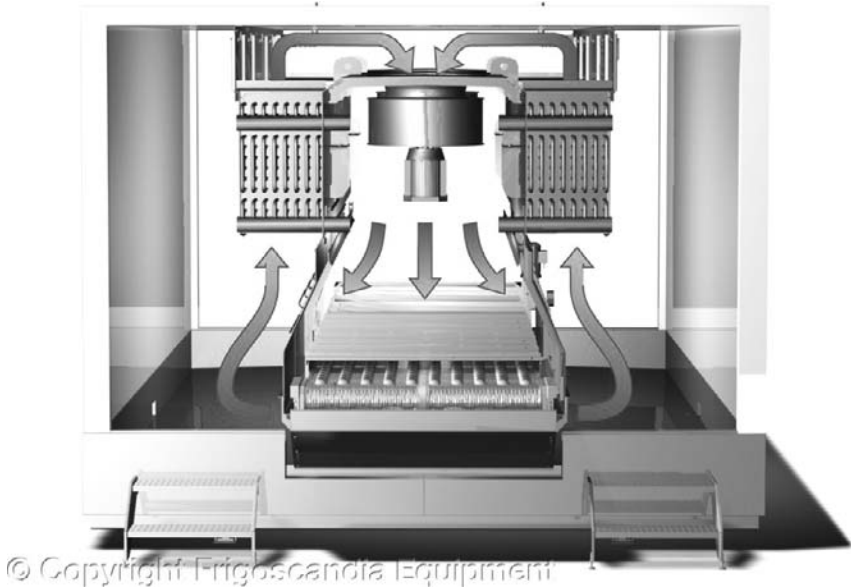
FIGURE 4.6   Operation of an impingement freezer (Courtesy, *Frigoscandia*, Equipment, Sweden)

conventional freezing. By increasing the pressure to 200 MPa, the freezing point of water is depressed, allowing the product to be cooled to about $-20°C$ without the water freezing. When the pressure is released, the water rapidly freezes with uniform nucleation of ice throughout the product (see Chapter 11). An innovative freezing process has been developed for freezing *sushi*. *Sushi* is raw seafood served as bite-sized pieces after topping the fish with sauce containing starch. During the freezing process, starch and water separate from the sauce toppings, causing loss of stickiness and leading to falling apart of the toppings when packed up. In the modified process, the IQF product is fed into a chamber. Then the sauce and liquid nitrogen are injected sequentially to freeze layers of sauce onto IQF foods, coating each piece of product completely and uniformly at a coating ratio as high as 50%. The freezing process is 20 times faster than the conventional quick freezing and five times faster than nitrogen freezing.[101] The system can use all different sauces and gravies having wide viscosity range.[102]

A general multifunctional processing line for fish, bivalves, and shrimp has been developed in the Netherlands.[89] The processing line is capable of pretreatment of different raw material, preservation, packing, and storage at a single site. One of the common operations is the cooking step, where mussels, clams, scallop, and shrimp can be cooked in the same machine. Another line of value addition, that is, battering and breading, can be used for all of the species mentioned earlier. The fishery products are subjected to IQF freezing in a multipurpose spiral, belt, or flow freezer. The flexibility in the production line will enable investors to utilize it more efficiently to the maximum capacity.

4.4.6 Packaging for IQF Products

The packaging system for IQF products is not standardized, which vary from processor to processor. IQF shrimp and other items are not well protected compared with their glazed, block-frozen counterparts against dehydration and oxidative rancidity during storage. Hence, there is a need for good-quality packaging material. In general, the package system followed by processors is plastic-film pouches (monofilms or co-extruded films) that may be printed to indicate the details regarding the product. When laminated to print surface it will brighten the colors appreciably and enhance the sales appeal of the package as a whole. Besides, lamination prevents moisture affecting the cartons. Compression strength of 500 kg has been specified to provide reasonable safety to the product. Generally, the product is placed in the primary container/pack along with the code slip and weighed. The unit pouch carries product weighing 500 g, 1, 2, or 4 kg. Since the unit-packaging material is in direct contact with the food, it should have adequate barrier properties to protect the product from dehydration, oxidation, discoloration, and off-flavor to offer shelf life extension, ease of distribution and presentation. An accepted packaging material for IQF shrimp consists of 12 μm plain polyester laminated with 250-gauge low-density polyethylene.[62] The individual packages may either be placed in intermediate cartons or directly in master cartons. The intermediate cartons are made from duplex board or 3-ply corrugated fiberboard. The cartons must maintain their structural rigidity all the way to the market. It is usually made out of much more insulating, but stronger, corrugated-wall paperboard.[62] Table 4.12 gives requirement for packaging frozen fishery products.

TABLE 4.12
Requirements for Packaging Material for Frozen Shrimp

Wrapping material	
Low-density polyethylene	100 gauge (0.025 mm)
HM-HDPE	60 gauge (0.015 mm)
LLDPE	60 gauge (0.015 mm)
Pouches	
Low-density polyethylene	200 gauge (0.05 mm)
HM-HDPE	120 gauge (0.03 mm)
LLDPE	120 gauge (0.03 mm)
Cartons	
Grammage	300 GSM
Caliper	0.4 × 0.02 mm
Wax content	10 GSM on each side
Polyethylene coating	20 GSM on each side
Ring stiffness	270 N
Bursting strength	400 Kpa
Cobb 30° value	10

Source: Adapted from Gopakumar, K., *Seafood Export J. (India)*, 2, 7, 1996. With permission.

4.5 TRANSPORTATION OF FROZEN SEAFOOD

For transportation of the frozen material, a proper cold chain maintaining the required temperature is an essential necessity.[104] Because of inadequacy of proper cold chain facility, there is a tendency in some countries to transport frozen foods in insulated vans without any active refrigeration. This involves serious quality problems and perhaps loss of the product. There are several mechanically and cryogenically refrigerated transportation systems available. Cryogenic transportation systems provide a number of advantages such as simplicity of use, nonrequirement of precooling, light weight, adaptation on all isothermal vehicles, and additional cargo space and have potential for adoption in countries where there is a shortage of mechanical refrigeration and trained personnel. Spraying of liquid nitrogen has been examined to provide a technique for transportation of frozen material. This system has been examined for transportation of 12.5 t of frozen shrimp (temperature, $-18°C$) at an ambient temperature of $30°C$ in an insulated truck.[80,81] After spraying the vehicle was ready for transit. Figure 4.7 shows temperature distribution inside frozen-shrimp block as a result of spraying the cryogen for different duration. The treatment lowered the core temperature of the product depending upon the thickness and spraying time. Termination of the spraying resulted in rise in core temperature during transit, as shown in Figure 4.8.
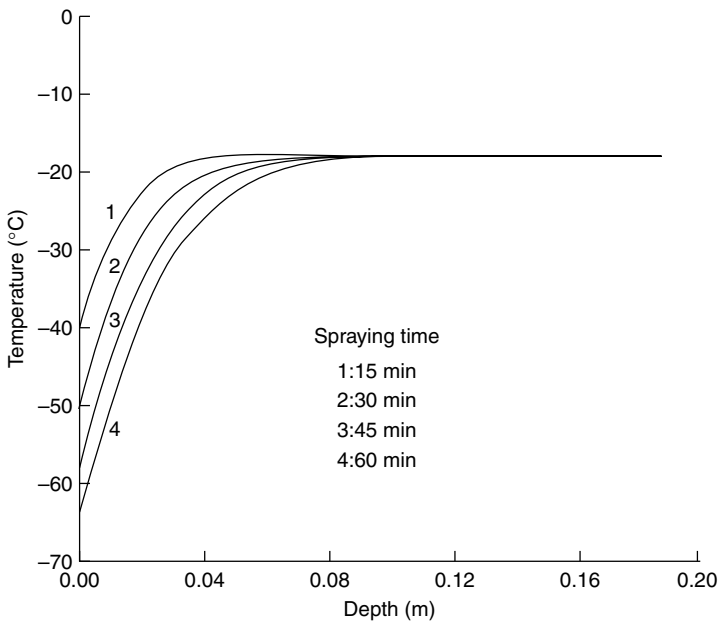
FIGURE 4.7 Temperature profiles after spraying liquid N_2 at a rate of 3 kg/min on the side (1.88 m) of the frozen shrimp block (12.5 t) for varying time. Initial temperature of the product, $-18°C$; ambient temperature, $30°C$ (Reprinted from Goswami, T.K., *Indian Food Ind.*, 21, 27, 2002. With permission from Association of Food Scientists and Technologists (India), Mysore)
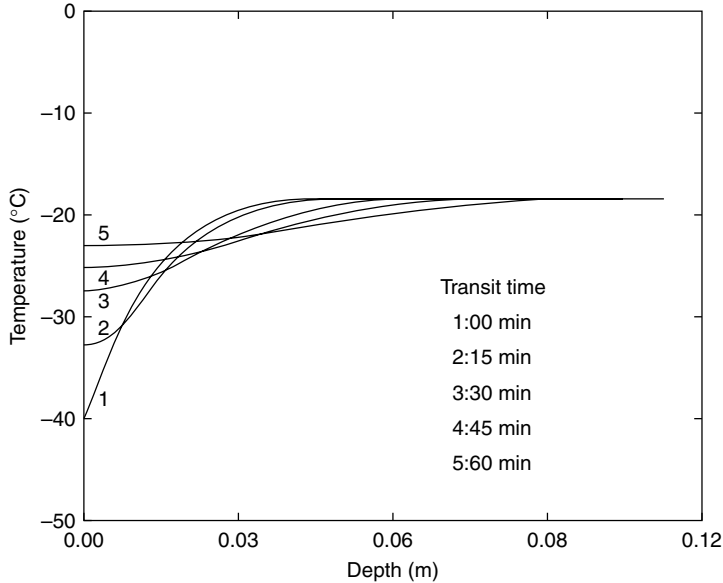
FIGURE 4.8 Temperature profiles in the frozen shrimp block along a length of 1.88 m during transit: (1) immediate after spraying, $t = 0$; (2) after 3 min of spraying, $t = 3$ min; (3) $t = 15$ min; (4) $t = 30$ min, and (5) $t = 60$ min (Reprinted from Goswami, T.K., *Indian Food Ind.*, 21, 27, 2002. With permission from Association of Food Scientists and Technologists (India), Mysore)

It was therefore important to spray the gas intermittently to maintain the product temperature to a minimum of $-18°C$. It was calculated that 90 kg of liquid nitrogen was required to transport the material under the conditions for a duration extending up to 11 h.[80] However, care should be taken to avoid fluctuations in core temperatures in order to maintain the product quality.

In summary, freezing and frozen storage offer a practical method for value addition of fishery products. There are several advantages with respect to quick freezing, particularly individual quick freezing of fishery products. Innovations in freezing techniques and their judicious applications have significant scope in production of a variety of convenient frozen fishery products to the expanding markets.

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5 Cook-Chill Processing

5.1 INTRODUCTION

The increased demand for convenient, fresh-like, ready-to-eat, or ready-to-prepare products has encouraged applications of combination of preservation techniques such as heating and refrigeration in food processing.[1] Chilling in conjunction with minimal processing treatments such as heating can retain freshness and build in convenience in a number of food products. Foods that fall into this category are designated as minimally processed foods, which include fresh-cut vegetables, prepared sandwiches, ready-to-eat meals, and chilled prepared foods.[2]

5.2 "MINIMALLY PROCESSED FOODS"

International bodies have defined the criteria for "minimally processed foods." According to European Economic Commission notification,[3,4] minimally processed foods are characterized by the following criteria, namely:

1. A mild preservation process where product temperature during processing fall within 0 to 100°C.
2. Reliance on refrigerated storage and distribution.
3. Water activity (a_w) higher than 0.85.
4. pH higher than 4.5.

The limiting values of pH and a_w are based on the growth limits of *Clostridium botulinum* (pH > 4.5) and *Staphylococcus aureus* (a_w, 0.85). This definition is consistent with that of the European Chilled Food Federation.[4]

The Codex Alimentarius Commission definition is slightly different.[5] The Commission classifies "minimally processed foods" as:

1. Those intended to be refrigerated during their shelf life to retard or prevent proliferation of undesirable microorganisms.
2. Those that have an extended shelf life of more than 5 days.
3. Foods that are heated or processed using other treatments to reduce their original microbial population.
4. Low acid type foods (pH > 4.6) and those that have high water activity (>0.92).
5. Items that may use hurdles in addition to heat or other treatments and refrigeration in order to retard or prevent proliferation of undesirable microorganisms.

6. Packaged foods, not necessarily hermetically, sealed before or after processing (heat or other preservation treatments).
7. Those that may or may not require heating prior to consumption.

By the above definitions, minimally processed foods which need time/temperature control may be designated as TCS (Temperature Controlled for Safety) foods. The Institute of Food Technologists, United States in a report to the U.S. Food and Drug Administration evaluated potentially hazardous foods and recommended a definition of TCS foods as "foods that require time/temperature control to limit pathogen growth or toxin formation that constitutes a threat to public health."[5]

5.3 COOK-CHILL PROCESSING

"Cook-chill" products are minimally processed foods, processed by mild heat treatment followed by chilling for the purpose of limited storage and distribution. "Cook-chill processing" is defined as a "catering system based on the full cooking of food followed by fast chilling and storage at controlled low temperatures above freezing point (0–3°C) and subsequent thorough heating close to the consumer before consumption."[6–8] The term, "pasteurized chilled foods" is also used in this context to these foods that have received a heat treatment or have been processed using other methods to reduce their initial microbial population. Therefore, they are "minimally processed foods" according to the above definitions.

The important steps in the "cook-chill" processing are cooking or mild heat treatment required to pasteurize the product followed by packaging and chilling. The technique can be used as a hot-fill system for liquid foods such as soups, stews, and gravies, which are prepared, pasteurized, and pumped directly into barrier-plastic casings at the pasteurization temperatures. The process is also employed for prepackaged foods that are subjected to pasteurization treatment.[6] Whereas chilling has long been used as a method to extend the shelf life of foods, the heating step was incorporated to reduce the initial microbial population in the product to favor a shelf life of a few days at refrigerated temperatures. The cook-chill process has been developed with a view to market precooked, ready-to-prepare, consumer friendly, convenient foods. Since minimally processed foods, including cook-chill items, are produced using techniques that are not intended to completely inactivate all the contaminant microorganisms, they are not sterile. The safety of the products during storage depends on an appropriate refrigerated storage for specified periods, which should prevent the growth of any hazardous microorganisms that can jeopardize food safety.[4] In order to ensure consumer safety, the refrigerated storage period is limited normally to 5 days. However, products for extended storage period could be developed by modification of the process (see Section 5.3.6). Interest in this processing technology was recognized in 1984 by the European Cooperation in Science and Technology program.[4] Several European countries have shown interest in using the technology in food service.[3] The major product,

cook-chill meals, is popular in many hotels in western countries. The method has also potential for use in hospital, school, and factory feeding programs.

5.3.1 Design and Operation of Cook-Chill System

The minimum unit operations of the cook-chill catering system are cooking, chilling, packaging, and chilled storage, as depicted in Figure 5.1. Additional operations may be introduced as per the product requirement. Equipment has been developed for these operations such as air cooking, brine immersion, and chilling.[9] The preparation of the raw material is comparable to those in the conventional processing systems. The raw material is subjected to cooking in order to inactivate the vegetative cells of microorganisms including pathogens. There may be variations in heating and chilling procedures depending upon the countries where the technology is used. Pasteurization temperatures usually range between 65 and 95°C.[3] The cooked product is rapidly chilled to 0 to 3°C, preferably within 30 min of leaving the cooker. The product is stored at this temperature. The cook-chill products require reheating to at least 70°C immediately before consumption. Several European countries have stipulated requirements for heat treatment conditions, particularly the internal temperature of the product, time period for chilling after the heat treatment, and the minimum chilled temperature. However, variations are noticeable in their specifications among the countries. For example, Belgium and the Netherlands stipulate the cooling should be immediate after heat treatment, while Finland and France permit 120 min for the product to be cooled to 10°C after heating. Denmark has allowed 180 min for bringing down the temperature of the heated product from 65 to 10°C, and in Norway, 240 min is the time limit to bring down the temperature from 60 to 7°C.[4] While the conventional heat pasteurization may take up to 30 min, a novel method for minimal processing using radio-frequency (RF) heat treatment is under development for fast pasteurization of vacuum-packed salmon and cod. In this, the packed products are immersed in deionized water of very low electric conductivity and exposed to RF waves, followed by fast cooling or freezing. Preliminary results suggest that heating time of 1–2 min may increase the temperature to 75°C and the products have a shelf life beyond 10 days at 4°C.[10]

5.3.2 Packaging

Packaging is an integral part of the cook-chill process. Usually, flexible packaging is provided for the product, which performs three functions, namely: (i) control of the local environmental conditions to enhance storage life, prevent moisture vapor transfer from the product, and prevent microbial contamination, (ii) display the product in an attractive way and convey information to the potential buyer, and (iii) protection of the product during transportation and storage. Ideally, all the required functions of packaging could be met with a single layer of polymer, but sometimes several layers are required, each designed for a different purpose.[7,11]

Raw material

↓

Processing/preparatiom

↓

Cooking   Initial cooking treatment
                 ensures destruction of vegetative
                 cells including pathogens

↓

Chilling   ←   Commences as soon as possible
                 after completion of cooking

↓

Chilled storage ← Storage period, not more than
(between 0 and 3°C)   5 days

↓

Distribution

↓

Reheating   ←   Immediately before
                 consumption

↓

Consumption
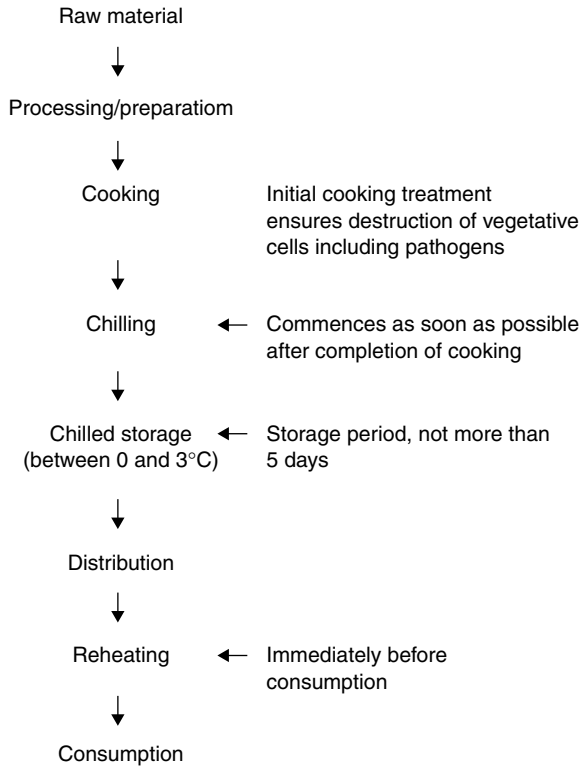
FIGURE 5.1   Process for cook-chill systems (Reprinted from Hill, M.A., *Food Chem*., 49, 131, 1994. With permission from Elsevier)

For example, for cooked meals, the packaging includes a dual ovenable shrink-wrap bag, transparent dome, and a paperboard   hatband incorporating a poly-coating for moisture resistance.[11] The type of packaging may be unit-portion packs and vacuum-barrier bags for main course items, flexible pouches, bag-in-box products, as well as modified atmosphere packaging (MAP).[7] A special barcode with a time/temperature indicator that changes color can give information on temperature abuse during storage.[7] In the case of polymer films, inclusion of an outer layer with enhanced printability is often necessary to display the product characteristics.

5.3.3 Shelf Life

Shelf life of minimally processed foods including cook-chill products is defined as the duration the product maintains its microbial safety and sensory qualities including visual appearance under expected storage conditions.[3] Safe product shall mean any product, which under normal conditions of use does not present any risk

or presents only the minimum risks compatible with the product's use considered as acceptable.[12] In the standard cook-chill, the products have a shelf life of not more than 5 days. The food may be subjected to reheating, generally to 70°C for 2 min, immediately before consumption. Most products will retain high quality from the sensory, nutritional, and microbiological viewpoints during this period.

5.3.4 Merits of Cook-Chill Technology

There are several merits associated with cook-chill technology. As a result of the minimal processing and the limited use of salt and other preservatives, cook-chill products, particularly cook-chill meals have enhanced sensory and nutritional quality in comparison with processed meals.[13] The technology offers facility for bulk processing of the raw material at a central production unit employing careful and standardized methods required for controlled heat treatment, chilling and packaging, and refrigerated transport to consumer centers. Provisions for automation and quality assurance including Hazard Analysis Critical Control Point (HACCP) protocol could be incorporated in the production line. Process modifications as per consumer demand and employing research data could also be made possible at the central production unit. The cook-chill process offers a solution for dissociating food preparation from service. The use of steam for heat treatment for decontamination employed in cook-chill processing is advantageous, as it does not require the use of chemicals. Further the wastewater from the process does not require any specialized treatment. In addition, the system can be designed to be self-sterilizing as well as automated. However, disadvantages of cook-chill catering include microbiological risks, product instability during extended storage, environmental issues with respect to packaging material and need for high capital investment. Some of the disadvantages are also part of conventional processing technologies. The merits of cook-chill technology are summarized in Table 5.1.

5.3.5 Classification of Cook-Chill Foods

There is no internationally recognized classification of cook-chill foods. "Project Harmony," one of the initiatives of the Food Linked Agro-Industrial Research, European Economic Commission, provides an inventory in order to bring uniformity.[3] The Australian Codes, distinguish two types of cook-chill systems, standard *short life* cook-chill (traditional or "open tray," shelf life, 5 days) and *extended* shelf life cook-chill (shelf life, 28 days).[14]

5.3.6 Extended Shelf Life Cook-Chill Processes

Consumer interests and marketing requirements have resulted in attempts to modify the conventional process in order to achieve extended shelf life for cook-chill products. The limited chilled shelf life of 5 days of cook-chill products could be extended by incorporation of certain additional processing steps. These

TABLE 5.1
Advantages and Disadvantages of Cook-Chill Technology

Advantages
Processing
   Central production unit
   Production is separate from consumption point
   Bulk-buying power
   Higher productivity
   Better equipment
   Lower storage costs, since temperature is not below freezing
   Heating is rapid. Microwave oven can be used
   Facility for HACCP
   Less equipment needed
   Less space
   Less skilled and unskilled staff
   Less waste of raw material
   Possibility for varied food product formulary
Packaging
   Less food waste (flexible packaging size)
   Convenience and flexibility
   Protection from recontamination
   Vacuum retards spoilage processes
   "Sealing in" juice and flavors
   Labeling information
Disadvantages
   Microbiological risks require strict process and storage control
   Product instability during prolonged storage
   Environmental issues with respect to packaging material
   Limited chilled distribution channels
   High energy requirement for storage
   Need for capital investment

Source: From Rodgers, R.S., *Trends Food Sci. Technol.*, 15, 366–372, 2004. With permission from Elsevier.

processes include *sous-vide* (cooked under vacuum), hot-fill and also hygienic, and aseptic packaging. Additional treatments such as low-dose irradiation and MAP could be employed. Catering production units often use a combination of the methods.[12] Such efforts resulted in the development of convenient meals incorporating items such as cooked fish or meat with vegetables and others. The modification of the cook-chill process for extended shelf life can be of different nature, depending upon the raw material. These are discussed in Sections 5.3.6.1 to 5.3.6.4.

Raw material
(Cleaned fish, meat, poultry, or vegetables)

↓

Preparation
Adding seasonings and other ingredients,
mixing with raw material

↓

Packaging in laminate pouches,
impermeable to air

↓

Vacuum sealing

↓

Pasteurization at controlled temperatures

↓

Quick chilling

↓

Storage at 0–3°C

↓

Reheating
(10–15 min in boiling water/4 min in microwave)

↓

Serving
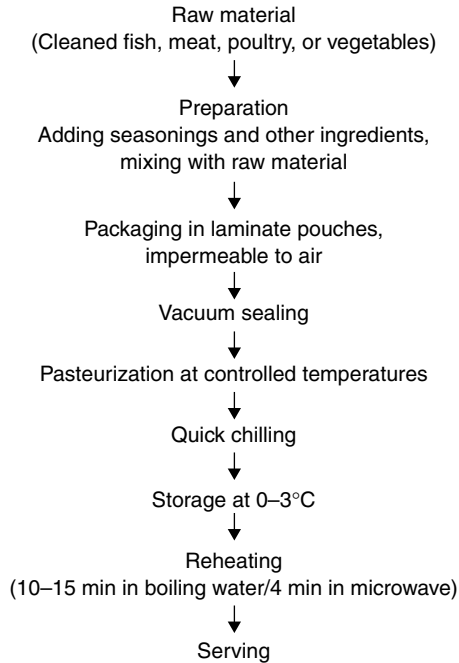
FIGURE 5.2 Flow chart of *sous-vide* processing (Reprinted from Rodgers, R.S., *Trends Food Sci. Technol.*, 15, 366, 2004. With permission from Elsevier)

5.3.6.1 *Sous-vide* technology

The *sous-vide* (meaning, "under-empty") cooking is defined as cooking of raw materials under controlled conditions of temperature and time, inside heat-stable vacuumized pouches. The major difference of *sous-vide* processing with conventional cook-chilling is vacuum packaging of the food before cooking with respect to the former. The food is vacuum packaged in laminated pouches followed by heat pasteurization at carefully controlled temperatures (to achieve internal temperatures between 70 and 80°C for meat products) followed by rapid chilling (often in ice bath) to between 0 and 3°C and stored chilled.[15–18] The European Union has recommended heat treatment equivalent to 90°C for 10 min in the center of the products to obtain a substantial reduction in numbers of the pathogenic microorganism *C. botulinum*.[3] A flow chart of the *sous-vide* process is given in Figure 5.2.

The flavor and texture of *sous-vide* foods are comparable to those of conventional cook-chill and conventionally cooked foods.[13,19] Cooking the food in the pouches prevents loss of volatile flavor compounds and the controlled temperature process prevents overcooking of delicate products such as fish. Furthermore, leaching and oxidation of nutrients are avoided.[12,15] Nevertheless, the heating regime needs to be standardized since it is of vital importance to obtain the required

sensory properties as well as a microbiologically safe products.[17] The European Chilled Food Federation's (ECFF's) "Botulism Working Party" determined a need for additional effective preservation factors for *sous-vide* foods, in which at least a 6-D reduction of nonproteolytic *C. botulinum* can be guaranteed.[20] Applying the technology, red meat products were found to retain their sensory quality for 23–35 days, white meat products for 14–30 days, and shorter shelf life for fish and vegetable products.[21–23] A recent study has shown that rainbow trout processed by *sous-vide* method that involved heating to maintain a core temperature 90°C for 3.3 min resulted in substantial microbial reduction in the product. The *sous-vide* product stored at 2°C had lower counts of mesophilic and psychrotrophic microorganisms. *Bacillus cereus*, *Clostridium perfringens*, and *Listeria monocytogenes* were not found in the samples during chilled storage up to 45 days.[17]

The reasons for limited acceptance of *sous-vide* products may be, in part, because of the safety concerns associated with these types of processes. *Sous-vide* products are not shelf stable because they are not formulated with preservatives and employ minimal thermal processes. Besides vacuum packaging provides an anaerobic environment in the pouches. Mild heat treatment in combination with vacuum packaging may selectively favor growth of *C. botulinum* increasing potential hazard due to botulism.[12,24,25] In view of the disadvantages, *sous-vide* processing has not received wide acceptance from the food industry, in several parts of the world.[24]

5.3.6.2   Modified atmosphere packaging

Another way to extend the shelf life of cook-chill products is application of MAP. This technology has been discussed in detail in Chapter 6. The potential benefits of use of MAP in the production of cook-chill ready meals have been recognized.[26] There is also good potential to employ MAP to significantly extend the shelf life of cook-chill ready meals including pasta meals, cooked meats, breads, pastries etc. by delaying microbial spoilage and inhibiting the development of oxidative "warmed-over flavors." However, the U.K. government's Advisory Committee on the Microbiological Safety of Foods (ACMSF) considered the risks of botulism from foods in MAP and recommended limitation of the shelf life of MAP cook-chill ready meals to periods less than 10 days. The potential benefits of using a combination of MAP with natural antioxidants and an oxygen scavenger or of using noble gases in MAP need to be evaluated.[26]

5.3.6.3   Radiation treatment

Cook-chill products, because of the limited heat treatment, harbor some microorganisms including spoilage-causing and pathogenic organisms. Several of these organisms are, however, sensitive to low doses of ionizing radiation emanating from radioisotopes, electron beams or x-rays.[27,28] Controlled exposure of the processed products, therefore, further reduces the incidence of these microorganisms

in the food; thereby facilitating enhanced shelf life. Effect of low-dose irradiation and chilled storage on the microbiological and sensory qualities of a ready meal has been examined.[29] Irradiation (2 to 3 kGy) reduced the initial number of microorganisms in meals to less than 100 per g. Therefore, significant microbial growth did not occur during storage. Growth of *Pseudomonas* spp. caused obvious spoilage of nonirradiated meals after storage, whereas, spoilage was not apparent in irradiated meals. The radiation treatment, however, has some limitations. For example, all the components in a meal may not be equally amenable to radiation. Changes in radiation-induced flavor characteristics may limit the potential of irradiation to extend the shelf life and enhance the safety of processed foods including ready meals and dairy products. In addition, although food irradiation technology is sound, it is still facing some consumer resistance.

5.3.6.4 Frozen storage

While chilled storage is part of the cook-chill process, freezing and frozen storage, instead of chilled storage, has also been examined for the cooked products.[30] Cooked-frozen foods can have significantly longer shelf life. Cooking prior to freezing may alleviate some of the problems associated with frozen storage of fresh raw material, such as fish. Cooked-frozen foods have enhanced food safety, because growth of surviving microorganisms is arrested under frozen conditions. The frozen state also prevents water migration in the product. However, the products require thawing at the consumer end, hence, convenience in its handling is lost. While some caterers trade in precooked frozen food, many may not be interested in the long shelf life because of economic reasons.

5.4 QUALITY OF COOK-CHILL PRODUCTS

5.4.1 Nutritional Aspects

The cook-chill process being mild does not significantly affect the nutritional value of the food. The changes are comparable to those in conventional cooking done at home. A study on the vitamin retention in microwave cooking and cook-chill foods showed that the length of time of cooking the food (e.g., at 80°C) has a direct effect on ascorbic acid retention. Rapid chilling reduces loss of the vitamin.[31]

5.4.2 Sensory Aspects

There is a dearth of information on the sensory quality of foods produced by cook-chill methods. In a review on the limited sensory data on foods produced by conventional and enhanced cook-chill methods it was observed that many variables affected the end result, which was often product and process specific.[8] Lack of standardization, both in the catering industry and in experimental methods, made comparisons between the results difficult.[8,23,74] Sensory quality and consumer acceptance of some *sous-vide* meat-based dishes have been reported.[22,23]

A bologna-based meat sauce and a chicken tikka masala were filled into high barrier-plastic pouches, vacuum sealed, and heated at 70 or 90°C for 90 min. The packs were chilled to 3°C in less than 2 h, and then stored in air at 1.5°C up to 40 days. Products were reheated to 75°C (to simulate final service conditions) within 5 min and served to panelists. It was observed that the crucial attribute that determines consumer acceptability for stored *sous-vide* meat-based dishes was the appearance. In general, products retained their sensory quality and acceptability for 40 days of storage. It was concluded that *sous-vide* technology applied to meat-based dishes has the capability to satisfy consumer demands for extended durability and sensory quality.[22] Development of some warmed-over flavor in meat and poultry products has been noticed.[32] The problem is not significant in fish-based products. Sometimes, the flavor of the product could be enhanced in extended shelf life products, depending upon the added ingredients. For example, apart from inhibiting microorganisms, salting enhances flavor and texture of foods.[33] The sensory characteristics of commercial samples of a variety of extended-life cook-chill vegetable and muscle foods including hot-filled items and products in cook-in-a-bag type packaging, stored at 0 to 3°C have been reported.[12] Shelf lives of these products ranged between 28 and 56 days. In the majority of foods, changes in quality attributes were detected before the microbial count exceeded specified standards. In general, hot-filled products were less stable during storage than cooked-in-a-bag products. There were no significant sensory changes in cook-chill processed white pomfret and shrimp, even after 3 weeks[34] (see Section 5.8).

5.4.3 Microbial Inactivation

The cook-chill products, being not sterile, harbor microorganisms. The products are "unprotected" due to negligible contents of salt and other preservatives. Preservatives are usually avoided in order to convey a fresh or homemade appeal to consumers. The majority of them have high water activity above 0.97 and pH 6.5 and above. In addition to microorganisms surviving the heat treatment, some viable, but not culturable (VBNC) cells can pose problems during the course of storage (see Chapter 2). Possibilities of survival of pathogenic microorganisms including *Vibrio* spp., *Clostridium* spp., *Listeria* spp., and *Salmonella* spp. can pose potential threat to consumer safety.[35,36] It is therefore of utmost importance that the raw material should be fresh so that the initial level of contaminant microorganisms can be as minimum as possible. The process design for thermal inactivation assumes that the initial level of microorganisms will never go above a predetermined maximum expected level and that to deliver a specific level of safety, a known number of log reductions of the organism of concern must be delivered by the process. The U.S. Food and Drug Administration recommends a 5-log reduction of *Salmonella enteritidis* for in-the-shell pasteurization of eggs.[36] The ECFF has determined a need for additional effective preservation factors for *sous-vide* foods, in which at least a 6-D reduction of nonproteolytic *C. botulinum* cannot be guaranteed.[20] It should be, therefore, cautioned that under the conditions

stipulated for development of cook-chill product microbial hazards encountered in the food cannot be underestimated.

Substantial inactivation of initial microbial population in the product takes place during the heating regime while preparation of the food. The behavior of microorganisms in the product is comparable with any other food with respect to conventional heat-treatment conditions. It is well known that inactivation of microorganisms follows first-order kinetics. It is characterized by a single rate constant "k" or its reciprocal, the linear "D-value," considered a measure of resistance to an applied lethal agent, such as temperature. This approach allows for simple straightforward calculations and comparison of thermal process equivalencies. Process engineers have been standardizing the process based on these data. However, the survival curves for thermal inactivation of microorganisms are characterized by deviations, accompanied with shoulders and tails in the log-linear models.[37] Substantial recent evidence indicates that microbial inactivation, usually is *not* a process which follows first-order kinetics, because cells or spores do not have identical heat resistance, as assumed earlier. This bio-variability of microorganisms is also reflected in several other areas, such as lag times in *Salmonella* cells, germination times of *C. botulinum* spores and variations in injury of *Lactobacillus* spp.[37,38] A recent research summit under the aegis of U.S. Institute of Food Technologists considered the various aspects of microbial inactivation with respect to processing treatments. The summit concluded that the survival curves might not follow first-order kinetics, essentially due to biodiversity and variations in responses of individual cells to various processes.[36] It was suggested that microbiological models should not be used outside the range of the factors used to create them or extrapolated to predict results. Therefore, thermal inactivation kinetics of several microorganisms under mild heat treatment, as in cook-chill processing, have been examined.[39]

5.4.3.1 Hazards

Since cook-chill products harbor microorganisms, it is important to examine the influence of storage temperatures on their growth and toxin formation, if any. The growth characteristics of 29 bacterial strains representing six recognized pathogenic genera have been evaluated.[38] Based on minimum growth temperature (MGT), they could be divided into two groups, namely, mesophilic and psychrotrophic. Organisms belonging to the former group consisted of *Salmonella* sp. (MGT, 5.1 to 8.7°C), *Escherichia coli* (*E. Coli*) (7.1°C), and *S. aureus* (MGT, 9.5 to 10.4°C). Psychrotrophic pathogens included *L. monocytogenes* (MGT, −1°C to −0.4°C), *Yersinia enterocolitica* (MGT, −0.9 to −1.3°C), and *Aeromonas hydrophila* (−0.1 to +1.2°C). Table 5.2 shows growth characteristics of food-poisoning bacteria important in seafood processing. It may be noted that many of the pathogens are capable of growth at pH values as low as 4.6. They can also tolerate appreciable levels of salt, with *L. monocytogenes* and *Vibrio* spp. tolerating salt up to 30 and 10%, respectively. These data suggest that many pathogens are capable of growth in the products during chilled storage, if they are not eliminated during the processing stage.

TABLE 5.2

Growth Characteristics of Food-Poisoning Bacteria Important in Seafood Processing

Bacteria	Growth temperature (°C)			Lowest pH for growth	Maximum tolerable NaCl (%)
	Minimum	Optimum	Maximum		
C. botulinum types A & B	10.0	35.0	48.0	4.6	10.0
C. botulinum type E	3.3	30.0	45	4.8	6.0
Vibrio spp.	5.0–7.0	35.0–35.2	42.2	5.0	9–10
Salmonella and *Shigella* spp.	5.5	37.2	49.1	5.0	8.0
S. aureus	5.6	35.0	47.2	4.8	17.0
C. perfringens	15.0	49.0	50.0	5.0	5.0
L. monocytogenes	2.8	30.0–37.2	45.0	4.9	30.0

Source: From Lee, J.S., Hilderbrand, S. Jr., Hazard analysis and critical control point applications to the seafood industry, Oregon Sea Grant, ORESU-H-92-001, Corvalis, OR.

Contamination of fishery products with *L. monocytogenes* is quite common in fishery products. Presence of this bacterium could be a health hazard, since the organism is capable of growth even at chilled conditions.[40–42] A recent survey of fish processing plants showed that the bacterium was present in 16.7% of 234 raw fish samples, 9% of 253 finished products, and 27.3% of 553 environmental samples.[40] *L. monocytogenes* was present in 18% of retailed chilled meals (mainly poultry), possibly through contamination.[41] However, since the bacterium could not survive the initial cooking process, its presence in the cooked product indicates cross contamination after cooking.[42,43] In another exhaustive survey, 43 Public Health Laboratory Service (PHLS) laboratories examined 1301 samples of cooked poultry and chilled meals during 1988 and 1989. *L. monocytogenes* was isolated from 63 of 527 (12%) samples of ready-to-eat poultry, 13 of 74 (18%) chilled meals, and 10 of 627 (2%) main course items from cook-chill catering units.[44] Low-dose irradiation and incorporation of chemical preservatives including some herbal extracts can alleviate listeriosis.[45,46]

Another microbiological hazard in foods of this type is growth of and toxin production by nonproteolytic *C. botulinum* (types B, E, and F). Spores of these organisms are capable of survival of the comparatively mild heat treatment. Further, the spores are able to outgrow, if the storage temperature is above 3.3°C and produce lethal toxin.[14,45,46] It was suggested that in view of potential survival of pathogenic microorganisms at chill temperatures, refrigeration alone could not be depended upon to completely ensure microbial safety. Nevertheless, a storage temperature between 0 and 3°C and a relatively short storage period up to 5 days should not jeopardise the safety of the products. Table 5.3 shows some typical microbial survivors in cook-chill products and their heat sensitivities.

TABLE 5.3
Microbiological Survivors in Cook-Chill Products

Microorganism	Some characteristic features
C. botulinum	Nonproteolytic, $D_{80°C} = 21.6$ min
C. botulinum	Proteolytic, $D_{121°C} = 0.23–0.33$ min
C. perfringens	$D_{98°C} = 21–31$ min
B. cereus	$D_{100°C} = 2.7–3.1$ min

Source: From Rodgers, R.S., *Trends Food Sci. Technol.*, 15, 366, 2004. With permission from Elsevier.

5.4.4 Enhancement of Safety of Cook-Chill Foods

The safety of cook-chill products has been the major concern with respect to their commercial value.[46–51] Flaws in the heat treatment may adversely affect uniform temperature distribution in the food.[46] In addition, the pH and water activity of the products are favorable to the growth of microorganisms and these organisms are capable of survival at chilled storage temperature.[50,51] High standards of hygiene and temperature control are essential to ensure safety in chilled products. The cooking process should be adequate to kill these bacteria and other pathogens. There should be no contamination between cooking and packaging. Once a food leaves the processing stage, its keeping quality as well as safety and the extent to which it will retain its intended attributes are a function of the environment inside and outside the package. The important parameters are temperature, gas composition, relative humidity, pressure or mechanical stresses, and light. To maximize quality, control of internal and external conditions should be maintained to the point of consumption. The U.S. National Food Processors Association in a position paper on "Safety Considerations for New Generation Refrigerated Foods," addressed basic considerations related to preparation, handling, and distribution of refrigerated foods.[52] The Association recommended incorporation of multiple (at least two) barriers or hurdles (in addition to refrigeration) into the product formulation to ensure microbial safety. Examples of such microbial barriers include acidification, reduced water activity, preservatives, protective cultures, and MAP. Several efforts have been initiated to enhance the microbiological safety of these products.

5.4.4.1 Reduction of surface bacteria on the products

Methods to eliminate surface bacteria from food are known. Sanitization of the surface with aqueous solutions of preservatives such as acetic or lactic acid is one method. Alternative method is postpackaging thermal processing of processed meats submerged in hot water near boiling. The process is not suitable for cooked

products. Modification of the process is exposure of the processed product to steam for a short time. Exposure of *L. monocytogenes*-inoculated salmon to heat in a pilot steam-treatment system for 8 sec resulted in elimination of the bacterium.[42] In many cases, a short duration steaming may not be effective, since the surface bacteria may be residing in rough surfaces of the food in air pockets. It is critical that the transfer of heat to the bacteria be extremely fast so that only the bacteria are affected and not the food. A recent vacuum/steam/vacuum (VSV) process has been developed for the purpose. It involves application of vacuum to the food to remove the air and moisture, followed by rapid steaming to kill the bacteria in the pores, and then vacuuming again to remove the condensed water. This also helps evaporative cooling of the surface. The time of exposure must be as short as possible. Employing saturated steam at 138–143°C and 2–3 cycles of operation, 1–5-log reductions in bacteria have been achieved depending upon the product.[53] The potential of this technology in cook-chill processing is worth examining. The heat treatment could be integrated into the packaging step at the last possible moment before sealing.

5.4.4.2 Inclusion of additional microbial barriers

Modification of the process to include additional microbiological barriers, through incorporation of salty, acidic, spicy, or other ingredients with antibacterial agents can help achieve microbiological safety of the products.[9,14,20,46,52,54,55] These barriers can enhance safety of the foods through combination and synergistic effects, through "hurdle technology" (see Chapter 6). Whereas the effects of these inhibiting factors on the food are mild, synergistically they result in improvement in product quality. Potentials for improving safety and shelf life of cook-chill products through incorporation of microbial barriers such as irradiation, hydrostatic pressure, MAP, low pH, addition of seasonings, sodium lactate, and other agents have been examined.[47,48,54] Some of these treatments, however, have limitations with respect to cook-chill products. For example, chemical preservatives, though cheap, easy to apply, and heat stable, are not compatible with the "freshness" image of the cook-chill products and often affect taste. Physical methods, such as irradiation, although does not leave any residue in the product, require government approval and complex expensive equipment besides having poor consumer appeal. MAP and hydrostatic pressure have limitations against spore formers. A novel approach of "biopreservation" through introduction of protective cultures in the product has been suggested.[48] It was observed that bacteriocin generated from *Lactobacillus* spp. and *Pediococcus* spp. inoculated in the package at concentration of 10^7 to 10^8 colony-forming units per g product inhibited pathogenic microorganisms including *C. botulinum.* The technique offered temperature-responsive preservation and "natural" image. However, there can be practical difficulties in large-scale handling of protective cultures by the industry. Further, new regulations are required before they are commercially applied. Table 5.4 depicts the advantages and disadvantages of additional hurdles for preservation of extended cook-chill products.

TABLE 5.4
Advantages and Disadvantages of Additional Hurdles for Preservation of Extended Cook-Chill Products

Hurdle	Advantages	Disadvantages
Low dose irradiation	Inactivates vegetative microorganism	Microbial spores, resistant
		Consumer resistance
	In-package treatment	Capital costs
	Additional shelf life	
Modified atmosphere packaging	Reduces oxidation and microbial spoilage	No significant effect on pathogens
Freezing	Longer shelf life	Thawing required
		Higher costs
High hydrostatic pressure	In-package treatment	Spores resistant
		Possibility of textural changes
Chemicals (pH, salt, spices)	Low cost	Impact on sensory quality
Protective cultures	Effective against spore formers	Cost of handling cultures, heat sensitivity
Bacteriocins	Many are heat stable	Inconsistency of inhibitory effect, decompose during storage
	Effective against spore formers	

Source: From Rodgers, R.S., *Trends Food Sci. Technol*., 15, 366, 2004. With permission from Elsevier.

5.4.4.3 Monitoring of temperature of cook-chilled products

As discussed earlier, cook-chill products are defined as TCS foods, where "TCS" implies "Temperature Controlled for Safety." The term is explicit in its requirement of strict control of temperature for the purpose of safety.[36] Monitoring of temperature must be stringent at all points in the chill chain, from raw material, processing, storage, handling, transportation, to retailing and consumption. While temperature of these foods has been recommended as "the closer to $0°C$, the better," the requirement may be abused at one or more points in the production and distribution chain unless sufficient care is taken in the operations. It has been pointed out that unlike food manufacturing, catering businesses often do not have sufficient food-safety expertise or a culture of strict process and hygiene control. During transportation and storage, temperature conditions are often prone to abuse.[48]

Temperature audits have shown significant refrigeration inadequacies at both retail and home level, with a substantial portion of refrigerated foods being exposed to temperatures as high as $10°C$. A survey indicated that in the U.S. food distribution chains, temperatures are more probably in the range of $45°F$ ($7.2°C$) and $55°F$ (about $13°C$), well out of the range need to ensure microbiological safety and quality.[7] In developing countries the situation may be worse due to lack of sufficient infrastructure for refrigeration. The difference between a nearly ideal

31°F (−0.5°C) and 41°F (5°C) is a factor that increases the deterioration rate by a factor of 1.5 to 2.0. It is therefore, quite natural that the accelerated quality losses due to the exposure to higher temperature can result in unacceptable products at the retail level. Therefore, strict control of storage temperature is absolutely necessary to provide safety to new-generation extended-shelf life-refrigerated products that are minimally processed products including controlled atmosphere packaged MAP fish, meats, poultry, pasta, and salad.[54] Temperatures lower than 4.4°C have been advocated for these foods.[52,56] The U.S. FDA 1999 Food Code advocates maintenance of a temperature of 41°F (5°C) (7.2°C is acceptable) at the warmest location of the food, including the defrosting cycle. Under certain circumstances, time alone at a specific temperature can be used to control product safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen(s) of concern in the product, since the lag time and generation time of a microorganism depends on temperature.[36]

Measurement of temperature could be made using the Time-Temperature Indicator (TTI) devices. These devices exploit a change in physical or physico-chemical property to produce irreversible evidence of exceeding a predetermined temperature threshold or record the cumulative time-temperature history. The rate of change is temperature dependent, increasing at higher temperatures in a manner similar to most physico-chemical reactions.[7] TTIs integrate, in a single measurement, the full time-temperature history from time of activation and can be used to indicate an "effective average" temperature during distribution (see also Chapter 3). Some of the popular TTIs include *Fresh-Check*™ and Vitsab® labels, which can be attached on surfaces of packages for products such as fresh meat, fish, salads, and other products. Similarly, several specific freshness indicators are available, which determine volatile or nonvolatile compounds in food. *Fresh Tag*® reacts with volatile amines, when the amines pass through a wick containing a reagent.[7] The tag has found commercial applications. In addition to TTI elements, almost of equal importance are the *Product-Process-Package* (PPP) factors and their interactions with the TTT elements.[4]

5.5 GUIDELINES

Several guidelines have been stipulated by international organizations in order to build safety into food-product development. The Codex Alimentarius Commission has prepared a Code for Refrigerated packaged foods (ALINORM 99/13, APPENDIX III, CAC/RCP 39–1993).[57] According to the Code, a Food Safety Objective (FSO) has to be established, which is achievable through Good Manufacturing Practices/Good Hygienic Practices (GMP/GHP) and HACCP. The FSO scheme can be used domestically to control food production within a country and internationally to assess the equivalency of food produced in other countries. The framework recognizes the importance of process and product design as the most effective means to ensure food safety. Together with the FSO, there is a need to define maximum frequency or concentration of a microbiological hazard in a

food at the time of consumption that will provide an appropriate level of protection. A hypothetical example of an FSO is that the level of *L. monocytogenes* in ready-to-eat foods must not exceed 100 cfu/g at the time they are consumed. In practice, the expected level of control is achieved through the use of process and product criteria. Process criteria appear commonly as critical limits in HACCP plans. Product criteria relate to attributes of the food that must be controlled and may involve chemical, physical, and sensory values.

Association of U.S. Food and Drug Officials and European Chilled Food Association have provided guidelines for minimally processed foods including cook-chill products.[3,56,58,59] These are intended for retail marketing of refrigerated foods, such as cook-chill, packaged under reduced oxygen atmospheres vacuum packaging, *sous-vide*, and modified and controlled atmosphere packaging. Guidelines are also available for other process requirements (e.g., maintenance of low a_w, low pH, high contents of nonpathogenic competing organisms), labeling, and refrigeration. In 1995, a protocol for the validation of shelf life for chilled foods was drawn up by the French trade organization, SYNAFAP.[4] The guidelines are specific in their definition regarding heating, packaging, and possibility of postprocess contamination and risks of survival of pathogenic bacteria in various food categories. In addition, advice and recommendations for the safe manufacturing and handling of MAP foods have also been provided. The guidelines, which emphasize the use of HACCP, have helped establishment of a relationship between the growth and death of target microorganisms with processing, hygiene, and validation research. The U.S. National Food Processors Association has also advocated application of HACCP protocol to these foods.[52] According to the European Union Directive (EU Directive 79/112/EEC), like other foodstuff and prepacked foods, seafood must bear a "data of minimum durability" or a "use by date" warning. Compliance of the industry with these guidelines has resulted in developments in the technology of pasteurized foods including *sous-vide* products, particularly in Europe.[59]

5.6 Consumer Attitudes Toward Chilled Foods

Chilled foods have received a good quality image, which are perceived by the consumer as freshly harvested/prepared/cooked/processed, and containing no additives.[4] Cook-chill products offer convenience as well as freshness. Freshness in a food product is termed as newly produced, gathered, not stored, not artificially preserved, or products that retain the original qualities not deteriorated or changed by lapse of time.[55] Apart from convenience and freshness, the popularity of precooked, refrigerated foods is because consumers consider these foods more nutritious than fast foods and frozen items. Consumers have indicated that at a comparable price they would choose the fresh product over the frozen.[9] An added advantage with refrigerated foods is that since these products are marketed through the self-service refrigerated outlets, consumers are able to purchase

the entrees during normal food shopping without making a special trip to fast-food outlets.

5.7 COOK-CHILL PROCESSING OF FISHERY PRODUCTS

Compared to other food cook-chill processes for fishery products are sparse. Processes involving multiple microbial barriers including chill temperature has been reported for extended refrigerated shelf life of two commercially important fishery products, namely, white pomfret (*Stromateus cenereus*) and shrimp (*Penaeus indicus*).[34] These processes provide the fish and shellfish with an extended refrigerated shelf life, convenience in handling, and distribution to the consumer in ready-to-prepare conditions. The processes were developed incorporating the conventional salting as an additional microbial barrier in the products.[55] Cooking and salting are widely employed in preservation and culinary preparation of fish and shellfish, which arrest autolytic reactions, kill a significant portion of contaminant microorganisms, and impart acceptable texture and flavor.[8,33] The experimental protocol for these products was designed to give an extended storage life by brining to enhance flavor and texture and to maintain an additional antimicrobial barrier. The products were also given a dip in tripolyphosphate (TPP) solution to improve water-holding capacity and to function as a bacteriostatic agent.[55,58,60,61] The brining step can also sensitize the contaminant microorganisms to heat inactiviont.[75] To develop the products, peeled and deveined shrimp (*Penaeus indicus*) or white pomfret (*Stromateus cinereus*) were dipped in 10% brine for 1 h, drained, and treated with 5% TPP for 5 min. The salted samples were exposed to open steam for 15 min and then rapidly chilled to $3\pm0.5°C$, packaged in polyethylene pouches and stored at this temperature. The products had a shelf life of 25 days, as shown by microbiological and sensory evaluations. The process for pomfret and shrimp are indicated in Figure 5.3 and Figure 5.4, respectively.

Inoculated pack studies have a role in determination of the safety of the process. In the case of white pomfret and shrimp, the effectiveness of the process was verified through inoculated pack studies, using salt tolerant and psychrotrophic *Aeromonas hydrophila*.[61] Fresh pomfret steaks and peeled shrimps were dipped in cell suspension (10^5 cells/ml 0.9% saline) of the bacterium for 15 min. They were then processed into cook-chill products. Microbiological examination indicated that the product had a count of 100 per g immediately after processing. The count increased only to 1.2×10^3 per g after storage of the products for one month at $3 \pm 0.5°C$. The data are comparable with a recent report on elimination of *L. monocytogenes* from salmon after a steam treatment for 8 sec.[42] The sensory attributes showed little deterioration of the product after 25 days storage at $3\pm0.5°C$. In terms of all the sensory parameters, the TPP treated samples were preferred over brined counterparts. TPP treatment is known to enhance acceptability of catfish patties and thermally processed mullet.[62,63] Microbiological growth during storage was also much less, possibly due to inhibition by a combination of barriers including NaCl, TPP, and low temperature. Table 5.5 shows the sensory and microbial qualities of the products after 21 days of chilled storage. A shelf life of

Fresh white pomfret

↓

Remove head and viscera

↓

Wash, drain, cut into steaks

↓

Immerse in equal volume
aqueous 10% (w/v) sodium chloride solution
for 1 h

↓

Drain

↓

Gently stir for 5 min in 5% (w/v) aqueous
sodium tripolyphosphate (TPP) solution
(Ratio of fish to solution, 5:1)

↓

Drain

↓

Steam for 15 min

↓

Cool to 0 to 2°C for 15 min

↓

Package in polypropylene pouches

↓

Store at 3±0.5°C
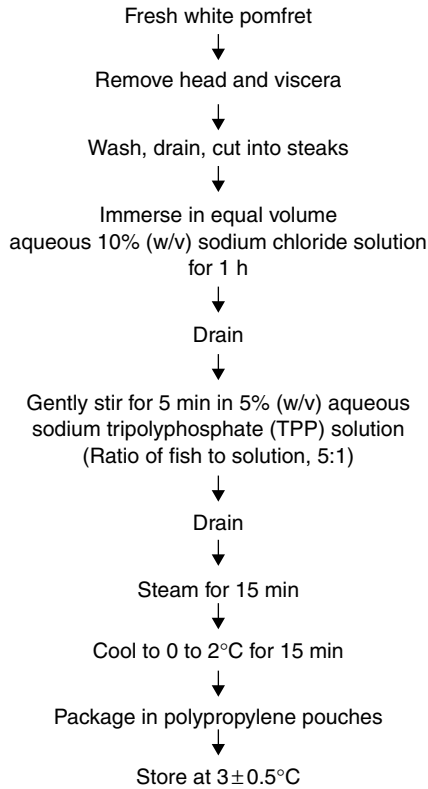
FIGURE 5.3 Cook-chill process for white pomfret (Adapted from Venugopal V., *Int. J. Food Sci., Technol.*, 28, 273, 1993. With permission from Blackwell Publications)

25 days was suggested for the processed products to provide a reasonable time for their distribution under chilled conditions.

Cook-chill process could extend the shelf life of oysters up to 3 months. The freshly shucked oysters were chlorinated in water, drained, vacuum sealed in flexible pouches, and subjected to heating in water bath to an internal temperature of 82°C followed by incubation at 72°C for 8 min. The heat-treated pouches were immediately cooled in chilled water and stored at 0.5°C. The treatment resulted in drastic reduction of both aerobic and facultative aerobic microorganisms.[64] *Sous-vide* processing of fishery products, such as salmon, has been examined.[21] However, sensory properties of *sous-vide* salmon were substantially affected during storage. After 2 weeks' storage at 3°C the product exhibited separation of fat and loss of fresh flavor and appearance.[15] *Sous-vide* process for farmed blue mussels (*Mytilus edulis*) has been reported.[19] The mussels were vacuum packed in pouches and pasteurized in an autoclave at 100°C for holding times between 17 and 35 min. The time/temperature data were recorded in

Fresh shrimp

↓

Peel, devein

↓

Wash, drain

↓

Immerse in equal volume
of aqueous 10% (w/v) sodium chloride solution
for 1 h

↓

Drain

↓

Gently stir in 5% (w/v) aqueous
sodium tripolyphosphate (TPP)
solution (Ratio of shrimp to solution, 5:1)

↓

Drain

↓

Steam for 15 min

↓

Cool to 0 to 2°C for 15 min

↓

Package in polypropylene pouches

↓

Store at 3°±0.5°C
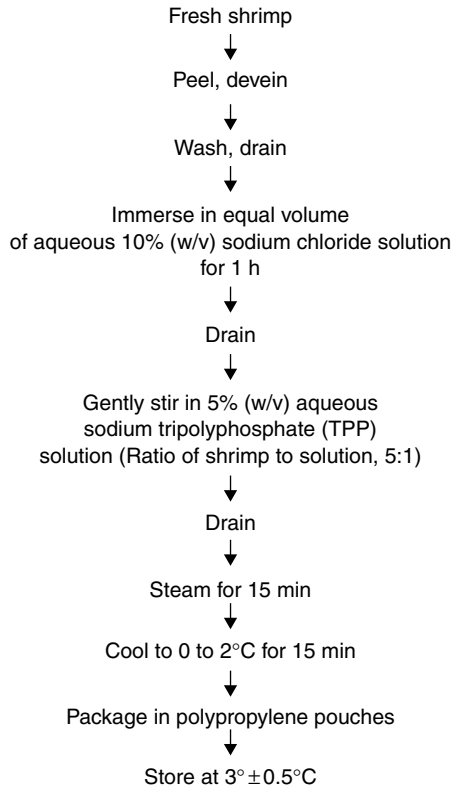
FIGURE 5.4 Cook-chill process for shrimp (Adapted from Venugopal, V., *Int. J. Food Sci., Technol.*, 28, 273, 1993. With permission from Blackwell Publications London)

the coldest spot of the mussels (which was identified using x-ray analysis of thermocouple attached pouches). The heat transfer in the product was found to be influenced by changes in pressure of retort and vacuum in the pouches. The product had a shelf life up to 21 days at 4°C. In another process, mussels are de-clumped, precleaned, and graded in a rewatering/depuration station. Rewatering enables the mollusk to recover from stress caused by harvesting and handling and also removes sand grit and silt from the bivalve. The cleaned mussels are filled in pouches, mixed with desired sauces and packaged under vacuum. The pouches containing the mussels are cooked in specially designed cookers followed by immediate cooling. The product has a shelf life of 14 days at 0 to 5°C. Instead of chilled storage, the cook-chill seafood can also be frozen at −18°C. Vacuum-packed, cooked, frozen mollusks are gaining popularity as a gourmet item.[65] The shelf lives for *sous-vide* processed cod, cod fillets, and salmon stored at 0, 3, and 4°C were 28, 21, and 15 to 21 days, respectively.[3] The process could be extended for other fish species also with suitable modifications to retain quality.

TABLE 5.5
Sensory Scores of Cook-Chill Processed White Pomfret and Shrimp

Parameters	White pomfret			Shrimp		
	Fresh, no TPP	**Fresh, with TPP**	**Stored for 25 days**	**Fresh, no TPP Added**	**Fresh, with TPP**	**Stored for 25 days**
Appearance	6.5±1.6	8.1 ± 1.2	7.7 ± 1.2	6.6 ± 1.0	8.5 ± 0.5	7.5 ± 1.3
Flavor	5.9 ± 1.1	7.8 ± 1.4	7.2 ± 1.6	6.8 ± 1.2	7.9 ± 0.7	7.3 ± 1.2
Texture	5.4 ± 0.6	7.4 ± 0.8	7.4 ± 0.8	6.8 ± 1.3	7.6 ± 0.5	7.4 ± 1.2
Overall acceptability	—	7.9 ± 1.6	7.3 ± 1.8	—	7.8 ± 1.5	7.4 ± 1.1

The products were stored at $3.5 \pm 0.5°C$. TPP, sodium tripolyphosphate dip treatment. Values represent panel scores $\pm$ standard deviation. Scales, 10 = fresh, high quality; 1, deterioration, total loss of quality. Scale for overall acceptability: Maximum, "9"= I would eat this at every opportunity; minimum, "1" = I will eat this if I were forced to.

Source: Reprinted from Venugopal, V., *Int. J. Food Sci., Technol.*, 28, 273, 1993. With permission from Blackwell Publications, London.

As discussed in Section 5.4.4.3, temperature monitoring is important in distribution of processed, chilled fishery products. The minimally processed seafood requires the same strict control of chill temperatures during distribution as for fresh seafood. At the retail outlet, fishery products are generally held in display cabinets of supermarkets. Temperature control of these cabinets is of utmost importance in product safety. Apart from the use of TTIs, thermographic imaging of food within display cabinets offers rapid assessment of the active temperature of the food product. Installation of such temperature controllers reduces costs, saves energy, and helps to comply with existing and pending regulations and guidelines to aid food safety. The time-temperature signals from packages may be electronically linked to the distribution system monitoring software to provide the status of the contained food. A pocket size, precise temperature logger that can record up to 8192 times stamped readings during transit or storage of food has been introduced recently. The equipment can measure temperatures ranging from −55 to +125°C with 0.1°C resolution and ±0.5°C accuracy.[66] The concept of *relative rate of spoilage* (see Chapter 2) and specific spoilage organisms (such as *Photobacterium phosphoreum* in modified-atmosphere stored products) has been the basis for development of shelf life models for some fishery products. Based on these parameters, a Seafood Spoilage Predictor software has been developed for fresh temperate and tropical water fish and also modified-atmosphere stored fish. These programs can read temperature logger files and predict shelf life as a function of temperature profile of product in distribution chains. Another Food Spoilage Predictor (FSP) software to predict the effect of temperature and water activity on growth of *Psuedomonas* spp. has also been developed. The model has been successfully validated with a few seafood items and is commercially available.[67]

5.8 IDENTIFICATION AND TRACEABILITY OF FISH SPECIES IN PROCESSED PRODUCTS

With more and more fishery resources being processed into secondary products, information on the fish species used is essential for labeling and marketing. Substitution and adulteration are not unusual because of either variations in name for the same species in different countries or intention to make a higher profit by substitution of high-value species with cheaper ones having similar characteristics. The legislation in most countries demands correct labeling and specifications of the composition, nutritional value, and safety of processed foods including fishery products. The labeling on products available to the consumer has to include the species used in their manufacture. In Europe, since 2002 fish retailers have been obliged to give additional product information on fish and seafood when offering them for sale to the final consumer. This applies to smoked fish, frozen, dried, and salted products. However, at present cook-chill products, breaded fish products, marinades, canned products, fish fillets with topping, breaded crustacean or mollusk products, and caviars are not covered by this requirement.[68]

Identification of the species is carried out by comparing the electrophoretic protein pattern of an unknown material with that of a known species.[69] Recently protein patterns of about 100 fish species have been compiled in an electronic database available on the World Wide Web as the Regulatory Fish Encyclopedia. It contains gel images and tabulated PI values of proteins.[70] An alternative approach involves the use of antibodies directed against a species-specific protein or protein fragment. The methodology is dependent on an even supply of antibodies of constant reactivity. Species identification methods using genomic information have gained importance in food in recent years. Restriction fragment length polymorphism (RFLP) is a reliable and commonly used technique for differentiation of genera or species. The RFLP method consists in extraction of DNA from the sample material, amplification of sequence of species genome by use of polymerase chain reaction (PCR), digestion of the generated amplicon by the base sequence-selective restriction endonuclease and separation of the resulting DNA fragments corresponding to the length obtaining species-specific pattern. The last step is usually performed by gel electrophoresis followed by visualization with fluorescent dyes or staining with silver salts. Because of low costs and high automation, standard nondenaturing agarose gels are used in most cases.[71,72]

Traceability is defined as the ability to trace the history, application, or location of an entity by means of recorded information. The need for a quick and reliable traceability system has been felt for identifying the sources and content of aquatic foods, as well as the fish species used, in view of the continuing focus on food safety and the periodic occurrences of foodborne illnesses. The system translates into the ability to trace and follow food and food producing animals throughout all stages of production and distribution. Traceability of fishery products has been attracting attention of the regulatory agencies.[66,69] Recently, an Internet based traceability system has been developed for fresh fish supply chains in the Danish domestic market.[73] A software, designated as *FishMonger*™ and also

other software solutions for seafood distributors, brokers, and trackers including accounting are available.[66]

In summary, cook-chill processing helps in the development of seafood products in convenient, consumer friendly, ready-to-use, or ready-to-prepare conditions. The technology has potential for efficient domestic distribution and marketing of the commodity. Processing centers of such products could be positioned ideally near the landing centers to avail fresh raw material and should adhere to national and international guidelines and protocols including GMP and HACCP. Advances in packaging, refrigerated transport, techniques in temperature monitoring, and product identification can boost development of the technology.

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6 Modified-Atmosphere Packaging

6.1 INTRODUCTION

Modification of the gas atmosphere inside a food package has attracted attention of food technologists from the 1930s. This was based on the recognition that the perishability of the products could be retarded by this technique. The process was used commercially in 1935 to ship chilled fresh beef under carbon dioxide (CO_2) from Australia and New Zealand to the United Kingdom. In the case of seafood, fresh haddock, cod, sole, whiting, and plaice, were successfully preserved under atmospheres having 20 to 100% CO_2, with optimal concentrations ranging between 40 and 50%.[1] Ever since a great deal of research has been conducted on MAP of food items including fishery products. In recent times, MAP is employed for preservation of home-meal replacement foods, curd and fresh cheeses, case-ready fresh meats, and a host of other foods.[2]

Modified-atmosphere packaging involves replacement of air in the package with a different gas or gas mixture. There are basically two ways to achieve this, namely, CAP and MAP. In both systems, the packages containing food products are flushed and filled with nitrogen (N_2) or gas mixtures. CAP is enclosure of food in a gas impermeable package, inside which the gaseous environment with respect to CO_2, oxygen (O_2), and N_2 is changed and selectively controlled throughout the storage period to provide optimum storage conditions. This system is mainly used for bulk storage or transport. MAP, on the other hand, is used for bulk as well as retail handling. While under CAP the gas composition is maintained throughout the storage, in the case of MAP once a gas mixture is introduced there is no further control of the gas composition. Recent studies have shown that there is no special advantage associated with CAP as compared to MAP in the case of fishery products.[3]

As a consequence of change in gas atmosphere, the shelf life of the product is increased significantly because the modified atmosphere slows down the degradative processes, particularly microbial spoilage reactions. During storage the gas composition may change as a consequence of permeability of packaging material and chemical and biological activities of the packaged food. A decrease in CO_2 level in packages has been reported when lobster was stored under modified or controlled atmosphere due to dissolution of the gas in the lobster tissue. Whereas MAP envisages no subsequent control in gas composition once introduced, a weekly change of gas mixture has been attempted to maintain the gas
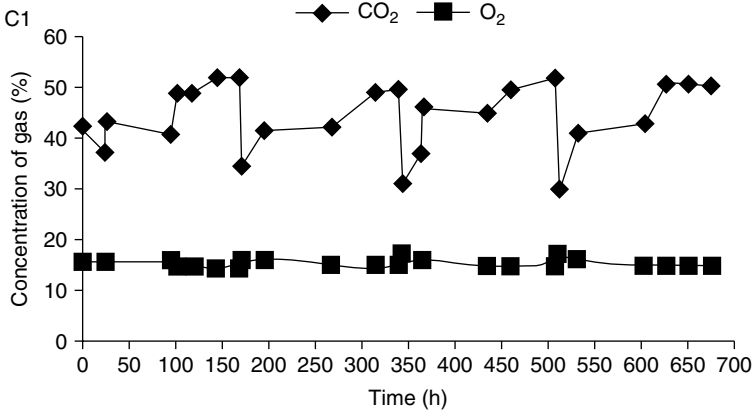
FIGURE 6.1 Evolution of concentration of CO_2 (%) and O_2 (%) in Norway lobster during controlled (CI) and modified (MI) storage with mixtures of CO_2, O_2, and N_2 in the ratio 60:15:25 (Reprinted from Ruiz-Capillas and Moral, *Food Chem.*, 86, 85, 2004. With permission from Elsevier)
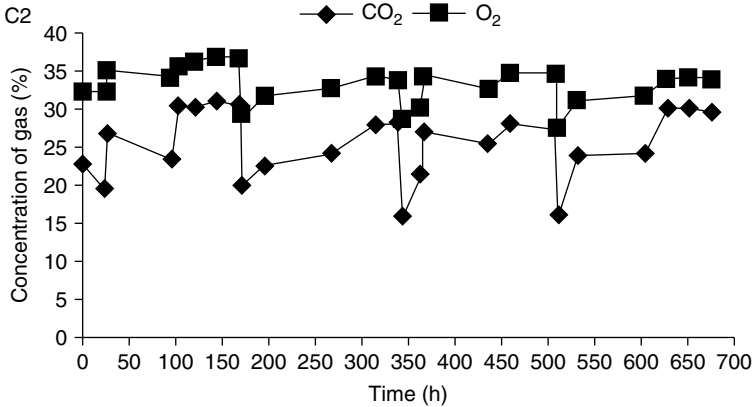
FIGURE 6.2 Evolution of concentration of CO_2 (%) and O_2 (%) in Norway lobster during controlled (CI) and modified (MI) storage with mixtures of CO_2, O_2, and N_2 in the ratio 40:40:20 (Reprinted from Ruiz-Capillas and Moral, *Food Chem.*, 86, 85, 2004. With permission from Elsevier)

atmosphere in MAP Norway lobster.[3] Figure 6.1 and Figure 6.2 depict changes of gas composition during storage of Norway lobster under controlled and modified atmospheres of two different compositions. Whether it is MAP or CAP, the gas mixtures are more effective in controlling spoilage when the storage is done under strict temperature control.[4]

Apart from the two major ways of changing the package atmosphere, a third method is through vacuum packaging, which is being applied to a limited extent

for some foods. The choice of the type of packaging atmosphere is restricted by certain considerations such as its influence on food color, oxidative rancidity, etc. Although vacuum packaging prevents oxidative rancidity, it favors proliferation of anaerobic pathogenic microorganisms, particularly under conditions of temperature abuse. At chilled storage temperatures, the shelf life of vacuum-packaged fish may be comparable with that of MAP samples such as salmon and trout and is doubled compared with air-stored fish.[5]

6.2 COMPONENTS OF GAS MIXTURES IN MAP

6.2.1 Carbon Dioxide

Carbon dioxide is the important gas used in MAP of fish, because of its bacteriostatic and fungistatic properties on contaminant microflora in the fish. It inhibits growth of aerobic spoilage-causing bacteria, the extent of inhibition increasing with increasing concentrations of the gas in the package. The preservative action of CO_2 was initially thought to be due to displacement of some or all of the O_2 available for bacterial metabolism, thus slowing their growth. However, since even 100% replacement of air by N_2 offered no major advantage, the preservative effect of CO_2 was investigated in detail.[6,7]

According to Henry's law, the concentration of CO_2 in the food is dependent on the product's water and fat contents and of the partial pressure of CO_2 in the atmosphere.[6] CO_2 is soluble in water and fat and the solubility increases greatly with decrease in temperature (Figure 6.3). The solubility of the gas in water at 20°C and normal atmosphere is 1.73 g/kg water, whereas at 0°C, it is 3.38 g/kg water.[7] Inhibition of the growth of microorganisms by CO_2 is determined by the concentration of the gas dissolved in the product.[8] Due to the higher solubility of the gas at lower temperatures, the effectiveness of the gas is increased as the storage temperature is lowered.[4,5]

The solubility of CO_2 leads to dissolved CO_2 in the food product to give carbonic acid, according to Equation (6.1)

$$CO_2 \text{ (gas)} + H_2O \ \leftrightarrow \ HCO_3^- + H^+ \ \leftrightarrow \ CO_3^{2-} + 2H^+ \qquad (6.1)$$

For pH $<$ 8, typical of seafood, the concentration of carbonate ions may be neglected.[9]

$$CO_2 + H_2O \ \leftrightarrow \ HCO_3^- + H^+ \qquad (6.2)$$
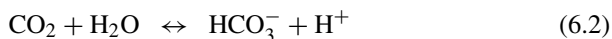
Due to formation of H^+, a drop in surface pH is observed in MAP products. However, the acidic effect may not completely explain the bacteriostatic action of CO_2. In addition, the inhibitory effect of CO_2 was suggested to result from one or all of the following four mechanisms.[9,10] These are: (i) alteration of cell membrane function affecting nutrient uptake and absorption, (ii) penetration of bacterial membranes leading to intracellular pH changes, (iii) direct inhibition of enzymes or decreases in the rate of enzyme reactions, and (iv) direct changes in the physico-chemical properties of the proteins.
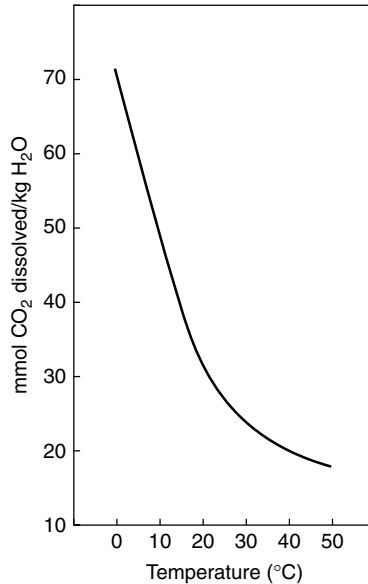
FIGURE 6.3 Solubility of CO_2 as a function of temperature (From Ronner, U., in *Food Preservation by Combined Processes*, Leistner, L. and Gorris, G.M., Eds. Final Report, FLAIR Concerted Action, No. 7 Subgroup B, Food-Linked Agro-Industrial Research, EUR 15776 EN, 1995, The Netherlands. With permission)

Once the package is opened, the product slowly releases the CO_2. However, it continues to exert preservative effect for some more time. Since a certain amount of gas dissolves in the water, it is important to keep the gas volume higher than that of the food, in order to prevent collapse of the package. Usually, a gas to product ratio (G/P) between 2 and 3 is ideal. It is advisable to mention the G/P ratio on the pouch to indicate the care that has to be taken to prevent pouch collapse.[5] In many cases, 100% CO_2 has been used for bulk transportation of fishery products without any negative effect.[5]

6.2.2 Nitrogen

Nitrogen is an inert tasteless gas, and is mostly used as a filler gas in MAP because of its low solubility in water and fat.[11] N_2 is used to replace O_2 in packages to delay oxidative rancidity, inhibit growth of aerobic microorganisms, and also as an alternative to vacuum packaging. Nevertheless, O_2 in MAP fresh fish can influence reduction of trimethylamine oxide (TMAO) to trimethylamine (TMA).[8,12]

6.2.3 Oxygen

Oxygen is a critical mass transfer component in a number of deteriorative reactions that can have an effect on shelf life of many packaged foods. Of major concerns are

TABLE 6.1

Recommended MAP Conditions for a Variety of Nonrespiring Food Products

Product	Gas mixture	Gas volume (ml/100 g product)	Shelf life (days) Air	Shelf life (days) MAP	Storage temperature (°C)
Fatty fish	60–70% CO_2, 30–40% N_2	200–300	3–5	5–9	0–3
Cooked fish products	80% N_2, 20% CO_2	50–100	2–4	28–35	4–6
Poultry meat	50–80% CO_2, 20–50% N_2	100–200	7	16–21	2–3
Sausages	80% N_2, 20% CO_2	50–100	2–4	28–35	4–6
Hard cheeses	80–100% O_2, 0–20% N_2	50–100	14–21	28–70	4–6
Rye–wheat bread	20–40% CO_2, 60–80% N_2	50–100	2–3	14	20–25
Pizza	30–60% CO_2, 40–70% N_2	50–100	7–14	14–35	4–6

Source: Ronner, U., in *Food Preservation by Combined Processes*, Leistner, L. and Gorris, G.M., Eds. Final Report, FLAIR Concerted Action, No. 7 Subgroup B, Food-Linked Agro-Industrial Research, EUR 15776 EN, 1995, The Netherlands. With permission.

lipid oxidation and subsequent development of rancid flavors, O_2-induced changes in color and nutritional changes, and also nonenzymatic browning. The presence of O_2 during processing and storage favors spoilage due to growth of aerobic microorganisms and promotes various enzyme-catalyzed reactions. On the other hand, depletion of O_2 can lead to other deteriorative effects such as spoilage by anaerobic microorganisms. The shelf life of O_2-sensitive packaged food products depends on exposure to oxygen during processing, the amount of O_2 incorporated in the sealed package and the amount of O_2 ingress with the package during storage. The O_2 in MAP is normally set as low as possible to inhibit the growth of aerobic spoilage causing bacteria. However, a certain concentration of O_2 is required to maintain the red color of certain fish fillets such as tuna. It has also been recognized that development of rancid off-odors in fatty fish species in O_2 containing modified atmospheres has not been a major problem.[13] Table 6.1 gives the recommended MAP conditions for a variety of food products.

6.3 MAP OF FISHERY PRODUCTS

In MAP fresh fish or shellfish, which may be gutted, filleted, steaked, or shelled, are placed in a bag or tray made of thin special plastic suitable for retail display.

The air in the bag is replaced by a mixture of N_2, CO_2, and O_2. The sealed packages are maintained between 0 and 3°C. The change in the gas environment suppresses the activities of aerobic microorganisms, which are mostly responsible for spoilage of the fish under natural conditions. In addition to the bacteriostatic effect of the modified gas atmosphere, some changes in the visual appearances of certain fish, particularly fillets, could take place. Therefore, according to the type of the fishery product composition of the gas may have to be varied to derive maximum benefits. Once the gas mixture is introduced in the package, there is no further control of the gas composition throughout the storage period. As a result of the activity of surviving microorganisms and also other reactions such as autolytic enzyme activities in fresh muscle, the gas composition will inevitably change during storage. Figure 6.1 and Figure 6.2 show the profiles of CO_2 changes during storage of MAP Norway lobster during chilled storage. A large volume of information is available on MAP of fishery products.[4,5,14–18]

6.3.1 Effect of MAP on Fish Quality

6.3.1.1 Microbial changes and specific spoilage organisms

As discussed in Chapter 2, spoilage of aerobically stored chilled fish has been attributed to certain gram-negative microorganisms.[19–21] These bacteria grow with a doubling time of approximately one day at chilled temperatures, and after 2–3 weeks, reach 10^8–10^9 colony forming units per g flesh or cm^2 skin. Spoilage potential and rates of spoilage of different species of microflora can be assessed in model systems using sterile raw fish juice.[20,22] A qualitative test for the ability of the bacteria to produce hydrogen sulphide (H_2S) and reduce TMAO may also be used when the spoilage flora is screened for potential spoilage bacteria. Based on these screening tests, the specific spoilage organisms (SSOs) in various fish products can be identified. The numbers of SSO and the concentration of their metabolites can also be used as objective indices of spoilage in shelf life determination. With knowledge on the microorganisms responsible for spoilage, a dose relationship between log numbers of SSO and remaining shelf life can be expected.[21] In chilled aerobically stored cod, the bacterial species responsible for spoilage are *Shewanella putrefaciens*, *Alteromonas putrefaciens*, and certain *Pseudomonas* spp. Therefore these organisms are the SSOs in aerobically stored chilled fish.[8,23]

In foods packed under modified atmosphere containing CO_2, the gas exerts bacteriostatic and fungistatic properties on aerobic microorganisms. The bacteriostatic effect of CO_2 is reflected in extended lag phase and growth period. Combination of elevated CO_2 concentrations and restricted quantities of O_2 produces a synergistic effect on the growth of *Pseudomonas* spp. and *S. putrefaciens* by effectively extending the lag period of growth and enhancing the growth of gram-positive microorganisms.[23,24] Other spoilage causing microbial species affected include *Acinetobacter*, *A. putrefaciens*, *Flavobacterium*, and also *Staphylococcus* spp.[17] Consequently they do not cause spoilage and associated formation of

TABLE 6.2

Dominating Microflora and Specific Spoilage Bacteria During Storage of Cod Under Various Temperature and Packaging Atmospheres

Storage temperature (°C)	Packaging atmosphere	Dominating microflora	SSOs
0	Aerobic	Gram-negative psychrotrophic, nonfermentative rods (*Pseudomonas* spp., *S. putrefaciens*, *Moraxella*, *Acinetobacter*	*S. putrefaciens*, *Pseudomonas* spp.
0	Vacuum	Gram-negative fermentative rods with psychrophilic character (*Photobacterium*) Gram-positive rods (*Lactobacillus* spp.)	*P. phosphoreum*
5	Aerobic	Gram-negative psychrotrophic rods (*Vibrionaceae*, *S. putrefaciens*)	*Aeromonas* spp., *S. putrefaciens*
5	Vacuum	Gram-negative psychrotrophic rods (*Vibrionaceae*, *S. putrefaciens*)	*Aeromonas* spp., *S. putrefaciens*
5	MAP with CO_2	Gram-negative psychrotrophic rods (*Vibrionaceae*)	*Aeromonas* spp. *P. phosphoreum*
5	MAP with high CO_2 (>90%)	*Lactobacillus* spp.	*S. putrefaciens*

Source: Adapted from Huss, H.H., *Quality and Quality Changes in Fresh Fish*, Rome, FAO Fisheries Technical Paper No. 348. Food and Agriculture Organization of the United Nations, Rome, Italy, 1995. With permission.

metabolites including histamine typical of the fish stored under chilled aerobic atmospheres. Lactic acid producing bacteria such as *Streptococci* and *Lactobacilli* spp. are less affected by CO_2. Therefore these organisms predominate in seafood during storage.[25] Other tolerant organisms include *Photobacterium phosphoreum* and *Brochothrix thermosphacta*. Modified-atmosphere stored marine fish from temperate waters are spoiled by the CO_2-resistant *P. phosphoreum*, whereas, gram-positive bacteria are likely spoilers of CO_2-packed fish from tropical waters.[20,21] In CO_2 atmosphere, *P. phosphoreum* has been identified as the SSO of cod and salmon.[5,20,21] In the case of vacuum-packed fish, *S. putrefaciens* and *P. phosphoreum* function as the SSOs. In the case of freshwater fish aerobically stored at ambient temperature, motile aeromonads are the SSOs.[26] Table 6.2 shows dominating microflora and specific spoilage bacteria during storage of cod under various temperatures and packaging atmospheres.

The spoilage potentials of SSOs in fishery products stored under different conditions have been compared in order to assess spoilage patterns of MAP fish. *P. phosphoreum*, *Vibrio*, and *S. putrefaciens* are capable of significant reduction of TMAO to TMA. *P. phosphoreum* generates 10 to 100 times more of TMA than that produced by the commonly known specific spoiler, *S. putrefaciens*. Similar to

Pseudomonas spp., *S. putrefaciens* and *P. phosphoreum* are also capable of production of hypoxanthine, a characteristic compound indicative of fish spoilage, as a result of nucleotide catabolism.[26] *S. Putrefaciens*, present in chilled fish spoiling under aerobic conditions, produce TMA, H_2S, methylmercaptan (CH_3SH), dimethyl sulfide $(CH_3)_2S$, and hypoxanthine and *Pseudomonas* spp. produces ketones, aldehydes, esters, and H_2S. However, *P. phosphoreum* is not capable of producing foul smelling H_2S, another index of spoilage. Therefore, there is a certain change in spoilage pattern of MAP fish as a result of shift in microbial profiles and potential of surviving flora. As discussed in Chapter 2, *K*-value is a good quality index for the quality of fresh fish, which is calculated from concentrations of the nucleotide breakdown products, hypoxanthine, inosine, and inosine monophosphate present at a given time. However, little relationships between *K*-values and sensory qualities of MAP stored tilapia and sardines have been reported.[14] It must be emphasized that many of the bacterial species predominating in MAP fish do possess a certain degree of spoilage potential. Therefore, the extension in shelf life obtained by MAP of fishery products is limited.

6.3.1.1.1 *Targeted inhibition of SSOs*

A new approach for the development of mildly preserved seafood with extended shelf life originates from the SSO concept. There is scope for extension of practical shelf life of MAP fish integrating with additional processing techniques to reduce the growth of SSOs. Mild preservation procedures that reduce the growth of these specific spoilers can extend shelf life, even if the treatment does not influence the other microflora present. This approach of targeted inhibition of SSOs has been successfully used to extend the shelf life of MAP cod fillets. The SSO, *P. phosphoreum*, was inhibited by low levels of antimicrobials. The technique could be applied not only to fresh, but also to frozen fish. Compared to fresh MAP cod fillets, shelf life of thawed MAP cod fillets was extended from 11 to 12 days to more than 20 days at 2°C.[20]

6.3.1.2 **Safety of MAP fishery products**

Whereas MAP results in a shift in spoilage causing microorganisms it has no significant influence on growth parameters of most pathogenic microorganisms.[2,27] It is possible in some cases that the change in atmosphere could even encourage growth of certain pathogens such as *Clostridium botulinum*.[24] This means that food can have acceptable organoleptic quality but could still be unsafe for consumption. In order to achieve safe shelf life objectives, it is, therefore, essential to have good handling practices, proper chilled storage, and also gas-barrier packaging.[2] Refrigeration is essential both to control spoilage and to protect the product from the growth of potential food poisoning organisms. Pathogens such as *C. botulinum* and *Listeria monocytogenes*, which have minimum growth temperatures of 3.3 and 0–2°C, respectively, can present hazards under normal refrigeration. These organisms pose greater potential risks in MAP products since normal spoilage

microflora are suppressed. Such hazards are manifold in products intended for consumption without further heat treatment. These products include ready-to-eat products, *sashimi/sushi*, smoked salmon, and cooked shellfish. Safety of MAP fishery products is, therefore, of primary concern during commercial production of these foods.

Several studies on safety aspects of MAP fishery products have concluded that storage of these items may not increase the risks from pathogens such as *Salmonella*, *Staphylococcus*, *C. perfringens*, *Yersinia*, *Campylobacter*, *Vibrio parahaemolyticus*, and Enterococci including *Escherichia coli* O157:H7 above those expected for air-stored products.[27] For example, no difference in survival of *E. coli*, *S. aureus*, *V. parahaemolyticus*, and *C. perfringens* was observed in jack mackerel during storage under air, 100% N_2, or in a CO_2:N_2 ratio of 40:60.[28] It has been generally concluded that only three major microbial species are responsible for safety hazards in MAP fishery products. These are *L. monocytogenes*, *C. botulinum* type E, and nonproteolytic *C. botulinum* type B.[5]

Listeria monocytogenes is capable of growth even at chill temperatures. Packaging under enhanced CO_2 and chilled storage are not sufficient to control the growth of this pathogen in some products. However, inhibition of the bacterium by 100% CO_2 or in an atmosphere of 50% CO_2 with additional hurdles like bacteriocins, and salt and lowered pH has been reported.[24,29] Alternately, 1% lactic acid and MAP containing 75% CO_2, 15% N_2, and 10% O_2 can extend the lag phase of the bacterium up to 8 days, compared with air- or vacuum-packaged samples. Therefore, it appears that along with MAP and chilled storage, additional hurdles may be required for control of *L. monocytogenes*.[30]

Nonproteolytic type strains of *C. botulinum* in MAP fishery products represent another potential risk.[4,5,31] The reasons for this are many. The bacterium is widely distributed in freshwater, estuarine, and marine environments. They can grow and produce toxin at temperatures as low as 3.3°C and even at 3.0°C. The inhibition of the normal aerobic spoilage bacteria by MAP reduces bacterial competition favoring growth and toxin production by the bacterium, particularly during temperature abuse. Furthermore, MAP products contaminated with *C. botulinum* may become toxic and yet remain acceptable with respect to odor and appearance.[30] Composition of fish has some influence on the growth of pathogens in the products. The safety margin (days from sensory spoilage to toxin detection) is less for fatty fish such as salmon and catfish, when compared with lean fish such as tilapia and cod, suggesting the influence of fat content on the margin of safety. Nevertheless it has been reported that irrespective of the actual minimum growth temperatures, production of neurotoxin generally requires weeks at the low temperature limit.[32] The safest packaging atmosphere with regard to toxin production was found to be a mixture of equal parts of O_2 and CO_2 in conjunction with strict control of storage temperature.[30] A computer program has been developed to assess the risk of food pathogen growth under various packaging and storage conditions.[30]

The Advisory Committee on the Microbiological Safety of Food (ACMSF), U.K., in a report on *Vacuum Packaging* and *Associated Processes* published in 1992, observed that if the storage temperature and the shelf life were less than

$10°C$ and 10 days, respectively, the risk due to *C. botulinum* and other pathogens was low.[5,14,29] The ACMSF introduced stricter recommendations for the MAP foods having a shelf life of longer than 10 days but are not subjected to a heat treatment to inactivate the spores of psychrotrophic *C. botulinum*.[5,14] It was suggested that when chilled storage was the only controlling factor, storage at temperatures between 5 and $10°C$ should be limited to 5 days, while shelf lives could be up to 10 days for storage temperatures at $5°C$ or below. Optimization of parameters is essential to obtain a meaningful and safe shelf life of MAP fishery products.

6.3.1.3 Flavor changes

Many of the free amino acids such as alanine, glutamic acid, and glycine, which are abundant in the nonprotein N_2 fraction in crustacea, are responsible for flavor and taste. Alanine and glycine have sweet taste and glutamic acid has the umami taste typical of crustacea.[33] Free amino acid contents in relation to freshness were examined in the muscle of Norway lobster during bulk storage in controlled and modified atmosphere containing two different gas mixtures, having a composition of $CO_2:O_2:N_2$ of 60:15:25 or 40:40:20, respectively. The essential amino acids in the highest concentrations were threonine, leucine, valine, lysine, and arginine, all above 40 mg/100 g. Levels of threonine, valine, lysine, and arginine decreased significantly during storage. These changes were more in samples packaged in CO_2-rich atmosphere than O_2-rich packages. The decrease was observed in both CAP and MAP samples. The nonessential amino acids were glycine, alanine, and glutamic acid, in the range of 31 to 58 mg/100 g, along with the dipeptide anserine. Ornithine and tryptophan increased significantly during storage, suggesting these amino acids could serve as freshness indices for the gas-packaged and iced Norway lobster.[3]

6.3.2 Fish Species Examined for MAP

A number of commercially important fish species have been examined for their suitability for MAP. These include catfish, cod, crayfish, flounder, haddock, hake, herring, hybrid striped bass, mackerel, rockfish, salmon, sardines, shrimp, snapper, swordfish, tilapia, trout, and whiting. The product forms included whole fish, fillets, as well as steaks, which may be fresh or subjected to minimum processing such as cooking, salting, or smoking. In several studies reported, there have been wide variations in the parameters employed. Researchers have used storage temperatures ranging from 0 to $26°C$. The composition of the gas atmospheres differed significantly. CO_2 was the major component of the gas phase; in most cases the percentage of the gas was in the range of 75–100% (with some exceptions where the gas was 25%). In exceptional cases, CO_2 was as low as 2–3%. Some studies reported included N_2 as high as 98%. Usually, a G/P of between 2 and 3 is ideal.[5] However, most of the reports do not mention the G/P ratio. Furthermore, changes in the CO_2 and O_2 levels inside the package headspace or the amount of dissolved CO_2 during storage were also seldom measured. Vacuum packaging has also been

employed for shelf life extension of fish. There have been wide variations among the packaging materials employed. As a result of wide fluctuations in processing, packaging, and storage parameters, there is a wide difference in the shelf life extensions obtained even in the case of a single fish species. These variations are visible in the voluminous data summarized.[4,5,34,35] These make comparison between different studies difficult. Notwithstanding the variations, the use of MAP or vacuum packaging for bulk shipment with appropriate temperature control is an accepted practice. When the bulk packages are opened for retail packing, storage, and distribution, the product will undergo its normal spoilage.[36]

Salmon, because of its consumer importance, is one of the fish extensively examined for MAP. A shelf life of 40 days for commercially processed chilled salmon fillets stored under CO_2 and N_2 mix has been reported, in contrast to a shelf life of 20 days for fresh whole salmon in ice.[37,38] The extended shelf life of 20 days provides sufficient time to distribute salmon within markets outside the salmon-farming industry. For markets farther away, expensive airfreight, frozen distribution, or inclusion of more preservatives are practiced.[39] In scaling up of MAP processes for commercial applications, there is a need for ensuring strict control regarding the composition of the gases used, the volume and surface area of the product, the volume of the gas, and the storage temperature.[38] It was not just the volume of the CO_2 that was important in giving an acceptable product, but the ratio of gas to product was also critical. Higher concentration of CO_2 could give a less acceptable product due to the effect of the gas on the delicate nature of salmon flesh. Figure 6.4 depicts extension of shelf life of salmon steaks under modified atmosphere.

The best result of MAP storage on shelf life has been obtained with fish from warm waters.[26] Voluminous data collected at the Icelandic Fisheries Laboratories have clearly established the potential of MAP in preservation of temperate water fishery products.[40] Shelf life increases of 28–52% for retail-packaged and 32–73% for bulk-packaged haddock fillets have been reported. Retail-packaged cod fillets had a shelf life extension of 32–52%.[41] In spite of wide variations in the data on cod, a shelf life of 14–20 days at 0–3°C was probably a more realistic estimate of shelf life.[5] A 42–74% increase in shelf life was observed for ocean perch fillet. Bulk storage of mackerel and salmon fillets in airtight bulk containers at 0°C under 90% CO_2 atmosphere has been reported to have an acceptable sensory shelf life of 21 days for each fish.[42,43] Atlantic mackerel stored under subzero temperatures and modified atmosphere remained up to 21 days. Packaging gas composition did not change during the storage period. Sensory evaluation showed decrease in seaweed odor and increase in sourness due to lactic acid bacteria at terminal storage life. Yellowness and Hunter *b* value increased. Coliform, yeasts, and molds were negligible at the end of the shelf life.[43] Sardine (*Sardina pilchardus*) packaged in 60% CO_2 and 40% N_2 had a shelf life of 12 days at 4°C, while vacuum- and air-packaged samples gave a shelf life of only 9 and 3 days at this temperature. After 15 days storage, the highest concentration of TMA was detected in air-stored sardine, followed by vacuum-packaged and MAP fish. Gas packaging suppressed formation of histamine in sardine. The concentrations of histamine were 20, 13,
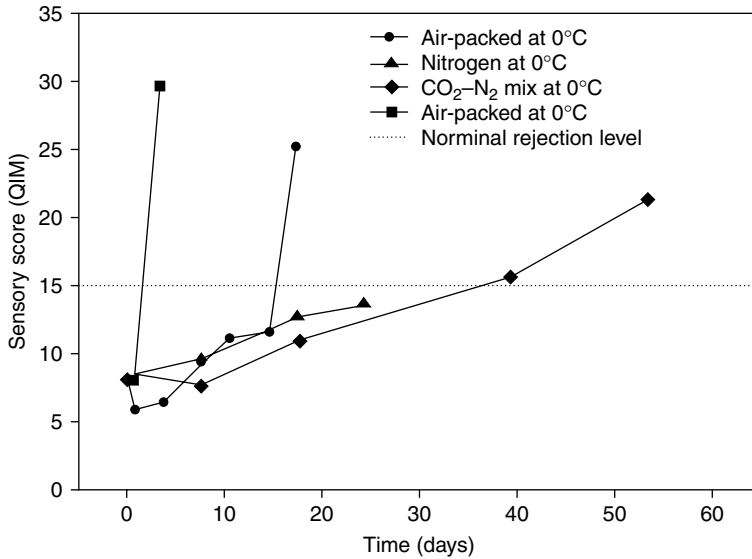
FIGURE 6.4 Extension of shelf life of salmon steaks under modified atmosphere (From Bremer, P.J. et al., Enhancing the quality and shelf life of chilled seafood products: the integration of capture, processing, handling, and packaging regimes. International Food Information Service, Online features, 11 December 2002 [http://www.foodsciencecentral. com/library.html#ifis/11590]. With permission)

and 10 mg/100 g, respectively, in the samples after 15 days storage.[44] Some typical examples of shelf life of MAP fishery products stored at chilled temperatures are given in Table 6.3.

6.3.3 MAP of Fish Onboard Fishing Vessels

Modified atmosphere or vacuum packaging for bulk packaging of freshly caught fish at sea under frozen conditions could be employed. When the bulk consignment is broken down for retail packing, the product will undergo normal spoilage.[45] Sea-frozen fillets could also be retail packed in modified atmosphere at centralized factories and distributed globally in the frozen state independent of season and distance from the fishing grounds. These products could be thawed in shops and sold in the same way as fresh MAP fillets. The fish must be of high quality, properly headed, gutted, and washed. For the best quality, a temperature of $-25°C$ or lower is recommended. Blast freezers or plate freezers can be used for freezing operation.

The methodology generally employed for onboard MAP of cod has been described.[46] The fish is headed and gutted immediately after catch. The fish is then bled in seawater for about 30 min at 4°C, and then filleted and skinned, using a BAADER 190 and BAADER 51 filleting and skinning machines, respectively. The fillets are trimmed manually to free from bones, parasites, and bloodstains

TABLE 6.3
Shelf Life of Some Fishery Products Stored Under Modified Atmospheres

Fish product	Temperature (°C)	Composition of gas atmosphere	Shelf life (days) in air	Shelf life (days) under MAP
Catfish fillets	4	$CO_2:N_2:75:25$	13	38–40
	4	Vacuum	13	20–24
Channel catfish fillets	0–2	CO_2:air 80:20	—	28
Cod fillets	3	100% CO_2	14	10
Cod fillets	1	$CO_2:N_2:O_2:40:60:0–11$		
Cooked crayfish	4	$CO_2:N_2$ 80:20	14	>21
Hake slices	0–2	$CO_2:N_2:O_2$ 40:45:5, 60:30:10, or 40:30:30	7–8	14
Herring fillets, Baltic	2	$CO_2:N_2$ 20:80	—	3
Herring (*C. harengus*) fillets	0	$CO_2:N_2$ 60:40	12	14
Pearl spot	0–2	$CO_2:O_2:N_2$ 40:30:30	11	18
Rock cod	4	80% CO_2:20% air	7	21
Rockfish fillets	1.1	100% CO_2	14	25
Cooked and brined shrimp	0–2	50% CO_2:50% air	12–16	7 months
Salmon	2	$CO_2:N_2:O_2:10:0:0$	8	18
Tilapia fillets	4	$CO_2:N_2$ 75:25	9–13	>25
Trout, whole	0	$CO_2:N_2$ 60:40	—	8
Trout, fillet	1.7	$CO_2:N_2$ 80:20	10	20
Whiting	3–4	$CO_2:N_2$ 100:0	4	15

Source: Adapted from Stammen, K., Gerdes, D., and Caporaso, F., *Crit. Rev. Food Sci. Nutr.*, 29, 301, 1990; Sivertsvik, M., Jeksrud, W.K., and Rosnesa, J.T., *Int. J. Food Sci. Technol.*, 37, 107, 2002; Bhaarti, A. and Sahoo, J., *Indian Food Ind.*, 18, 299, 1999, Manju et al., in Food Safety, Surendran, P.K. et al. (eds)., Society of Fishery Technologists, India, 2003, p. 144; Dalgaard, P. and Jorgensen, I.V., *Int. J. Food Microbiol.* 35, 431, 2000.

and the boneless fillets are manually interleave packed in plastic films, each block weighing about 7 kg. The blocks are frozen in a horizontal plate freezer until core temperature reaches −25°C. Frozen cod blocks are packed in cardboard boxes and placed in the cold store maintained at −30°C onboard the trawler. After 10 weeks, when the frozen blocks are landed, they are sawed into pieces of about 100 g and the pieces are placed in trays with absorbent drip pads and packed under modified atmosphere with 40% CO_2, 40% N_2, and 20% O_2, at a fish to gas ratio of 2.0. The packed fillets are then thawed at 5°C for 20 h and transferred to chill storage at 2°C. By this process, the shelf life of thawed MAP Baltic Sea cod was extended up to 20 days as compared with fresh MAP cod. It was observed that frozen storage up to 12 months had no significant effect on the quality attributes and the shelf life of thawed fish at 2°C. Another advantage is that freezing inactivates parasites,

the presence of which normally affects the quality of fresh fish.[46] In contrast to fresh MAP cod, no significant production of TMA and changes in taste and odor were detected during the chilled storage of frozen MAP thawed fish.[47] However, a slightly increased dimethylamine level, a higher drip, and detection of weak frozen-storage flavor were observed in the thawed samples. It was concluded that the use of frozen fish as a raw material not only provides a stable product in MAP but also allows much greater flexibility in production and distribution. The thawed MAP fish products, however, should be labeled to indicate the process. Fillets and steaks not chilled prior to packaging or not properly refrigerated during distribution could lose several days of quality shelf life during shipment. In some parts of Europe, some consumers are showing reluctance to this process because freezing and thawing deprive the product of its freshness (Dalgaard, P., pers. comm.).

Modified-atmosphere packaging is applicable to aquacultured fish as well. Shelf lives of fresh cultured gilthead seabream fillets (*Sparus aurata*) in over-wrap, vacuum, and gas-mixture packages stored for 27 days at $1 \pm 1°C$ were assessed. The quality of packaged fillets was measured in terms of total volatile bases (TVB), TMA, thiobarbituric acid (TBA) value, psychrotrophic bacterial counts, and sensory evaluation. The inhibitory effect of CO_2 on bacterial growth was highly pronounced, maintaining very low counts throughout the storage period. Gas packages without O_2 and also vacuum packaging reduced oxidative rancidity. TVB and TMA were poor freshness indicators for the products. Sensory quality of fillets in over-wraps deteriorated faster than in vacuum and gas packages. MAP of filleted gilthead seabreams with a gas mixture consisting of CO_2 and N_2 in equal amounts resulted in extension of shelf life compared to over-wrap packaging. This was due to suppression of microbial growth, rancidity development, and hypoxanthine formation during storage.[48]

Modified-atmosphere packaging can have potential for transportation of live seafood. Live mussels (*Mytilus galloprovincialis*) packaged under 75% O_2 in the absence of CO_2 gave high survival, with a storage life of 6 days at 2–3°C. In contrast, the air-packaged mussels had a stability of only 3–4 days at this temperature. Sensory quality of cooked products of mussel stored under MA storage was high throughout the storage period.[49] The shelf life of fresh crustacean shellfish increased up to 20% at 0°C under a $N_2:CO_2:O_2$ ratio of 30:40:30 and controlled melanosis. However, higher concentrations of CO_2 resulted in pack collapse, drip loss, bleaching, and development of metallic odors.[50] MAP storage also helped to extend shelf life of other products such as fish cakes, raw squid, and white octopus.[5] Care should be taken to maintain the product under chilled conditions, ideally below 3.3°C to prevent growth of *C. botulinum*.

6.4 Advantages of MAP of Fishery Products

The advantages of MAP of food products include significant extension in shelf life of perishable commodities including fishery products, reduction in economic losses, and better distribution of products over longer distances with fewer deliveries at the consumer centers. Consumers are also provided with good-quality

products that can be handled with ease. MAP controls melanosis, namely, the development of black spots in crustacea and also reduces the formation of histamine in scombroid fish species during storage. However, there are certain disadvantages with respect to MAP such as added cost, special equipment requirement, and also shift in microbial patterns which influence the food safety necessitating stringent temperature control during storage.[24] The poor conductivity of the gaseous mixture in a modified atmosphere, such as the mixture of $O_2/CO_2/N_2$, makes cooling more difficult than with unpacked or vacuum-packed fish.

Amenability of MAP storage depends upon the type of fishery products, gas composition, and storage temperature. Formation of copious quantities of drip or exudate is a problem in some MAP items. The problem is more with fillets or steaks cut from fish previously frozen and thawed. Depending upon the circumstances, up to 5% of the initial weight may be lost. Nevertheless, the problem can be corrected by dipping the fillets or steaks briefly (1–2 min) in 5–10% aqueous solution of polyphosphates such as sodium or potassium pyrophosphate, sodium or potassium tripolyphosphate, or sodium hexametaphosphate.[51] These phosphates act by swelling the outer surfaces of the fillet, thereby partially sealing them. An additional benefit of this treatment is the glossy translucency imparted to the cut surfaces of fillets. The problem of drip can be also solved to a great extent by dipping fish fillets in NaCl solution prior to packaging.[37] However, this may not be practical always, because the fish often become too salty.[51] MAP has been shown to cause softening of texture of trout associated with excessive drip formation. Whether this is due to high amount of dissolved gas and drop in pH is not certain.[52] In the case of whole fish items, the color of the belly flaps, cornea, and the skin may be altered when stored under high CO_2 concentrations.[13]

6.5 COMBINATION OF MAP WITH OTHER PROCESSING TECHNIQUES

6.5.1 Hurdle Technology

The microbial stability and safety of most foods is based on a combination of several barriers (hurdles) that should not be overcome by the microorganisms present. These hurdles in a stable product control microbial spoilage and food poisoning. Hurdle technology is the integration of different microbial barriers in synergy with each other to prolong the initial quality of the food product. The application of this concept (referred to as combined processes, combination preservation, barrier technology, or hurdle technology) has proved very successful since an intelligent combination of hurdles secures the microbial stability and safety as well as the sensory, nutritive, and economic properties of a food.[30] For each stable and safe food a certain set of hurdles is inherent, which differs in quality and intensity depending on the particular product. These hurdles must keep the normal population of microorganisms in the food under control without allowing them to overcome ("jump over") the hurdles present. In foods preserved by hurdle technology, the possibility exists that different hurdles in a

food will not just have an additive effect on stability but could act synergistically. Therefore, employing different hurdles in the preservation of a particular food should have advantages because microbial stability could be achieved with a combination of gentle hurdles. About 40 hurdles including temperature, water activity, acidity (pH), redox potential (Eh), competitive microorganisms and preservatives, O_2 tension, smoking, modified atmosphere, pressure, radiation, and other physical processes, microstructure of the food, and packaging (selective permeable films, advanced edible coatings) have been identified.[30,50] In lightly preserved products such as cold smoked salmon and low-heat treated products, growth and toxin production by *C. botulinum* is a potential hazard as a result of abuse of storage temperature.[53] It has been recommended to include additional barriers to enhance the safety of these fishery products.[54] MAP could be coupled with other processing methods such as freezing, cooking, irradiation, superchilling, smoking, and application of edible coating containing antimicrobials. If the intensity of a particular hurdle in a food is too small ("minor"), it should be strengthened ("optimized"). On the other hand, if it is detrimental ("negative") for the food quality, it should be lowered ("avoided"). By this adjustment, the hurdles in foods should be kept in the optimal ("positive") range considering safety as well as quality.[30,50] Examples of microbial hurdles in a food are given in Figure 6.5.

6.5.1.1   Superchilling

MAP can be combined with superchilling to further extend the shelf life and safety of fresh fish. In this technique, the temperature of the fish is reduced to its initial freezing point of -1 to $-2.5°C$ before MAP.[55] MAP of fresh Atlantic salmon (CO_2:N_2, 60:40) and superchilling to $-2°C$ resulted in a good quality product having negligible bacterial growth for 10 days, whereas MAP, chilled ($+4°C$) samples were acceptable for 7 days only.[55]

6.5.1.2   Smoking

Smoking can be combined with MAP. MAP combined with smoking resulted in doubling of shelf life of blue cod.[41] Hot smoked mackerel under MAP had a shelf life of 8 days (see also Chapter 12).

6.5.1.3   Feed supplementation in aquacultured fish

Recently, the effect of dietary supplementation of antioxidant, α-tocopheryl acetate on shelf life of aqucultured Atlantic halibut under modified atmosphere or vacuum packaging was examined. The fish were fed 249 and 544 mg α-tocopheryl acetate per kg diet for 2 months. After slaughter, the fillet portions were stored for 11 days at 2°C in MAP and at $-20°C$ for 6 weeks in vacuum package. The higher level of dietary α-tocopheryl acetate resulted in significantly less malonaldehyde in MAPs and in lower total bacterial count (TBC) in vacuum packaging. Moisture loss was less in MAP samples compared with vacuum-packaged samples.[56]

FIGURE 6.5 Examples of microbial hurdles in a food. If the intensity of a particular hurdle in a food is too small ("minor"), it should be strengthened ("optimized"). On the other hand, if it is detrimental ("negative") for the food quality, it should be lowered ("avoided"). By this adjustment, the hurdles in foods should be kept in the optimal ("positive") range, considering safety as well as quality (From Leistner, L., *Food Preservation by Combined Processes*, Leistner, L. and Gorris, G.M., Eds. Final Report, FLAIR Concerted Action, No. 7 Subgroup B, FLAIR, Food-Linked Agro-Industrial Research, EUR 15776 EN, 1995, p. 1. With permission)

6.5.1.4   Treatment with antimicrobial compounds

A number of antimicrobial compounds could be incorporated in the food product to provide additional hurdles to microbial growth. These include ozone, organic acids, suphites, phosphates, ethanol, and enzymes such as lysozyme and lactoperoxidase.[30,57] In a commercially adopted process in Northern Ireland for prawns (*Nephrops norvegicus*), they are headed at sea and the tails are blast frozen on landing. After peeling the larger tails are refrozen, dipped in water to provide a glaze, and then packed for sale. A method to extend the chilled shelf life up to 14 days was developed for value addition and better distribution of the prawns. In this, mild acid treatment was used to extend the shelf life. A combination of acetic acid, either as cold soak or as blanch, followed by MAP in at least 40% CO_2 and remaining N_2 was found to suppress the spoilage flora. For a cold soak, 4 min treatment with 0.1% (v/v) acetic acid was optimal. The treatment prevented black spot formation too. Prolonged extension in shelf life was however difficult due to autolytic reactions in the muscle.[58]

6.5.1.5   Edible films

Edible films in combination with MAP could be advantageous. The last few years saw considerable progress in the development of edible coatings from polysaccharides, proteins, and also lipids, which have potential to extend shelf life of foods. These coatings can retain quality of fresh, frozen, and processed muscle foods including fish items by retarding moisture loss, reducing lipid oxidation and discoloration, enhancing product appearance in retail packages, and functioning as carriers of food additives such as antimicrobials and antioxidant agents.[59] These compounds include bacteriosins, chitosan, ethanol, allyl isothiocyanate, or precursors of chorine dioxide. Although these have been currently tested only at the laboratory scale there is a possibility of development of packaging material capable of controlling microorganisms.[2] Edible packaging films based on fish myofibrillar proteins have been developed.[60,61] It has been shown that coating of the fish protein film over fishery products could prevent quality loss in chilled freshwater and marine fish as well as in frozen marine-fish mince.[62,63] Combination of edible coating along with appropriate food additives and MAP can exert significant effect in extending shelf life of fishery products. An edible film, preferably from fish proteins itself, impregnated with acid can lower the pH on the surface of a seafood and reduce the growth of microorganisms (Chapter 13).

6.5.1.6   Low-dose irradiation

Combination of low-dose radiation treatment with modified atmosphere storage has been found beneficial to enhance the shelf life of cod fillets.[64] Low-dose irradiation of cod fillets packaged in 60% CO_2–40% N_2 enhanced chilled shelf life of the fish fillets as compared with unirradiated counterparts. Irradiation also helped extension of shelf life of the fish fillets packaged under vacuum[64] (see Chapter 10).

6.5.1.7 Heat treatment

Modified-atmosphere packaging of cooked shrimp has resulted in 200% increase in shelf life.[5] Additional benefits could also be realized by combination of MAP with natural antioxidants and an O_2 scavenger or by using noble gases in MAP.[5,14] MAP can have other applications for products in which fish may be a component of the processed food products such as meals.[65,66]

6.5.1.8 Light-pulse treatment

Light-pulse technology is an emerging nonthermal processing method and involves exposure of foods to short-duration pulses of intense broad-spectrum light. The spectrum of light includes wavelengths in the ultraviolet to the near-infrared region. Usually a wavelength distribution having 70% of the electromagnetic energy within the range of 170–2600 nm is used. These high-intensity flashes of light pulsed several times in a second can inactivate microorganisms on food surfaces with remarkable rapidity and effectiveness. The technology can also be used to sterilize packaging material too. The gerimicidal effect appears to be due to both the UV content and brief heating effects. The material to be treated is exposed to at least one pulse of light having an energy density in the range of 0.01–50 J/cm^3 at the surface. The effectiveness of light-pulse treatment depends on several factors such as intensity, treatment time, food temperature, and the type of microorganisms. Light pulses have the ability to inactivate enzymes in food as well. The technology has potential to replace or complement conventional thermal processing methods. However, at present, industrial implementation of light-pulse technology for food has been rather slow, despite its potential to produce safe, nutritious, and high quality foods.[67,68] Other physical methods that may be used in combination include pulsed electric fields and oscillating magnetic fields. Table 6.4 shows combination of MAP with other processing techniques, and Table 6.5 presents shelf lives of some packed and nonpacked seafood at different temperatures.

6.5.1.9 Other methods

Inclusion of protective cultures such as lactic acid bacteria inhibits undesirable flora through the production of low molecular weight antimicrobials, such as lactic acid, hydrogen peroxide, and bacteriocins.[30] Nisin produced by lactic acid bacteria is able to prevent the outgrowth of germinating bacterial spores.[30] Alternatively, sachets containing appropriate compounds could be incorporated in the pouch to arrest growth of specific microorganisms. Ethanol entrapped in silica and put in a sachet made with a film highly permeable to ethanol vapor can be used to extend the shelf life of semidried fish and other food items. The vapor deposits on the food surface, eliminating the growth of molds and pathogens. Humidity in the package can be controlled by including a desiccating film or sachet in the form of a pillow with entrapped propylene glycol. Keeping the sachet in contact with the surface of fish for several hours absorbs water and controls microorganisms including molds and yeast.[69]

TABLE 6.4
Combination of MAP with Other Processing Techniques

Technique	Product	Extension in shelf life at chilled temperatures
Cooking	Shrimp	14 days
Freezing	Cod	20 days
Irradiation	Cod	25 days
Superchilling	Salmon	3 weeks
Superchilling	Mackerel	21 days
Smoking	Cod	Doubling of shelf life
Edible coating containing antimicrobials/antioxidants	Various fish	Additional shelf life

Source: Summarized from Brody, A.L., *Food Technol.*, 55, 74, 2001; Sivertsvik, M., Jeksrud, W.K., and Rosnesa, J.T., *Int. J. Food Sci. Technol.*, 37, 107, 2002; Penney, N., Bell, R.G., and Cummings, T.L., *Int. J. Food Sci. Technol.*, 29, 51, 1994; Hong, L.C. et al., *J. Food Sci.*, 61, 646, 1996; Madden, R.H. and Bolton, G., *Proceedings, Commission C2 Chilling and Freezing of New Fish Products*, International Institute of Refrigeration, Paris, France, 1990, p. 161; Licciardello, J.J. et al., *J. Food Sci.*, 49, 1341, 1984.

6.5.2 Packaging Material

Packaging material plays a crucial role in the success of MAP technology.[70] A barrier material is important not only to keep O_2 from permeating into the pouches during storage, but also to prevent CO_2 and N_2 permeating out of the package. Generally, all MAPs are based on thermoplastic polymers. The permeability of gases such as O_2, N_2, and CO_2 through polymeric materials increases as temperature increases, but the extent of permeability varies for different polymers. Table 6.6 depicts representative O_2 and CO_2 permeability of some food packaging polymers. Packages made from such plastic materials allow some gas transmission, even at chill temperatures. Therefore, it is not possible to maintain the atmosphere initially introduced into the MAP for long, suggesting the importance of barrier properties of the films. For example, it was shown that snapper fillets doubled their shelf life from 3 to 6 days when vacuum packaged in high O_2-barrier bags compared with medium O_2-barrier bags, all other variables being the same.[71] Shelf lives of some seafood in combination with packaging and other treatments are given in Table 6.4.

Polymers such as polyvinylidene chloride (PVDC) can provide excellent barrier to O_2 permeation under both wet and dry conditions. However, hydrophilic polymers such as ethylene vinyl alcohol copolymer (EVOH), cellophane containing hydroxyl groups and nylon polymers containing amide groups exhibit large increases in permeability as the polymers absorb water. EVOH has superior gas-barrier properties under dry conditions, therefore co-extrusion of water

TABLE 6.5
Shelf Life of Some Packed and Nonpacked Seafood at Different Temperatures

Product	Storage temperature (°C)	Packaging	Shelf life (days)
Fresh seafood			
White-fleshed, average size, and caught in temperate and cold waters	0	Air	12–18
Cod	−2	Air	26
Cod	−2.5	MAP	36
Caught in warm waters	0	Air	18–35
Tilapia	4	MAP	>25
Large halibut, tuna, and similar fish	0	Air	21–22
Salmon	0	VP	19
Dark-fleshed, small fish			
Low fat	0	Air	6–9
High fat	0	Air	4–6
Shellfish, temperate and cold waters	0	Air	6–10
Pink shrimp	0	Air	3–4
Shellfish, warm waters	0	Air	8–12
Lightly preserved			
Cold-smoked salmon	5	VP	20–56
Hot-smoked cod (lightly salted)	0.5	Air	24–26
Hot-smoked mackerel (lightly Salted)	0	MAP	8
Brined roe (lump fish)	5	VP	75–90
Cooked and brined shrimp	0	MAP	230–308
Cooked and brined shrimp	25	MAP	4–6

Source: Adapted from Dalgaard, P., in *Handbook of Food Science Technology and Engineering*, Hui, Y.H., Ed. With permission from CRC Press, Boca Raton, 2005.

sensitive EVOH as an internal layer in structures consisting of water vapor-barrier polymers such as polyethylene, polypropylene, or polycarbonate can protect its O_2-barrier properties.[72] Materials commonly in use for MAP are laminated or co-extruded films of polyethylene with polyester or nylon with or without the addition of high-barrier layer of vinylidene chloride–vinyl chloride copolymer or ethylene–vinyl alcohol copolymer, depending on the barrier required.[69,73] If a layer of aluminum foil is introduced in the pouch it can provide complete barrier to transmission of gases including water vapor.[74] The packaging material should also have mechanical strength to withstand machine handling and subsequent storage, distribution, and retailing. Stand-up flexible pouches are now commercially used for preservation of all classes of food and beverage products, employing thermal sterilization, hot filling, aseptic/retort packaging, packaging of liquid/solid products including chilled and frozen meat, and seafood items.[75] These pouches

TABLE 6.6
Representative Permeability of O_2 and CO_2 in Some Food Packaging Polymers at 23 to 25°C and 75% Relative Humidity

Polymer	Permeability (cc m^2 day^{-1} atm^{-1})	
	O_2	CO_2
High-density polyethylene (HDPE)	73	228
Low-density polyethylene (LDPE)	196	284
Biaxially oriented polypropylene (BOPP)	59	216
Polyvinylidene chloride (PVDC)	0.04	0.098
Polyethylene terephthalate (PET)	1.88	9.44
Nylon 6, unoriented	2.6	4.0
Ethylene vinyl alcohol copolymer (EVOH), 27% *m* ethylene	0.012[a]	0.016[b]
Ethylene vinyl alcohol copolymer (EVOH), 44% *m* ethylene	0.028[a]	0.078[b]

[a] 20°C.
[b] 20°C, 65% relative humidity.

Source: Adapted from Brown, W.E., *Plastics in Food Packaging*, 1992, p. 292. With permission from Marcel Dekker.

can have good application for MA stored fishery products, which also calls for efficient distribution. The machines used for packaging depend upon the packaging material. The pillow-wrap or horizontal form-fill-seal machine employs a single reel of flexible packaging material, which is formed into a tube and the two edges are heat-sealed. The product is passed into this tube that is vented of air by flushing gas prior to sealing.[74,76] Thermoformed rigid or semirigid base material is also used for packaging. The base material is thermoformed into a tray. After loading of the product, the tray passes into a chamber in which the air is evacuated and the desired gases are introduced. A top-lid material is drawn over the tray and sealed to lip edge. This system has the advantage of being able to evacuate air from the package before flushing with gas and a low residual O_2 level in the package can be achieved.[18,75] A machine has been developed for MAP of fishery products that has facilities for variation in packaging sizes together with provision for precise vacuum and gas flesh control and low gas alarm. CO_2-sensitive labels that change color if modified atmosphere package is opened or damaged have been developed.[11]

6.6 QUALITY CONTROL

As discussed earlier, MAP results in a shift in the microflora and associated spoilage potential of the surviving microorganisms. Therefore, determination of quality of

MAP fishery products by conventional methods can have limitations at least for some of these products.[17,48,77,78] Nevertheless, sensory techniques may still be the reliable method for quality assessment of MAP fishery products. In addition, it was recently reported that free amino acid, β-alanine and 1-methyl histidine could serve in combination with anserine and tryptophan as quality indices for some modified atmosphere and chilled stored fishery products.[3]

An electronic nose with electrochemical gas sensors (CO, NH$_3$, SO$_2$, and H$_2$S) has been used as a rapid technique to monitor changes in the headspace gases of MAP whole ungutted redfish (*Sebastes marinus*) during chilled storage.[79] The precision of the electronic nose measurements was determined using standard compounds (ethanol, trimethylamine, acetaldehyde, and dimethyl disulfide). The response of the carbon monoxide sensor, which detected formation of alcohols and carbonyls, increased with time in both aerobic and MA storage and correlated well with sensory analysis based on the Quality Index Method. By this technique, slower spoilage rate, reflected by lower intensities of the sensors' responses, lower microbial counts, and less TMA production, was observed in MA-stored red fish, compared with aerobic stored counterparts.[79]

6.7 CHILLED DISTRIBUTION CHAIN

Cold chain is an integral part of the food production and distribution system for safe delivery of the chilled products (Chapters 2 and 5). This is also true for MAP flesh foods including fishery products. The increasing outbreaks of foodborne diseases have created a greater awareness on the need for temperature management in handling and distribution of processed foods. The rate of quality loss for refrigerated products generally increases by 2 to 8 times for a 10°C increase in temperature. Therefore, regulatory authorities have prescribed maximum limit for storage temperature for various products. The European Union Agreement on the International Carriage of Perishable Foodstuffs has specified storage temperature limits of 1°C and 1 to −5°C, for lightly preserved and semipreserved products, respectively.[80] Such temperature limits are prescribed on technological as well as hygienic considerations. Chilling foods after processing and maintaining a temperature below 5°C are not easy operations for food productions on an industrial scale. This is particularly so in MAP products due to the poor thermal conductivity of the gas mixtures. In addition, surveys have shown that serious breaches of the chill chain are not uncommon occurrences at the supplier level and the consumers are not aware of such abuse. Systematic temperature monitoring of chilled processed foods needs to be integrated into the quality assurance program, setting limits in accordance with national legislation and guidelines.[80,81] Some of the precautions that need to be taken include distribution of foods through temperature-controlled refrigerated trucks, systematic monitoring of temperature of deliveries at fresh food terminals, and deliveries in specially designed, locked cold stores. Further, outlets including display cabinets need to be submitted to regular hygiene inspections. While chilled aerobically packaged seafood during transportation should have an O$_2$ transmission rate not less than 10,000 cm^3/m^2/ day, seafood in reduced O$_2$

packages should be handled with strict temperature control and thermal history monitoring using time-temperature monitors for checking *C. botulinum* type E.

6.8 GUIDELINES FOR MAP FOODS

Commercial interest in this modified atmosphere packaging technology has resulted in the formulations of several guidelines by professional bodies and regulatory authorities in many countries to ensure the safety of these food products including seafood. According to European Economic Council (EEC) Directive of July 22, 1991 (91/493/EEC), vacuum packaged and MAP fish products are considered as fresh products. Consequently, CO_2 can be used for preservation of fresh fish products, when shelf life extension for only a few days is sufficient. The U.S. Association of Food & Drug Officials has prescribed guidelines for packaging foods in a reduced O_2 atmosphere.[82] These guidelines also provided definitions of types of reduced O_2 packaging (cook-chill, vacuum packaging, *sous vide*, and MAP and CAP). In addition, guidelines for labeling and refrigeration requirements for foods packaged in a reduced O_2 atmosphere (e.g., low water activity, low pH, high contents of nonpathogenic competing organisms) and for implementation of a hazard analysis critical control point (HACCP) program have also been given.[82] Guidelines for MAP have also been provided in the United Kingdom.[83]

In summary MAP has potential for distribution of fresh fish to consumers both in domestic and foreign markets. However, in spite of extensive studies, at present the technology has not been accepted well by the seafood industry, unlike in the case of red-meat products. For example, in New Zealand it is only recently, following developments in packaging and refrigeration techniques that this technology was found both technically and economically feasible for export of seafood products. While several reasons have been responsible for the poor acceptance of the technology, combination of additional techniques along with MAP can address some of the problems associated with fishery products. In addition adherence to guidelines and strict quality control can encourage use of MAP for better distribution of fishery products.

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7 Retort Pouch Packaging

7.1 INTRODUCTION

Retortable pouch packaging is receiving renewed interest in thermal processing of foods. The retort pouch is replacing the conventional metallic can to a great extent because of the ability of the pouch to withstand thermal processing and the convenience associated in handling the product. Food-canning companies are increasingly using pouches to replace the metallic can, glass jar, or aluminum foil, with a view to appeal modern generations of customers. The pouch, which is lighter in weight than the can offers better convenience in food handling. Unlike the metallic cans, the boil-in-bag facility offers warming the food in the pouch immediately before consumption.[1] The concept of the retort pouch dates back to the 1940s, when shelf-stable packaging was examined for military rations, although it lost its appeal until recent events, which include changes in consumer lifestyles, progress in packaging technology and shortage in metal cans brought it back to a position of prominence.[2] In the present times, retort pouches and trays are gradually penetrating the food markets as an option for convenient shelf-stable products.[2–5] The advent of the retort pouch-processing technology has also made availability of shelf-stable ready-to-eat (RTE) foods a reality. A variety of RTE foods are being manufactured by several multinational companies world over. In India, for example, production of RTE foods has reached an annual value of about US$20 million.[3] These products, as home meal replacement options, have high acceptability among middle-class consumers in the country. Some of these products, include vegetable curries, pudding in tubes, as well as coffee and dairy beverages in plastic bottles.[3] Developments in retort pouch technology have also helped advances in aseptic packaging.[2,3]

7.2 CHARACTERISTICS OF THE RETORT POUCH

7.2.1 Packaging Materials

Retort pouches are extensions of conventional pouches making them capable of withstanding thermal inputs required to achieve sterility and consequent ambient temperature shelf stability. Basically, a retort pouch is a flexible, laminated pouch (or tray, dish, bowl, or cup). The packaging material is a laminate of three materials, an outer layer of polyester, a middle aluminum foil, and an inner layer of polypropylene. The outer polyester layer gives the pouch strength, abrasion resistance, and a surface for printing information about the contents. The aluminum layer functions as a moisture, light, and gas barrier, while the inner layer is the heat-seal and food-contact material. For a prolonged shelf life of processed

product, the material for such packs must provide superior barrier properties, seal integrity, toughness, and puncture resistance and must also withstand the rigors of thermal processing. The thinner retort pack allows shorter thermal processes to retain flavors in the final product.

The pouch material is generally a biaxially oriented polypropylene/ nylon/polyethylene usually with a layer of aluminum as a barrier to light. Most common form of pouch consists of a 3-ply laminated material, consisting of polyester/aluminum foil/cast polypropylene. The outer polyester film is normally 12 μm thick, although foils having 7, 9, and 15 μm thickness are used. It serves to protect the foil and to provide the laminate with strength and abrasion-resistance. The core of aluminum foil is used to give the laminate the necessary barrier properties for water, gas, odor, and light. The primary function of the polypropylene inner ply is to provide strong heat seals and good product resistance required in the retort pouch. The layer also helps to protect the foil and contribute to overall pack strength. The thickness of the cast polypropylene layer will depend to some extent on the nature of the product being packed. For soft liquid-containing products, 50 μm polypropylene is normally used. This is increased to 70 μm for hard products. While it is possible that any of the three pouch components could contain an imperfection such as a pinhole, the probability that these would be coincident in the laminate is very remote. The three layers of the retortable pouch are combined by adhesive lamination. Conventional polyurethane adhesives, cured by tolyolidene-di-isocyanate-based catalysts, were originally used in the process. Although this adhesive is still being used, doubts about its safety for the polypropylene bond have resulted in the developments of two alternative bonding systems. One of these is a polyurethane adhesive, which is cured by an aliphatic isocyanate, and the other, morprime, is based on a dispersion of polypropylene. The latter does not require any adhesive at all.[4,5] Table 7.1 shows some polymers used for retort pouches.

The first step in the manufacture of laminate for pouches involves printing of the polyester film. The printed polyester film can be adhesive laminated to the aluminum foil and then laminated to the polypropylene. Alternately, the printed polyester film can be laminated to the premounted foil polypropylene-base laminate. In either case, the adhesive is applied to a substrate and then pasted through an oven, which sets the adhesive. The combining of the two webs is done on a heated roller by employing pressure.[4,5]

TABLE 7.1
Some Polymers Used for Retort Pouches

Polyester 12 μm/nylon 15 μm cast/aluminum foil 9 μm/cast polypropylene 70 μm
Nylon 15 μm/cast polypropylene 60 μm
Polyester 16 μm/aluminum foil 9 μm/cast polypropylene 60 μm
Nylon 15 μm/cast polypropylene 70 μm
Nylon 25 μm/cast polypropylene 70 μm

TABLE 7.2
General Requirements for Retortable Pouches

1. Resistance to temperature up to 133°C
2. Low gas permeability and no oxygen permeability
3. Inertness in interaction with food components
4. Low water-vapor transmission rate. Ideal moisture-vapor transmission rate, nil
5. Heat sealability. Seal strength, 2–3.5 kg/100 mm. Bond strength, 150–500 g/10 ml
6. Burst test, 7.5 kg/15 mm seal
7. Physical strength to resist any handling during manufacturing and distribution
8. Good aging properties
9. Printability

Source: Adapted from Devadasan, K., *Fish. Chimes (India)*, 20, 44, 2001. With permission.

7.2.2 Thermal Stability

The retort pouch is aimed at producing ambient temperature-stable, high-water activity (>0.85), low-acid (pH > 4.5) foods with high initial quality. Therefore, thermal conductivity is the most important criterion in selection of food packaging material. When using polymer packages, processing temperature must be kept below the glass transition temperatures of the polymer in order to maintain dimensional stability of the package. It is necessary that these pouches should be able to withstand a temperature up to 133°C for 1 h. In addition, it should also be stable to subsequent cooling to 40°C under counterpressure, which must be carefully maintained to avoid differential pressure on heat-weakened seals that would otherwise open the seal.[2] The ambient temperature shelf stability demands protection against microbiological growth in the product during storage and distribution. Since low-acid foods are vulnerable to growth and toxin production from heat-resistant spores of pathogenic microorganisms, the thermal sterility requires temperatures higher than that of boiling water (by using steam under pressures) to ensure commercial sterility of the product. Foods with pH < 4.6, such as fruits and most beverages derived from fruits may be microbiologically stabilized by time-temperature processes far lower than that required for low-acid foods. Newly developed polymers and blow-molding technologies have increased the maximum application temperature associated with these containers. Clear polyethylene terephthalate (PET) and polypropylene (PP) bottles and jars are being used for hot-fill and hold, shelf-stable acid foods (pH < 4.6) and pasteurized low-acid foods (pH > 4.6) stored below 4°C for extended periods of time.[3] General requirements for retort pouches are given in Table 7.2.

7.2.3 Permeability to Gases

Permeability of gases is an important criterion in determination of the property of retortable pouches. The permeability of gases such as oxygen, nitrogen, and

carbon dioxide through polymeric materials increases with temperature, but the extent of these changes varies for different polymers. Although, ideally the pouch should not have any oxygen permeability (Table 7.2), as compared with metal cans, the pouch even in combination with aluminum foil has some permeability of oxygen. This can adversely affect the packaged food during prolonged storage.

7.2.4 Adhesion of Food

Adhesion of food products to the packaging film is another property of the pouches. A study on meat adhesion to different kinds of plastic casings in retort sterilized fish sausages showed that the plastic casings of significant wettability such as polyvinylidene chloride, polyvinylidene fluoride, polyester, and polyamide brought about greater meat adhesion than polyethylene and polypropylene. Modification of polyethylene casing using ultraviolet radiation brought about greater meat adhesion. The meat adhesion of modified polyvinylidene chloride having ─COOH groups introduced to the surface of casings became greater. This suggested that the important factors of meat adhesion were not only the wettability but also the functional groups of the casings. There was also a good correlation between meat adhesion on polyvinylidene chloride and its breaking strength.[7]

7.3 PROCESSING OF FOOD IN RETORT POUCHES

7.3.1 Basic Operations in Canning

The unit operations in retort pouch processing are generally comparable to those of conventional canning. In traditional canning, the fish is filled into metal cans or glass jars, hermetically sealed mechanically, and subjected to temperatures in the vicinity of 121°C under pressure to ensure that the slowest heating point within the food reaches a preestablished time-temperature integral.[2] The unit operations include skinning, filleting, separation of fish parts after evisceration, and trimming of fins, scales and other inedible parts, brining, cooking, smoking, exhausting, hot filling, and sealing.[8–10] The main objective of brining is the enhancement of flavor of the final product. The filling medium accelerates heat transfer to the fish and avoids overcooking at points closest to the can walls. The cans are heated (exhausted) in steam to displace air, sealed and then heat sterilized. Sterilization is done at 121°C (a steam pressure of 2.0 bar would give a temperature of 120.2°C) for the required duration to attain commercial sterility. Cooling of the heat-sterilized cans is usually achieved by injecting chlorinated water into the retort after cutting off the steam. Thermal sterilization of fish in sealed cans eliminates bacterial as well as autolytic spoilage and gives products a shelf life of 1 to 2 years at ambient temperatures (Chapter 12). Figure 7.1 shows process flow sheet for canning of mackerel.

7.3.2 Process Operations in Retort Pouch Packaging

The process operations in retort pouch packaging are filling, air removal, sealing, traying, autoclaving, and cooling.[11] These are discussed in the following sections.

Fresh mackerel (thawing, if frozen)
↓
Beheading, gutting and skinning
↓
Washing
↓
Precooking at 90°C
↓
Cooling and separation of frames
↓
Filling into cans
↓
Adding fillers (brine, sauce or oil)
↓
Exhausting
↓
Double seaming
↓
Retorting
↓
Cooling
↓
Drying and labeling
↓
Canned product
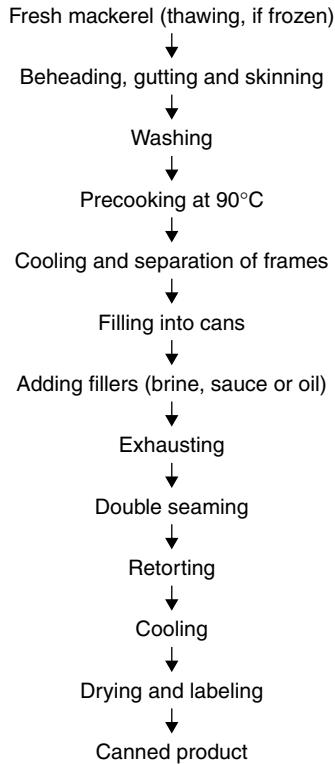
FIGURE 7.1 Flow sheet for production of canned mackerel fillets (*Source:* Venugopal, V. and Shahidi, F., *Food Rev. Int.*, 14, 35, 1998. With permission from Marcel Dekker)

7.3.2.1 Filling and sealing systems

The food for retort pouch packaging is prepared similar to canning. Filling of the food in pouches needs careful operation. Contamination of the seal area in flexible pouches can cause process failures. Systems used for filling retort pouches range from manual to fully automatic with variations and combinations of both. During filling, it is important that the pouch seal must be kept free from contamination with product since this could impair sealing and cause leakage at the seam. Systems capable of dispensing product components of various types of food (such as fresh food pieces, slices, and sauces) are available. Liquids and liquid-solid mixes can be closed using rotary-valve piston dosers. Solids can be dispensed using dry fillers. For filling products such as fish fillets, automatic systems are available. A simple technique has been devised to eliminate contamination due to spurting and accumulated drip as the delivery nozzle comes into contact with the inner surface of the pouches while withdrawing. In this, a double-walled delivery system is used. The outer pipe shields the seal area at the inner surface of the pouch, preventing it from coming into contact with the product. The inner pipe is

longer than the outer pipe to prevent any spurting from reaching the tip of the outer pipe. This delivery system is ideal for both manual and automated filling of fluids having viscosity up to 1000 cps.[11]

7.3.2.2   Air removal

Removal of air from the filled pouch is very important to ensure integrity of the package during retorting. This also helps to avoid bursting of the pouch and uniform heat transfer. Removal of air from the filled pouch is normally effected on an automatic line. If too much air is left in the pouch there is a danger that it will inflate burst during sterilization or reheating. Inflation of the pouch during sterilization will also impair heat transfer to the pouch contents. For liquid products it is possible to exclude much of the air by squeezing the pack, so as to raise the level of liquid to just below the line of seal. Packs containing solid products can be vacuum-sealed using chamber machines. Effective vacuum packs can also be made by injecting steam to displace the air in the packs prior to sealing. The steam is injected just prior to making the top seal. Condensation of steam minimizes the amount of headspace gas when cooled. Superheated steam is generally used because it causes less moisture condensation in the seal area. The residual air should be less than 2% of the volume of the pouch contents.

Residual gas in the package may have an adverse effect on the product. Influence of residual gas volume on physicochemical and sensory properties of wet-pack military rations of RTE pears packaged in retort pouches was examined involving four selected residual gas volumes, namely, 10, 15, 20, and 30 cm^3, in the pouches. The samples were maintained at three storage temperatures, namely, 4.4, 26.7, and 37.8°C. It was observed that residual gas volume of 30 cm^3 promoted faster darkening and higher ascorbic acid degradation than the lower gas volumes. Higher storage temperature accelerated consumption of the remaining oxygen in the pouches, degradation of ascorbic acid, formation of 5-hydroxymethyl-2-furaldehyde (HMF) and softening of pears. It was concluded that residual gas volume above 20 cm^3 adversely affected the desired shelf life of the product.[12] Similar adverse effect of residual gases on low-acid foods could also be contemplated, although no detailed studies on this aspect have been carried out.

7.3.2.3   Sealing

Hot-bar sealing is preferable to impulse sealing. A seal width of 5 to 10 mm is desired for good strength.[13] The pouch should be double sealed to reduce the risk of seal defects. If impulse sealing is used, it should provide two sides heating and the sealing elements should be 6 mm wide rather than the normal 3 mm. The overseal should extend over the mouth of the pouch to prevent mold growth in any package above the closure seal. For sealing conical retort pouches, the four-seal type design is always preferred over fine-seal design, since the latter features multiple seal junction and is prone to leakage. On automatic high-speed filling machines, various techniques such as curved-bar sealing, sliding tube nozzles

with suck back, and water flushing have been adopted. However, the seal in a retort pouch is weak as compared to mechanical double-seaming of metal cans.

7.3.2.4 Racking

The autoclaves used for sterilizing the packs require specially designed crates to support the pouches. The sealed pouches are carefully laid on racks or trays to maximize the heat transfer and minimize potential damage of seals or foil structures within the retort. Usually, on the tray, each pouch is accommodated in a separate compartment or slot. This ensures that the flexible pouches receive uniform exposure to heating medium. Ideally the trays should be positioned in such a way as to avoid pouch movement and superimposition during retorting. An additional mesh restraint over the retort trays could be used to restrict pouch inflation and distortion in the retort.

7.3.2.5 Retorting

Retorting is the most important operation in the process. The optimization of thermal processes for retort-pouch sterilization is comparable to those for conventional canning, and includes determinations of the following parameters:

1. *Thermal death curves (TDC)*: This is obtained by determining the survival of heat-resistant microorganisms, such as spores of *Bacillus stearothermophilus* or *Clostridium botulinum*, when exposed to a constant temperature (e.g., 121°C) for varying time intervals, which gives the '*D* value' of the organism. The *D* value is the time of heat treatment at a particular temperature for reduction of the bacterial load by one log cycle.
2. *F_0 value*: It is the heating (e.g., at 121.1°C) time required for the reduction of the initial population microorganism by a factor of 10^{-12}. For spores of *C. botulinum* types A and B, *D* value at 121°C is about 0.25, and therefore, F_0 value is 3.

Generally, the minimum process time recognized for prevention of botulism in canned fishery products preserved by heat alone is 3 min at 121°C (i.e., F_0 value of 3). For highly heat resistant spores of *B. stearotheromophilus*, the minimum thermal processing time should be 25 min at 121°C. Such a thermal treatment can also take care of *C. botulinum*. Processing to such high F_0 value will adversely affect the texture and palatability of the product. Hence it is important to use good-quality raw materials and ingredients that harbor only low bacterial levels. F_0 values for medium- and low-acid products, range from 6 to 14 in order to afford safety of the products. A F_0 value between 5 and 10 is generally employed for retortable pouch packaging of these foods. Methods for determination of heat resistance using computer-controlled thermoresistometer have been described.[14–16]

Unlike metallic cans, retortable pouches, because of their flexible nature and limited seal strength, are unable to support internal pressure developed by expansion of headspace gases during retorting at the required thermal processing conditions. Therefore, the retort pressure is controlled very carefully to counter steam pressure developed within the pouch from the heating and the residual internal steam pressure during the cooling regime of the cycle.[17,18] Steam/air mixtures and water immersion/overpressure retorts are commonly used because of their ability to maintain pressure in the retorts, which is greater than the saturated vapor pressure within the package during processing. Steam/air mixtures have been used successfully for the commercial sterilization of foods in flexible and semirigid plastic packaging as well as rigid packages such as metal and glass. This enables to overcome the tendency of the gases within the package to expand and retard heat transfer. Further, steam/air mixtures also help to counteract internal vapor pressure, which could otherwise tend to destroy or burst the container. In steam/air processing, heat is supplied primarily from latent heat of condensing steam, in contrast to sensible heat transfer in superheated water system. As a result, it is essential to have a homogenous steam/air mixture to reach all product locations. The steam/air retorts have many advantages in terms of achieving heat transfer by steam condensation at essentially constant temperature. The gaseous nature enables easy circulation in turbulent flow throughout a loaded retort.[18]

The rate of heat transfer from the steam through the package wall into the outer layer of food is high. From this point, penetration of heat into the coldest region of the food is controlled by the thermal properties of the food. While studies on heat transfer have been well conducted during heat sterilization of the metal cans, similar studies with respect to retort pouches are sparse. In one study, heat transfer during the sterilization of food packaged in cylindrical, retort pouch, and rectangular containers was examined. Optimal thermal treatment conditions for these packages were compared with theoretical data. For all packaging systems, theoretical and experimental values were comparable.[19]

Vertical or horizontal type retorts are used for pouch processing. Vertical retorts are more efficient than horizontal ones, in terms of the number of pouches per unit of retort volume and floor space. The retort vessel is equipped with modular steam inlet and exhaust capabilities, a liquid-ring vacuum pump capable of rapid evacuation of the vessel, stainless-steel construction, sanitary piping for cleaning, and also a data acquisition system used to monitor retort pouches. In agitated retorts, the pouches may be continuously agitated for better heating. However, nonagitating or still retort is the most widely used equipment. Automation in retort pouch packaging has been examined in detail.[20] A computer controlled evaluation program for steam-air processing of retort pouches has been studied using a flexible pouch ($18.8 \times 12.78 \times 2.54$ cm^3) filled with 600 ml of 10% bentonite solution, to simulate food. Results of the computer evaluation, during normal and deviant processes were compared with the general method of evaluation. Tests for normal process conditions were undertaken at 120°C and 137.8 kPa. Deviant test process conditions were 115°C and 103 kPa for 0–12 min

and then 120°C and 137.8 kPa until the end of heating. Process conditions were also evaluated with various steam-air mixtures at 105–123°C and air overpressures at 13.7–41.3 kPa above the steam pressure. Results from this study suggested the computer program could be used to monitor process conditions. No significant difference was observed between results from the computer evaluation of normal and deviant processes and those from recorded time-temperature data of the general method.[20]

7.3.2.6 Cooling

After the sterilization process, the pouch is rapidly cooled to avoid overcooking of its content. The requirement of the cooling cycle needs to be included in the thermal sterilization process. The water to cool the processed containers must be chlorinated to avoid any contamination of the product through the cooling water. The production of foods in these pouches requires identification of a "High Risk" area for handling/packing after autoclaving of the pouches.

7.3.2.7 Detection of defects in pouches

Techniques have been devised to detect defects in heat-seal of retort packaging systems.[21] A study was conducted using a high-frequency acoustic imaging system, namely, the scanning laser acoustic microscope (SLAM), operating at 100 MHz, to detect packaging defects. The purpose of the study was to detect and classify channel defects that would have potential for microbial contamination through visually undetected defects. Channel defects transverse to the heat-seal major axis were fabricated by sandwiching 10, 16, 25, and 37 μm wire between two layers of either polyethylene or plastic retort pouch laminated film, which were then heat sealed. The wire was then pulled out, leaving the channel filled variously with saline solution, air, or both. The channel defects were then assessed using the SLAM and validated with confocal microscopy. The study demonstrated that acoustic microscopy can nondestructively image micrometer-scale channel defects in heat seals.[21]

7.3.3 Reheating before consumption

Retort pouch packaged products, generally require reheating before consumption of the packaged food items. Various parameters need to be considered in developing an ideal reheating system for institutional catering. These include economical form for reheating (bulk in bags, in bulk out of bags, or in portions), equipment for reheating (convection, Infrared [IR], or microwave ovens, compartment steamer, or steam-jacketed kettle), and the influence of storage time. It was observed that microwave heating of food packaged in retort pouches consumed least energy, suggesting its suitability for warming the food prior to consumption. A study revealed that heating of 3 kg of beef stew in retort pouch to 74°C required 18,884 BTU.[22]

7.4 SAFETY AND CRITICAL FACTORS

Similar to canning, there are three essential rules for the safety of retort pouch-processed products, namely, pouch-seal integrity, adequate thermal processes to eliminate the most dangerous and heat-resistant microorganisms including *C. botulinum* spores, and postprocess hygiene. A number of critical factors are involved in the development of retort-pouched products. These include product consistency, maximum filling or drained weight, perfection in sealing, processing time, temperature distribution and control, container orientation, residual head-space gas, processing and racking systems, processing medium, pouch thickness, and the pressure applied.[23] Shelf life of the product depends on the nature of the product, packaging, and heating conditions. Color and flavor deterioration within a few months of storage may be possible. Some of these adverse changes could be obviated by careful control of the process.

7.5 COMPARISON WITH CONVENTIONAL CANNING

As pointed out earlier, retort pouch packaging and canning are comparable in many respects with respect to methodology. Cost of cans accounted for a larger percentage of the raw material cost as compared with the pouch. Retort pouches cost much less than the metal cans.[2] Nevertheless, from an economic point of view, retort pouch system required a larger fixed capital investment than the canning process. Energy costs in processing for both systems contribute very little to the total operating costs. The variable operating costs are higher in the pouch system than in the cans. Increase in product output increases the economic performance of both systems, simultaneously reducing product cost.[24] Capital investment for a retort stand-up pouch operation has been estimated to be nearly $4 million for an annual production of 85 million pouches. It has been pointed out that with distinct advantages associated with retort pouch packaging, the technology has potential to deliver food products in stand-up flexible pouches in the near future.[2] Table 7.3 gives comparisons of retort pouch technology with canning.

Thermal treatment required to achieve sterility of product in pouches takes lesser time than in the case of cans. This is because unlike the pouches, the ratio of surface area to volume is lower in the case of cans, because of which heat has to travel through a longer distance to reach the innermost part to attain the pre-determined temperature for sterilization. As a result, food product near the surface of the can is exposed to heat for longer duration causing appreciable loss of its sensory and nutritional attributes. Thermal loss of quality of food in pouches is less due to lower sterilization time. Figure 7.2. shows heat penetration in a can and a retort pouch of comparable volumes.

7.6 CONSUMER ATTITUDE

Retort pouch-packaged products are attracting increasing consumer interests. The reasons for these include change in lifestyles, lack of sufficient culinary knowledge, desire for products having exotic tastes, better hygienic quality, and convenience in

TABLE 7.3

Comparison of Retort Pouch Technology with Conventional Metallic Canning

Features	Retort pouch	Can
Feasibility	Highly suitable for delicate products such as seafood, sauces	Good for products having tough texture such as beef, pork, etc.
Product development	Slower filling, thermal processing more complex	Convenient production line including filling and thermal processing
Sterilization time	Less	More
Product quality	Superior product quality, with more natural color, flavor, and texture	Intense cooking results in loss of natural sensory attributes
Shelf life	Comparable with canned products	Comparable with retort pouch products
Convenience in handling	Less weight, needs less storage space	More weight, requires more space for storage
Convenience in consumption	Can be easily opened by tearing across the top at a notch in the side seal or by cutting with a scissors	Requires a can opener
Capital investment	Higher capital investment	Medium level of capital investment
Marketing	Trade and consumers need to be familiarized with handling the pouches	Established technology and hence, minimum consumer education needed
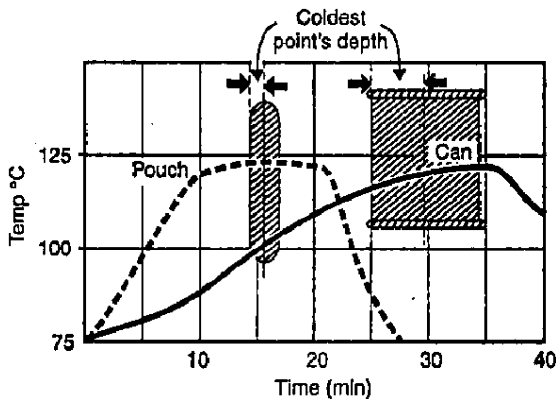
FIGURE 7.2 Heat transfer rates in retort pouch and can of comparable volumes. Source: Rangarao, G.C.P. et al., *Ind. Food Ind.*, 23, 21, 2004. With permission from Association of Food Scientists and Technologists, Mysore, India.

handling.[25] The comparable quality and cost advantages of retort pouch technology with the traditional canning process have also facilitated entry of exotic oriental culinary preparations in pouches in the western supermarket chains. Some of food items that are currently processed in retort pouches include meat balls, chicken and beef stew, meat chunks in curry, meat gravy and rice preparations, spaghetti and meat, beef products, barbecue chicken, and traditional Indian curries, among others.[3,26] An overview of the food products packed in aseptic cartons and retortable plastics developed for the United Kingdom markets has been presented.[27] Availability of these products was as a result of consumer interest in shelf-stable food. In addition, retort pouch technology has significant application in military ration essentially due to possibilities of convenient handling, which include lighter weight, boil-in-bag facility, and easiness in opening.[28]

7.7 RETORT POUCH PACKAGING OF FISHERY PRODUCTS

7.7.1 Basic Considerations

The quality is of foremost importance in selecting fishery products for retort pouch packaging. Fish quality is described in terms of nutritional, microbiological, biochemical, and physiochemical parameters (Chapter 2). However, it has been pointed out that none of these characteristics serve as adequate indices of quality. It was suggested that consumer acceptability must be regarded as an important parameter of seafood quality.[29] The delicate, inbuilt quality parameters of fishery products are too sensitive to withstand the harsh thermal treatments associated with canning. Considering the sensitive quality parameters, development of shelf-stable seafood products would prefer a process milder than the conventional canning. In this respect, retortable pouch packaging is ideal because of the less intense thermal processing required as compared with canning.

In the conventional canning methods, a certain degree of overprocessing occurred in the material close to the walls because of the thermal requirements to ensure that the contents at the center received adequate heat for sterilization. Therefore, often it has been found difficult to make a pleasing canned product from fish species, especially those with a low fat content, because on prolonged high-temperature cooking strong objectionable flavors are produced.[9,30] The pouch, on the other hand, needs much less time-temperature input due to much higher surface to volume ratio, which helps more rapid heat transfer from the pressure vessel into the package interior. The reduced thermal treatment, thus, ensures better product quality including flavor.[31]

In a comparative study, sexually mature chum salmon meat in retort pouches and also in conventional metal cans was processed to equivalent lethality. Evaluation of quality of the product by sensory and instrumental methods showed that the pouch product was firmer, more fibrous, drier, and chewier than their canned counterparts.[32] In another study, physical, chemical, and sensory properties of rainbow trout, pollock, and shrimp processed at equal lethality in cans and retort pouches were compared after identical storage periods. Pouched rainbow

trout, pollock, and shrimp required 34, 32, and 47% less thermal processing time, respectively, than their canned counterparts. The pouched products had a firmer texture and lighter color than the canned ones. In sensory evaluation, the products packaged in retort pouches scored higher in most cases, for color, flavor, and overall acceptability.[32] There was less impairment of texture and flavor due to reduced exposure to heat. These studies tend to establish the advantages of retortable pouch processing for fishery products. Furthermore, the advantages with respect to economics also favor adoption of this technology for seafood.[33]

7.7.2 Process Developments

In developing the retort process for seafood, an understanding of the heat transfer phenomena during thermal processing is foremost for optimization of the retorting conditions. This helps to maintain the required sensory properties for a safe and stable product. The heat transfer aspects have been examined in detail with respect to mussels (*Mytilus edulis*). Farmed-blue mussels were vacuum packed in pouches and pasteurized in an autoclave at 100°C using holding times ranging from 17 to 35 min. The time/temperature data were recorded in the coldest spot of the pouch. This spot was detected by the x-ray picturing of each of the pouches and thermocouples from different angles. Changes in heat transfer due to changes in pressure of retort and vacuum in the pouches were also investigated.[34] A common and serious problem with steam processing of blue crabs is the large temperature differences found within the retort during heat-up and cooling. This could lead to severe overprocessing, quality loss, and low yield. Therefore, a modified process involving evacuation of the retort following back flushing steam as a precook treatment has been developed. This technique greatly reduces the disparity in temperature distribution and also causes about 50% reduction in cooking time.[34]

Development of retort pouch-packaged fishery products including fish paste and fish curry has been reported from the Central Institute of Fisheries Technology, Cochin, India.[33,35] Fish curry is a popular preparation in which pieces of fish such as mackerel and sardine are suspended in gravy of spices. Normally, fish curries have only limited storage life of a day or two at ambient tropical conditions. Freezing or conventional canning is not suitable for the product because of significant loss in quality. Discoloration, desiccation, and rancidity are the common problems during frozen storage, while conventional canning of curry in metallic cans often resulted in an unacceptable flavor being imparted to the curry during storage.[4] Processes for retort pouch packaging of curries from fish species such as sardine, mackerel, herring, and seer have been developed. In a study, 220 g of mackerel curry, packed in retort pouch of $15.5 \times 17 \text{ cm}^2$ size, was heat processed in an overpressure autoclave fitted with a thermocouple. Time-temperature data were collected during heat processing using a data recorder cum cook-value integrator and the heat penetration parameters were calculated. Heat processing to F_0 values of 8.43 and 6.56 gave bacteriologically safe products. However, thermal treatment to give a F_0 of 8.43 was selected for better quality product. Influence of three types of retort pouch packaging on the curry was

Preparation of curry
↓
Filling gravy in the pouch
↓
Filling fish pieces to the gravy in the pouch
↓
Air removal
↓
Sealing
↓
Retort loading
↓
Heat processing
↓
Retort unloading
↓
Cooling and drying
↓
Storage
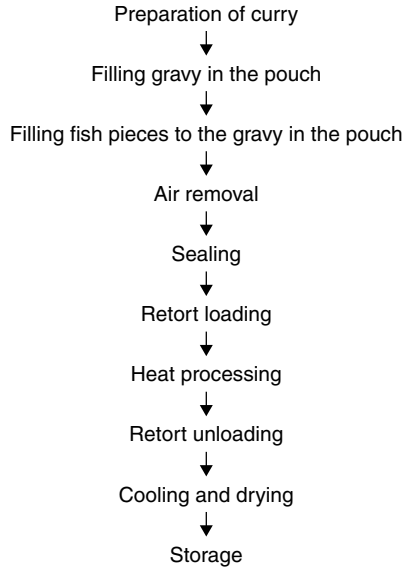
FIGURE 7.3   Flow sheet for the production of fish curries in retort pouches (Adapted from Devadasan, K., *Fish. Chimes (India)*, 20, 44, 2001. With permission)

also examined with respect to the product. The pouches were made of polyester/ aluminum foil/cast polypropylene having varying thickness (μm) of individual layers, namely, 12.5/12/87.5 (pouch A); 12.5/15/70 (pouch B); and 12.5/15/75 (pouch C). Samples stored in all the three pouches remained in good condition after thermal processing and storage for one year. Defects such as pinholes, wrinkles, leakage of fluids, and delamination were not detected. Flex cracks were observed in the aluminum foil component of pouches A and B, but the product remained sterile throughout the storage period at ambient temperature of 25 to 30°C. The thermal process time required was 38.4 min, which included 58% of the come-up time. The treated product had acceptable sensory characteristics, particularly texture. No undesirable off- flavors were imparted to the curry from the packaging material and sensory properties of the product remained acceptable throughout storage up to 12 months.[33,35] The process flow sheet for preparation of fish curry in retort pouch is shown in Figure 7.3.

A process for retort pouch packaging of seer fish (*Scomberomorus guttatus)* curry has been developed. The preparation was packaged in a retort pouch of polyester/aluminum foil/cast polypropylene. An amount of 220 g curry was filled in 17×15.5 cm^2 size pouch, exhausted by steam injection for 5 min, heat sealed, and heat processed at 121°C for 32 min giving F_0 value of 11.5. The curry remained sterile throughout the storage period at ambient temperature (25 to 30°C) and retained acceptable sensory characteristics up to more than one year.[36] A modification of the curry is fish moilee, where pieces of fish are suspended in a gravy consisting of coconut milk and spices. Fried seer fish (*S. guttatus*) (90 g) in 140 g

of moilee medium were packed in retort pouch of size 17×15.5 cm^2. Air inside the pouch was exhausted by steam injection, heat sealed, and processed at 121.1°C to a F_0 value of 8.15, with a total process time of 48.3 min. The product remained sterile throughout the storage period. The samples stored at ambient temperature (27 ± 1°C) were acceptable up to 18 months.[37] Some exotic oriental fish curries in retortable pouches are available in some supermarkets in India. The products include curries made out of sardines, mackerel, pomfret, and seer fish.

Retort pouch is a good packaging system for high-value items such as salmon. During the last two years, retort pouch packed tuna and salmon processed in Thailand or Ecuador have made successful entry in the U.S. market.[2,38,39] Several new salmon products such as flavored roasted fillets, smoked chowder as well as spread, pickled product, salmon pate, croquettes, lunch meat, pasties, low fat burgers and sausages, frozen salmon mornay, smoked salmon spread, and marinated salmon tenderloins have been examined for retort packaging. Salmon flakes were identified to have particular potential for the process.[40] Apart from salmon, other fishery products that have potential for retort pouch packaging include tuna, crab, clams, shrimp, and oyster products.[26]

Development of retort pouches has led to extension of the shelf life of oysters with less severe treatment.[41] Fish ball in curry with varying ratios were packed in pouches and their submergence were studied with different temperatures. An equal amount of fish ball and gravy gave a product in which the fish items were completely submerged. The rate of heat penetration in the preparation was observed to be much faster, in addition to good sensory attributes for the product.[39] Similarly, fish paste has also been processed in retort pouches. Fish paste was prepared by finely grinding textured cooked fish meat with starch, sugar, milk powder, food color, and flavor. It was packed in flexible pouches, heat processed under air/steam pressure in an overpressure autoclave, and stored at ambient temperature. The product was acceptable in terms of texture and spreadability up to 36 weeks. No peroxide formation was noticed during storage up to 36 weeks.[42]

Amenability of *surimi* and kamaboko to retort pouch packaging has been studied. Thermal treatment of *surimi*-based products could result in formation of certain volatile compounds. Methane thiol and dimethylsulfide, apart from hydrogen sulfide, were the major volatile sulfur compounds in retorted kamaboko. The contents of these compounds were less than one twentieth that of hydrogen sulfide. Both the compounds increased with rise in temperature above 80°C. These sulfides along with hydrogen sulfide were generated due to thermal degradation of cysteine and methionine residues of fish proteins. In addition to the sulfur compounds, trimethylamine (TMA) and dimethylamine (DMA) increased with rise in temperature above 100°C.[43]

Fish sausage has been examined for retort sterilization. The treatment can result in pressure increase in the plastic casings containing the sausage and have an impact on the quality. Internal pressure of fish sausage during retort sterilization at 100–120°C was measured with a pressure-measuring instrument equipped with a load-cell pressure sensor. As soon as heating was started, the internal pressure of fish sausage went up higher by 0.4–0.5 kg/cm^2 than the pressure

inside the retort vessel. The pressure difference decreased to 0.1–0.2 kg/cm^2 after reaching the highest pressure, and remained constant. No correlation was found between the internal pressure and the meat-adhesive strength to the inside of casing under the temperature condition of retort sterilization.[44]

7.8 Recent Developments in Packaging

The success of retortable pouch owes to advancements in packaging technology. While conventional pouches are generally pillow style, with fin ends that lie comfortably on their sides, the recently developed stand-up flexible pouches are flexible structures capable of erect positioning on shelves by virtue of a flat base and hence have better ability to display their contents. The bottom is produced by one of the several structures: a separate panel adhered to the body wall, a W-shaped gusset formed from part of the flexible face webs, and a foldable and adhered panel with side gussets to form a block-like shape. The bags have good seal integrity.[38,45] The stand-up flexible pouches are now commercially applied for all classes of food and beverage preservation. Uses of these novel packaging materials involve operations such as thermal sterilization, hot fill, aseptic, retort, liquid, solid pack for chilled, frozen, flowable powders, granules, meat, seafood, produced, and prepared foods.[2,38,45] Fish items that are currently being retorted in stand-up flexible pouches include tuna, salmon, and smoked fish. A boil-in-bag packaging system has also been developed. It consists of an elongated rectangular shaped boil-in-bag package for storing and cooking of food items.[46] The prepared products are shelf stable and have the convenience of frozen boil-in-the-bag products.

In conclusion, the advances in the field of packaging can enhance the prospects of commercial applications of retortable packaging to fishery products, so as to make convenient, RTE fishery products available to consumers.[47] Several countries, which import expensive metal containers, can adopt this technology as alternative to conventional canning process. On a comparison of total costs, including energy, warehousing, and shipping, the pouch looks more favorable. There is a 30 to 40% reduction in processing time compared to cans. Besides cost reduction, the other advantages associated with retort pouch packages, namely, boil-in-bag facility, convenience, ease of opening, reduced weight, and transportation costs, can favor growth of this technology for processing fishery products.

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8 Mince and Mince-Based Products

8.1 Introduction

Recovery of meat, perhaps, is the ideal means of utilizing many underutilized fish species, particularly pelagic fish. Mince separation techniques have also been used for the isolation of meat from fish frames generated during filleting of commercially important species, both of marine and freshwater origin. Fish mince offers immense scope for development of diverse products such as *surimi*-based seafood analogs, sausages, frozen battered and breaded products, dried fish flesh flakes, or powders, mince or soy blends, restructured, dried, and formulated products among others. The type of product prepared from the mince is dictated by the ingenuity of the processor as well as market demands.

8.2 Mince Separation Process

Meat can be separated from most fish species, shellfish such as krill and mollusk, as well as fish frames from the filleting operations. A wide range of meat separation machines is available to suit the raw materials. The principle is forcing the fish against a screened or slotted surface, when the flesh passes through the opening as a finely ground paste. A common type of machine is the belt and drum model. In this, the headed and gutted fish is passed between a counter rotating belt and a perforated drum. The perforated drum of the deboner usually has an orifice size ranging from 3 to 5 mm. Generally a 5 mm orifice is suitable for different species of fish, including whole bony fish. The dimensions of the orifice of the drum influence the quality of the mince, especially with respect to the content of the bones and scales. A smaller orifice may result in higher disintegration of the mince, which adversely affects the texture of the mince from fish such as sardine and mackerel. The fish muscle, fat, and blood are squeezed through the drum, while the bone and skin are retained outside the drum and are scrapped and removed. The operation is schematically presented in Figure 8.1.

Fish that are approximately 20–50 cm long can easily be deboned. Deboning of the whole fish may yield poor quality mince due to contamination by viscera and skin portions as well as the microorganisms from these portions and consequently require refining before use. For better mince quality it is advisable to cut open the fish along the backbone thus allowing removal of the exposed viscera as well as the head. The efficiency of deboning and the quality of the mince obtained depend upon the nature and the initial quality of the fish used. For larger fish, a crusher
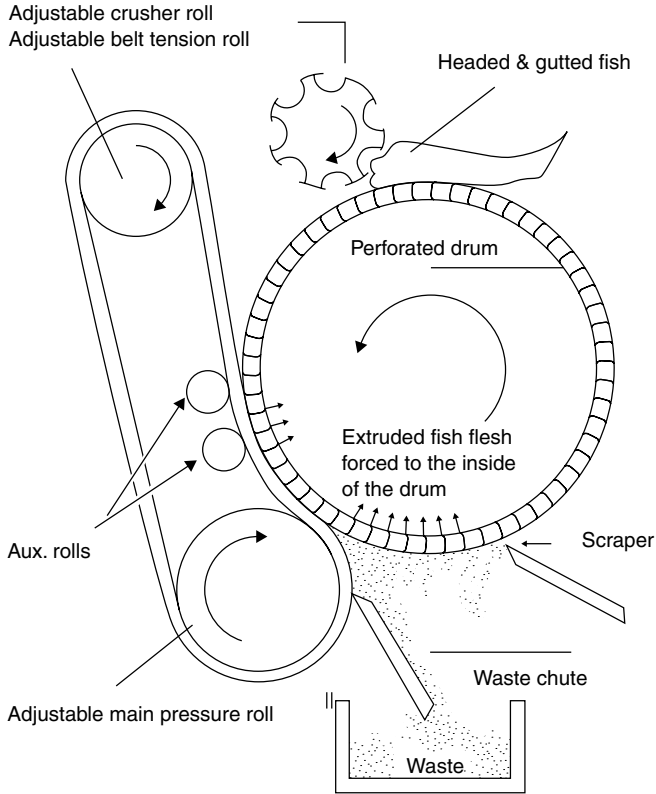
FIGURE 8.1 Schematic diagram of a deboning machine (From Venugopal, V., *Trends Food Sci. Technol.*, 31, 2–5, 1992. With permission from Elsevier)

may be required for size reduction prior to deboning. The commonly employed drum-belt type machines for the mincing operations are Baader 605, Baader 607 and Baader 694. These machines can yield up to 5 tons of minced fish per hour, depending upon the fish used. An average 50–60% (w/w) of the whole fish can be recovered as mince. Another machine used is the stamp type in which fish are compressed against a perforated plate in a continuous operation. The mince separation technology has been discussed by several scientists.[1–5] A new technology of water jet deboning has been reported recently. However, efficiency of this technique was not found comparable to that of the conventional mechanical deboning machine. Thus, when used to recover meat from Pacific whiting filleting frames (which forms 15–17% of the whole weight of the fish), the yield was only 39% in comparison to 43% mince recovery obtained by the conventional deboning process.[6]

8.2.1 Properties of Fish Mince

Mechanically deboned fish meat is more susceptible to quality deterioration during storage than the intact muscle tissue, since the mincing operation causes tissue

disruption and exposure of the flesh to air, which accelerates processes such as lipid oxidation and autolysis. Such reactions adversely affect the color and odor of the product. Lipid oxidation can be a significant problem on the surface layers of frozen blocks of fish muscle mince. The oxidized lipids interact with proteins causing denaturation, drip loss, and changes in the functional properties. Trimethylamine oxide, which is present in appreciable amounts in gadoid fish, undergoes enzymatic cleavage to dimethylamine and formaldehyde, the latter influencing protein denaturation and toughening. The quality of fish mince, as reflected by its color, flavor, and functional properties, is dependent on the species, season of harvest, and handling, as well as the method of processing.[7] The bone content is a quality factor of the mince. A low bone content in the range of 0.1 to 0.4% w/w, dry weight is desired for better functional properties.[3]

The flavor of products prepared from fish mince depends on the contents of free amino acids, nucleotides, volatile compounds of lipid origin, extent of lipid oxidation, and the Maillard reaction products generated during heat processing as also the presence of certain typical compounds such as urea in fish belonging to Elasmobranchs. Proteins serve as the principal functional and structural component of food items developed from mince. Functional properties of interest in fish mince, which influence the quality of specific foods, are water absorption, cohesion–adhesion (simulated meats), emulsification and fat absorption (sausages), flavor binding (simulated meats), viscosity, and solubility (soups). Rheological and flavor properties of fish proteins (which are important in restructured products) depend on their ability to form gel, which in turn, is influenced by intrinsic molecular properties such as size, shape, and conformation, as well as processing conditions such as temperature, pressure, pH, and salt contents. Table 8.1 shows typical functional properties performed by fish muscle structural proteins and their applications.

The mince quality is influenced by the quality of fish used for the process. In order to possess excellent functional properties, mince needs to be prepared from fresh fish. Prolonged ice storage of fish before deboning or frozen storage of the mince prior to secondary processing can adversely affect protein functionality, solubility, cooking loss, drip loss, and texture that adversely affects the quality of the secondary products. Pink perch (*Nemipterus japonicus*) mince processed out of the fish held in ice or frozen prior to mincing suffered significant loss of protein solubility (PS), emulsifying capacity (EC), water binding capacity (WBC), cooking loss, thaw drip, and textural loss.[8–11] Frozen storage of fatty fish such as sardine resulted in lipid oxidation that destabilized the fish mince.[10] Fish caught during and after spawning is high in moisture and relatively low in lipid and protein contents, as compared with those caught during intense feeding periods. These factors adversely influence the mince quality.[3,7]

Mincing results in greater exposure of the meat to microorganisms. The total microbial count is invariably higher in minced meat than in whole fillet due to larger surface area and the higher temperatures reached during mincing. This can cause rapid spoilage of the mince.[12,13] Fish mince from croaker (*Johnius dussumieri*) was examined for changes in the bacteriological profile before and after storage at 4°C. The composition of bacterial flora was found to vary with

TABLE 8.1
Typical Functional Properties Performed by Fish Muscle Structural Proteins

Functional property	Mode of action	Product
Solubility	Protein solvation	Dispersion, soup
Water absorption and binding	Entrapment of water through hydrogen bonding	*Surimi*, *surimi*-based products, sausage, fish balls
Viscosity	Thickening	Gravies, soups
Gelation	Protein matrix formation	*Surimi*, *surimi*-based products, sausage, patties
Cohesion–adhesion	Adhesion	*Surimi*, *surimi*-based products, sausage
Elasticity	Disulfide bonds	*Surimi*, *surimi*-based products, sausage
Emulsification	Formation of fat emulsion fat binding	Sausage, fish balls, soup Sausage, fish balls
Flavor-binding	Adsorption, entrapment, release	Seafood analogs

fresh fish, freshly prepared mince, and spoiled mince. *Acinetobacter* spp. and *Aeromonas* spp., which were dominant in fresh fish, decreased drastically upon mincing, washing, and storage. In fresh mince 71% of the bacterial population comprised of Gram positive species of which *Micrococcus* spp. was predominant. On the other hand, flora of the spoiled mince was dominated by Gram negative species (80%) comprising mainly of *Vibrio* spp., followed by *Pseudomonas* spp.[11]

Quality of the mince can be improved by some preliminary treatment of the fish before deboning. In the case of pelagic fish, removal of dark muscles containing high amounts of hemoprotein pigments and enzymes can greatly improve the mince quality. A process to bleach dark muscle using hydrogen peroxide has been developed. Bleaching is done at an ambient temperature in the presence of a mixture of sodium carbonate and calcium hydroxide to maintain a pH of 10.5 to 11.5. After the treatment, the meat is washed with a solution of sodium sulfite in order to remove excess hydrogen peroxide. The mixture is then washed with an ascorbic acid solution to bring the pH back to neutral.[14] However, a side effect of the process is oxidation of lipids by hydrogen peroxide.

Several studies have been reported on the suitability of different low cost fish as raw material for mince preparation.[3] Gulf of Mexico butterfish (*Peprilus buri*), a largely underutilized fishery resource, has been examined for its application as a food grade marine product. Mince prepared from fish, initially washed in aqueous 0.5% sodium bicarbonate before mincing had lower lipid oxidation and free fatty acid (FFA) content than unwashed mince. The washed mince also exhibited improved color as well as water retention properties. Addition of "Tenox 2"

or vacuum packaging further reduced lipid oxidation and FFA liberation. This also helped prevention of subsequent lipid–protein interactions, as indicated by increased water retention.[15] Proximate compositions and gel forming capacities of the meat mince from twelve low priced tropical fish from India have been determined. The color of the steamed mince cakes from jew fish, bull's eye fish, barracuda and lizardfish was white, whereas it was light gray or yellow in the case of goatfish, sardine, rainbow sardine, thread fin trevally, horse mackerel, catfish, and scad. These minces exhibited satisfactory gel strength, indicating that minces from all the twelve fish species were suitable for secondary products.[16]

8.2.2 Storage Stability

Depending on the type of fish used, mince can have a storage life up to 6 months without any appreciable quality deterioration.[3,17] Mince from Alaska pollock, which is used mostly for *surimi* production, has poor frozen stability, and therefore requires careful handling and storage. Generally minced fish is frozen in 1 to 2 kg blocks at −40°C and then stored at −20°C. Weight loss due to dehydration is a major quality loss during prolonged frozen storage of fish mince. Glazing of mince blocks with chilled water and oxygen impermeable packaging can prevent dehydration on the mince surfaces. However, water glazing may have limitation in controlling dehydration losses in frozen minces. Recently, a protein dispersion prepared by weak acid induced gelation of washed fish meat has been developed as a glaze instead of the conventional water glaze. Such a dispersion having an apparent viscosity of 1 Pa sec prepared from washed mackerel mince has been shown to retard dehydration loss during storage of mince of Indian mackerel (*Rastrelliger kanagurta*) at −17°C. After 40 days of storage, the dehydration loss in dispersion-glazed samples was only 17%, as compared with 35% loss in water-glazed mince (see Chapter 13). Protein glazing also controlled lipid oxidation in the mince during frozen storage.[18] A thermo-mechanical method to measure denaturation and aggregation of proteins in mince based on simultaneous and continuous measurement of torque and temperature has been reported for fish species including whiting, grenadier, horse mackerel, mackerel, and cod. This method can also identify differences in denaturation profiles of fatty and lean fish.[19]

8.2.2.1 Storage temperature

Storage temperature is as important for the stability of frozen mince as whole, dressed fish, fillets, or steaks. Lowering the temperature can significantly enhance the storage stability. Storage of mince and fillets of mackerel (*Scomber scombrus*) at temperatures in the range of −30 to −40°C showed lower FFA formation in all the product forms. Similarly, storage at −30°C gave better quality of white hake (*Urophycis tenuis*) fillets, as judged by sensory and chemical indices. In addition to storage temperature, ascorbic acid accelerated cohesiveness development of mackerel, mince, and fillets.[20]

8.2.2.2   Washing

Washing can enhance storage stability of fish mince. A study on the quality of mince prepared from mackerel, carp, horse mackerel, and Atlantic pollock showed that washing improved frozen stability of proteins, particularly in the presence of sucrose.[21] The bycatch of shrimp trawling can be used as raw material for mince. While washing removed the odor-bearing compounds, loss in protein functionality could be controlled by incorporation of polyphosphate and benzoate during washing.[22]

8.2.2.3   Additives

One of the main problems during frozen storage of mince is the denaturation of the proteins. This may be prevented by incorporation of cryoprotectants such as polyphosphates and other salts, sugars, and hydrocolloids like alginate, carrageenan, carboxymethyl cellulose, guar gum, and xanthan gum.[23] Minced trout, either untreated or treated with additives (3% sorbitol, 0.5% monosodium glutamate (MSG) and 0.02% butyl hydroxyanisole), was found to be acceptable even after storage for one year at either -12, -18, or $-24°C$.[24] Reducing agents, such as cysteine and ascorbic acid, help to preserve the reactive sulfhydryl groups in order to maintain protein functionality. Small amounts ($<2\%$) of herb and spice seasonings such as clove, which have antimicrobial and antioxidant properties, have been recognized to contribute toward stability of fish mince during frozen storage. Other spices such as cinnamon and pepper were less effective. A minimal level of spice extracts has to be used, since higher concentrations can adversely affect sensory properties.[15]

The carbohydrates such as sorbitol and sucrose are used as cryoprotectants to stabilize the proteins thermodynamically through their interaction with the surrounding water. The mechanism of cryoprotection has been explained in terms of the glass transition concept.[25] Polyphosphates function as plasticizers and promote the dissociation of actomyosin. These compounds slowed down decreases in PS, viscosity, and formaldehyde formation during storage at -20 and $-10°C$, the extent of protection being higher at $-20°C$.[26] Incorporation of phosphates up to 5% has shown to improve textural characteristics of mince from pink salmon (*Oncorhynchus gorbuscha*) during storage at $-40°C$ for a period of 4 months. Of the different parameters used, such as punch test, torsion test, texture profile analysis, and water binding ability, torsion test was found to be a reliable test for quality evaluation.[27] Washing with water followed by addition of polyphosphates and salt enhanced the storage stability of frozen-stored minced blue whiting muscle.[26]

Mince can be kept frozen for varied applications. The mince from different fish species could be combined to prepare composite fillets. Foaming machines are used for reshaping large sections of mince blocks, which are initially cut into smaller size and pressed into a single or multiple shape in a single shaping cavity. Keeping the temperature of the process below 3 to 5°C helps to preserve the desired flaky texture. These products can also be used for breading and coating.

8.3 *SURIMI* AND RESTRUCTURED PRODUCTS

Surimi processing and *surimi* seafood represents a vast field for value addition of fish mince. *Surimi* is the Japanese name for washed, preserved fish meat, which because of its characteristic ability to form gels, can be used to develop products that simulate the appearance, flavor, and texture of expensive products such as lobster tail, shrimp, scallop, and crab legs. Fresh *surimi*-based products have been familiar to the Japanese for many centuries as kamaboko, chikuwa, and satsuma-age, which differed in shape and preparation and were steamed, baked, or fried. *Surimi* can also be used in other products such as sausages.

8.3.1 The *Surimi* Process

Before 1960, *surimi* was manufactured and used within a few days as chilled raw material because the product was unstable to frozen storage. With the discovery of cryoprotectants, mainly sucrose and sorbitol, the *surimi* industry was able to produce frozen *surimi* with appreciable storage life. The modern *surimi* production began in Japan in the late 1960s. The first step in the process involves washing the fish mince in chilled water to remove water soluble and odor bearing compounds consisting of enzymes, sarcoplasmic proteins, blood, inorganic salts, and some lipids that enhances the gel forming ability of the washed proteins. The efficiency of the washing process in removing water-soluble components depends on a number of factors including the number of washes, the ratio of mince to water, the washing time, agitation time, and the temperature of water. Usually, washing of mince is done in cold water at a mince to water ratio of 1:2. Generally, a three-step washing process of the mince affords good-colored products, with improved frozen stability and negligible odor. The washing process helps concentration of myofibrillar proteins, which are primarily responsible for textural quality of *surimi* through formation of gel. There is some swelling of the washed muscle as a result of hydration of the proteins. The reason for swelling of the proteins has been explored. At the pH of the washed mince (about 6.4–7.0) the proteins have a net negative charge and repel each other. The repulsion allows for expansion and water is absorbed to minimize the repulsion by separating the proteins. The addition of a small amount of salt (0.1–0.3%) shields the negative charges, reduces the repulsive forces, and prevents swelling.[28]

The washed mince is generally refined by straining through a screen having an orifice size of 1 to 2 mm in diameter to remove ligaments, scales, and bones. After straining, the preparation is pressed in a screw press to reduce its water content. The *surimi*, thus prepared, has a high concentration of myofibrillar proteins, and generally contains 75% water, 16% protein, and 1% fat. The washed mince is mixed with cryoprotectants, which helps retain water-holding capacity and prevents drip loss storage. A generally used composition of cryoprotectants is 4% sugar, 4% sorbitol, and 0.3% polyphosphates. After mixing with these additives, *surimi* is extruded into metal trays lined with polyethylene bags and then kept frozen. The relevant technology for *surimi* production has been discussed by several scientists.[28–34]

8.3.2 *Surimi* from Alaska Pollock

Alaska pollock (*Theragra chalcogramma*) (World landing in 2002, close to 2.6 mt) has been the fish widely used for *surimi* making. Because of its soft muscle tissue it is not necessarily suitable for other fish products. However, its low fat content and white flesh quality favors its use as raw material for *surimi* having acceptable color and textural properties.[29] The process of *surimi* production from Alaska pollock is shown in Figure 8.2. Fresh Alaska pollock, which is caught by mid-water and bottom trawlers, is ideal for best quality product. After harvest, the temperature of the fish should be kept just above the freezing point prior to processing, by storing it in crushed ice or in refrigerated seawater. The fish is headed, gutted, the viscera and a major portion of the backbone are removed, followed by filleting with a filleting machine. Generally, the recovery of fillets after this stage accounts for about 60% of the raw material, on wet weight basis. The fillets are deboned to give a mince with an overall yield of 47%. The mince is subjected to washing in three or four separate tanks each filled with cold (<10°C)
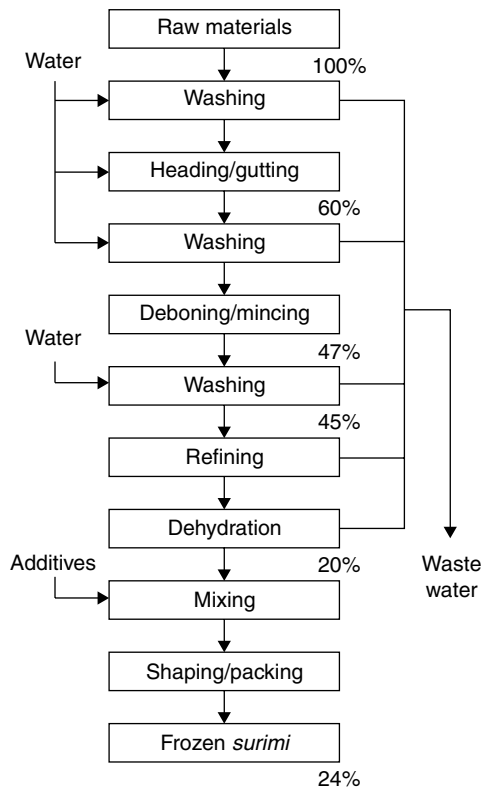
FIGURE 8.2 *Surimi* process from Alaska pollock (From Ohshima, T., Suzuki, T., and Koizumi, C., *Trends Food Sci. Technol.*, 4, 157–162, 1993. With permission from Elsevier)

freshwater. The washing process removes most sarcoplasmic proteins, enzymes, blood, and soluble nitrogenous compounds. Gentle stirring for 9–12 min with 3–4 repeated washings in separate tanks are commonly employed. The washed meat is passed through a refiner to remove any remaining small bones, skin tissues, dark muscle tissues, and scales. The refiner is a high-speed rotary spiral surrounded by a screen with many small pores of 1.2–3.2 mm in diameter. Excess water in the washed mince is removed using a screw press, to reduce the moisture content of the mince to 90%. It is then mixed well with 4% sucrose or 4–5% sorbitol in addition to 0.2–0.3% polyphosphate, as cryoprotectants. Sucrose may impart a sweet taste and hence sorbitol is preferred. The *surimi* is then packed in a freezing pan and frozen quickly to below −5°C using a contact freezer.[25] In the United States, the Alaska Fisheries Development Foundation has made a detailed project report on *surimi* processing from the Alaska pollock. The yields of mince and *surimi* from the fish were 47 and 24%, respectively, based on the weight of whole fish.[36] Since a large volume of wash water is consumed in the process, it is important that a modern *surimi* plant should have facility to treat the wash water both for the recovery of soluble nutrients and control of environmental pollution.[36]

8.3.3 Other Raw Materials

A decline in the landing of Alaska pollock has led to studies on the feasibility of using other species for *surimi* production. About 60 different species from tropical and cold waters have been examined in this context. These include hoki, threadfin bream, croaker, blue whiting, Pacific whiting, Atlantic cod, menhaden, sardine, mackerel, lizardfish, eel, barracuda, leather jacket, and capelin among others.[7,30,37,38] Scaling up studies on *surimi* production have been reported in the case of fatty fish such as mackerel.[38] Fish species having commercial potential for *surimi* include hoki, Atlantic cod, croaker, menhaden, jack mackerel, threadfin bream, and Pacific whiting.[39,40] Some subtropical bycatch species amenable for *surimi* processing are lizardfish, goatfish, catfish, and threadfin bream.[34] The Marine Fisheries Research Department of the Southeast Asian Fisheries Development Center has developed a method for producing *surimi* from low priced fish such as coral fish, dorab, bigeye snapper, threadfin bream, lizardfish, glassfish, small snapper, and croaker, which are trawled in the Southeast Pacific and Indian oceans.[25]

Irrespective of the nature of the fish used, the quality of *surimi* could be improved by using fresh fish and subjecting them to early heading and evisceration, since the spoilage rate is greater in whole fish.[36] While ice storage up to 3 days has negligible effect on *surimi* quality, prolonged storage may not be advisable because of the possible spoilage including degradation of the muscle by autolytic enzymes. This has been verified in the case of two species of bigeye snapper (*Priacanthus* spp.).[41] Nevertheless, in certain fish species, such as hoki (*Macruronus novaezelandiae*), a few days of ice storage may not affect *surimi* quality. This allows a longer duration of fishing voyage and convenient offshore

preparation of the product. Washed and unwashed hoki minces were prepared from ice-stored hoki. It was observed that even after 10 days, the strength of gels made from the fish minces still compared favorably with gels made from other commercial fish species processed without ice storage.[42] However, the influence of seasonal variations in the gel forming capacity of fish muscle has been studied.[43]

The technical problems associated with the dark flesh fish species are high lipid content, lipid hydrolysis, large portions of dark muscle, water soluble proteins, and rapid protein deterioration, which give rise to poor quality *surimi*, characterized by fishy odor and unappealing color.[38,44,45] These problems may be overcome by removing the dark meat, washing with bicarbonate solutions, and using an improved leaching process.[34] Lipid oxidation in fatty fish during *surimi* production can be controlled by vacuum grinding, use of nitrogen-purged water for washing, as well as cryoprotectants such as sugars and polyphosphates, and antioxidants, such as ascorbic acid, citric acid, erythorbic acid, and polyphosphates. Color could be improved using whitening agents such as titanium oxide.[35] *Surimi* prepared from mackerel by the process remained acceptable when stored at $-20°C$ up to one year.[45] Recovery and yields of minces from several tropical pelagic fish species including silver belly, mackerel, and sardine-like species have been reported.[30,46]

Freshwater fish species have been examined for *surimi* production (see Chapter 12). Gel strength, compressibility and folding characteristics of gels prepared from three Indian freshwater fish, namely, rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) have been studied. Rohu showed good gel strength after setting at 50°C, whereas catla and mrigal required heating to 60°C for optimal compressibility and folding characteristics.[47] The result, however, is not in concurrence with another study that found poor gel strength and setting of *surimi* prepared from freshwater species including common carp, grass carp, and silver carp as compared with that of Alaska pollock.[48] Further, while *surimi* from marine fish species is traditionally used for the Japanese preparation known as "kamaboko"; the product prepared from silver carp may not be acceptable to the Japanese essentially because of its odor.[49]

Fish frames and trimmings from filleting operations, which contain appreciable amounts of meat, is also a source of raw material for the *surimi* industry.[6] In some instances, it is possible to recover up to 60% of minced meat from the filleted frames. A study on the quality of meat obtained by deboning whole fish carcasses and parts of carcasses (belly flaps, collar bones, back bones, with or without swim bladder and kidney, crushed heads) and cuttings such as pin bone and collar bone cuts of *Pollachius virens* has been conducted. The meat obtained using Baader 694 bone separator (perforation of drum 5 mm) from these portions was evaluated in terms of bacterial quality, total volatile basic nitrogen, total nitrogen, moisture content, and hydroxyproline. It was observed that the mince collected from meat of pin bone cuts was acceptable for human consumption. The minces from belly flaps and collarbones were of inferior quality.[50] Filleting of aquacultured fish generates a significant amount of frames and trimmings, which is a source of meat, although currently such recovered meat is not used for direct human consumption.

Manufacturing high value products such as *surimi* and *surimi*-based products from processing discards of aquacultured fish could be worth examining.[51]

8.3.3.1 Modification of the process to suit fish species

Characteristics of *surimi* are its white color, bland taste, ability to form gel, appreciable emulsification capacity as well as water- and fat-holding capacities. Depending upon the fish species used, the *surimi* process has to be modified to provide the required *surimi* quality.[25] These modifications are intended to:

1. Ensure impurities like bone or skin are not introduced in the product during the mincing process
2. Improve the color
3. Achieve required moisture in the product
4. Provide required texture and gel strength

Contamination of the mince with impurities can be reduced using drums of deboning machines having suitable perforation sizes. Washing conditions may need modifications to suit the raw material depending upon its composition. Since excess of water can cause loss of water soluble proteins and reduce the yield, optimum water needs to be used. On the other hand, too little water can leave protein destroying enzymes in the meat. Washing conditions (temperature, 2–18°C), duration (1–7 min each cycle), and water mince ratio (2:1 to 8:1) have been shown to influence the quality attributes of fish. Presently, in order to optimize the washing process, the screw press and the centrifuge have been combined into one, called the decanter, where the screw conveyer is surrounded by a spinning steel bowl or centrifuge. The decanter allows processors to achieve a much higher yield than production lines that use separate screw press. The mince and water are pumped into the bowl, which spins to create centrifugal force, pushing the solids to the periphery of the bowl and leaving the water in the center. Whereas separate conventional operations of centrifugation and pressing in a screw press give a mince yield around 55%, the yield from the decanter can be as high as 70%. The color of *surimi* could be a limiting factor particularly when fatty fish are used as raw material. Therefore, whitening agents have been employed for color improvement. One such compound is titanium dioxide, which in small quantities enhances the color of *surimi* from big eye snapper and mackerel without affecting the gel forming ability.[35]

8.3.4 Gelation of *Surimi*

The most important quality parameter of *surimi* is its gel forming ability, since this property dictates the versatility of *surimi* to give the required texture in diverse products. Gel formation is essential for proper binding of ingredients required to

modify the textural attributes. The process occurs rapidly when *surimi* is heated at 80–90°C, but also takes place moderately at 40–50°C, and slowly when held 0°C. At 0°C it takes several hours for completion of gelation. *Surimi* paste that has initially been set (setting is denoted as "suwari" in Japanese) at 40–50°C gives a stronger gel, if subsequently heated to 80–90°C. However, at 60–70°C, the gel softens, a phenomenon, termed as "modori" in Japanese.

A gel is an intermediate between solid and liquid in which strands of chains of proteins are cross-linked to form a continuous three-dimensional network. Actomyosin is the major muscle protein responsible for gel formation, which consists of a complex of long filaments of actin, tropomyosin, and troponin in combination with myosin. A myosin molecule is a long rod with two globular heads at one end, which has a total length of 155 to 160 nm. The tail portion of myosin comprises of two polypeptide chains in the form of a α-helical coil, which constitutes about 70% of the total α-helix. Because of their chemistry, the myosins are able to form a wider range of cross-links to form gels having different characteristics. For production of gels, the important properties of these proteins are their flexibility, including their ability to denature and give extended chains, and the ability to form extensive networks by cross-links. The gel formation process involves partial denaturation of the proteins and some loss of their α-helix structure, subsequently followed by aggregation of the proteins through intermolecular associations. Such gels are opaque and are usually made by heating. As the temperature increases, the molecules partly unfold, exposing hidden reactive groups, which then react at intermolecular levels to form a continuous network. The aggregation phenomenon is through hydrogen bonds, electrostatic bonds, and hydrophobic and covalent (disulfide) interactions.[52,53] In food processing operations, partial denaturation is achieved by mild heating, that causes the unfolding of α-helices in the tail portion of myosin molecules. It must be noted that the control of temperature is important since high temperatures fully denatures the proteins, which in the absence of as α-helices are unable to aggregate. During the association step of the partially denatured proteins, water, oil, and flavoring compounds can be entrapped in the gel matrix. These are held together by hydrophobic and hydrogen bonds. Rigidity of the gel can be suitably changed by incorporating ingredients such as salt, starch, polyphosphate, or proteins from other sources into the gel matrix.

Optimum gel formation in *surimi* is assisted by 2 to 3% sodium chloride, which enhances water uptake and protein solubility. The salt improves the binding ability of proteins by increasing the amount of salt-extractable proteins as well as altering the ionic strength and pH of the medium, facilitating the formation of a coherent three-dimensional structure during the process of heating the mince. Polyphosphate increases the binding of meat particles in *surimi*. When the fish mince is mixed in the presence of sodium chloride and polyphosphate, a sticky exudate is formed on the surface of the mince that acts as cement, particularly during cooking. There is also strong evidence that myofibrils swell in the presence of sodium chloride and the polyphosphate can reduce the quantity of sodium chloride for the process by nearly half.[30]

In commercial practice, the thawed *surimi* blocks are ground in the presence of small amounts of common salt that results in a viscous paste in which myosin and actomyosin are dispersed homogeneously. The paste sets into a semitransparent gel after mild heating or even prolonged storage. The extent of setting differs among warm and cold water fish species. In the case of Alaska pollock, the setting reaction occurs within 2 to 4 h at 25°C, while it requires 24 h to complete at 0°C. The setting phenomenon in warm water fish mince takes place at higher temperatures and may be negligible under refrigerated conditions.[53] The strength of the set gel differs depending upon the fish species. Of the white-flesh varieties, croaker, barracuda, threadfin bream, lizardfish, cutlass fish, stripped mullet, leather jacket, sea bream, frigate mackerel, puffer, and red big eye give surimi having appreciable gel strength. Among the dark flesh species, Pacific blue marlin has the highest gel forming capacity, followed by flying fish, dolphin fish, scad, horse mackerel, yellow fin tuna, Pacific mackerel, and skip jack tuna. Among sharks, dog shark (*Scoliodon walbeehmi*) has good ability to form gel.[37]

8.3.4.1   Modification of gel strength using other food additives

Use of cryoprotectants such as sugars and polyphosphates help to induce protein–carbohydrate interactions favoring stabilization of the mince during frozen storage.[54] The gel forming capacity and specific temperature requirements for setting and functionality of the gel could also be modified using certain other food additives in small concentrations. Some of them include soybean, wheat gluten, porcine plasma protein, chitin or chitosan, hydroxypropyl methylcellulose (HPMC), curdlan, gellan gum, xanthan gum, carrageenan and pregelatinized starch, locus bean gum, etc. either alone or in combination.[32,55–60] Strong elastic gels are produced from unmodified starches like potato and wheat starches. But gels prepared with these unmodified starches have poor freeze-thaw stability and turn rubbery and rigid on extended frozen storage. Modified starches are seen to improve freeze-thaw stability, but gives gels of poor elasticity and firmness.[30] Hence, a combination of half-modified starch along with egg white has been recommended to produce desirable balance between gel strength and freeze-thaw stability.[29] NADPH-dependent sulfite reductase could effectively protect sulfhydryl groups of native myosin enhancing the gelation of *surimi*. Other protective agents were recombinant chicken cystatin and transglutaminase.[59] Polysaccharides play a key role in influencing food texture through their interaction with food components. Thickening, gelling, and the general hydrocolloid action are the most important widely used properties of polysaccharides in the development of food products.[61] Carrageenan is a linear polysaccharide derived from various species of red seaweed and is commonly used in the food industry for thickening and gelation. It consists mainly of the potassium, sodium, magnesium, and calcium sulfate esters of galactose and 3,6-anhydrogalactose copolymers. The hydrocolloid is available in three basic forms, *kappa* carrageenan, which gives a strong and brittle gel; *iota* carrageenan giving a soft and elastic gel, and lambda carrageenan, which adds viscosity and body to the product. A wide range of fancy fish products

have been developed incorporating various carrageenans in mince from fish such as salmon, tuna, and pollock.[62] Other additives useful for enhancement of gel strength include sulfhydryl blocking agents and calcium chloride.[63,64] The quality of *surimi* quality from Atlantic croaker and Northern kingfish was improved by small amounts of additives such as calcium chloride.[64]

The gel strength of *surimi* is affected by prolonged frozen storage.[65] Effects of various freezing methods on the biochemical and physical properties on Pacific whiting *surimi* showed that stress values increased up to 3 months and then decreased, whereas strain values significantly decreased over time. At 6 and 9 months of storage, proteolytic degradation was also noticed.[66] The limitations of the *surimi* from certain fish species could be removed by mixing it with the *surimi* of other fish to improve texture and color. For example, *surimi* from hairtail fish at 3 to 18% could be mixed with Alaska pollock *surimi* to get optimal gel properties.[67]

8.3.4.2 Influence of muscle proteases on gel strength

In *surimi* from some species there is a softening of the gel (termed "modori") during setting at temperatures in the range of 40 to 60°C. The softening of the gel is caused by heat resistant proteases such as cysteine endoproteases acting on the muscle proteins, particularly the heavy chain of myosin.[68] The proteolysis of myofibrillar proteins is a factor that adversely affects utilization of fish such as arrowroot flounder, pink salmon, and cephalopods for *surimi* preparation.[68,69] Figure 8.3
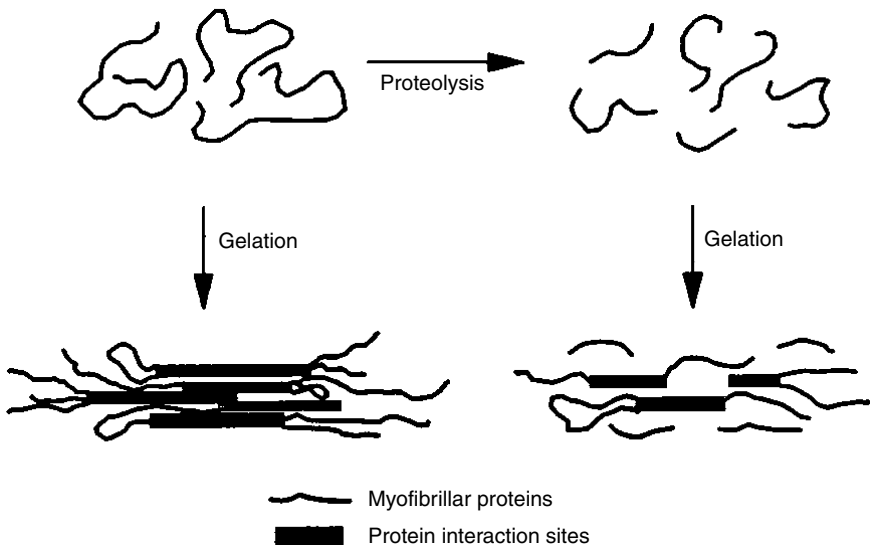
FIGURE 8.3 *Surimi* gelation and proteolysis of myofibrillar protein (Reprinted from An, H. Peters, M.Y., and Seymour, T.A., *Trends Food Sci. Technol.*, 7, 321, 1996. With permission from Elsevier)

depicts influence of proteolysis of myofibrillar proteins on *surimi* gelation. The problem of endogenous protease is indicated by the fact that when fish such as arrowroot flounder is cooked, it disintegrates into a mass of small particles as a result of proteolysis of the muscle proteins. In order to circumvent the problem acids such as vinegar or citric has to be injected into the muscle to reduce the severity of the proteases to get intact cooked fillets.[2] The problem of proteolysis in *surimi* and the associated softening of the gel can be reduced or prevented by the use of protease inhibitors.[70] Some of the protease inhibitors include beef plasma protein, egg white protein, whey proteins, and extracts of white potatoes; the most commonly used food grade inhibitors being beef plasma protein, egg white, and potato powder.[71–73] Cystein-protease inhibitor or bovine plasma powder has been successfully used for control of "modori" phenomenon in cephalopods.[74,75] Serine proteinase inhibitors from crude extracts of legume seeds including kidney bean, pea, chickpea, lentil, and soybean could be used to inhibit proteolytic degradation of myosin and to retain gel strength in *surimi* from Mexican flounder and Atlantic croaker.[59]

8.3.5 Assessment of *Surimi* Quality

The quality of *surimi* is determined in terms of its gel strength, water content, and color.[36,76] Gel strength of *surimi* is measured usually in terms of two parameters — the compression response (punch) test and the folding test. In the compression response analysis, the quality of *surimi* is determined by a standard method that involves producing a cooked gel for rheological testing. The gel is usually made in the form of a cylinder, 1 to 1.5″ in diameter, and is cooked in a water bath. A round-tipped plunger of 5 mm size in diameter is forced through the sample at constant velocity, when the equipment monitors the force on the plunger. The output data indicate the force exerted on the sample when failure occurs (stress at failure) and displacement (distance) of the plunger into the *surimi* when strain occurs (strain at failure). Many different instruments are available for measuring gel strength, the most common being the Instron Universal Testing Machine. This instrument measures force versus displacement (under either extension or compression).[53] In the folding test, disks of 3 mm are sliced from the *surimi* gel. The disks are folded into quadrants. The *surimi* is graded as 'AA', 'A', 'B', 'C' and 'D' on the basis of the cracks that form as the disk is folded. 'AA' Grade *surimi* is the one that does not crack after folding twice and 'D' grade *surimi* breaks by finger pressing even before folding. While behavior to large stress at failure is comparatively easy to study, the data are subject to variations depending upon the conditions of measurements. On the other hand, dynamic measurements involving small deformations under either a constant or a sinusoidal oscillating stress give more reliable information on viscoelastic characteristics of the gel. These measurements have been widely used to study the heat-induced gelation of fish muscle proteins.[53,75,76]

Other quality parameters for *surimi* are water content and color[36]. Water content of *surimi* is measured usually with an infrared moisture meter. The color of

surimi is important for its acceptability. The color is determined with a reflectance colorimeter on a Hunter scale, where, whiteness is reported as the *L* values in the "*L, a, b*" system. The "*L*" scale describes whiteness or blackness, the "*a*" scale describes red or green and "*b*" describes yellow or blue. Microbial quality of *surimi* is another parameter for its acceptability, since it is a good growth medium for microorganisms. It is important that care should be taken to reduce microbial contamination and their growth during *surimi* manufacture and during subsequent manufacture of products from *surimi*.[40]

More than 50 different fish species have been compared for gelling potential and setting rate of pastes prepared from their muscle. Based on the quality, ideal species for *surimi* production included black marlin, croaker, barracuda, threadfin bream, flying fish, bighead, dolphin fish, lizardfish, and cutlass fish. Species that met minimum quality standards included little tunny, carp, snakehead, and sardine.[77] Table 8.2 shows the quality of *surimi* prepared from several fish species.

TABLE 8.2
Quality of *Surimi* from Various Fish and Shellfish Species

Species	Very good	Good	Poor
		Surimi quality	
Marine	Alaska pollock	Barracuda	Angel fish
	Black marlin	Bonito	Anchovies
	Cod	Carrangids	Big-eye tuna
	Croakers	Eel	Flounder
	Flying fish	Pomfret	Lantern fish
	Hammerhead shark	Rays	Mackerel
	Horse mackerel	Ribbon fish	Rock fish
	Lizardfish	Sciaenids	Silver belly
	Pacific hake	Seer	Spiny dogfish
	Perches	Yellowfin tuna	Trout
	Puffer fish		
	Shads		
	Threadfin bream		
Freshwater	None	Carp	Snake head
		Tilapia	
		Mullet	
Shellfish	None	Cuttlefish, squid	Prawns, krill

Source: Adapted from Gopakumar, K., *Tropical Fishery Products*. Oxford and IBH Publishing Co., New Delhi, India, 1997; and Putro, S. Technical Handbook. 2. Processing of *surimi* and fish jelly products, *Infofish*, Malaysia, 1989.

8.3.6 Production of Seafood Analogs

Several value-added seafood analogs can be made from *surimi*. These include imitation shrimp lobster tails breaded scallops, imitation breaded crab claws, *sushi* products, *sushi* sticks, imitation crab shreds, minced sticks, filament sticks, and others. These seafood analogs possess the accepted texture, flavor, and appearance of the authentic products. Table 8.3 indicates a typical formulation of shrimp analog. Preparation of *surimi*-based imitation crab legs is outlined in Figure 8.4. For production of seafood analogs, the *surimi* blocks are chopped to create a paste. The paste is combined usually with additional amounts of cryprotectants and other additives such as salt, soy protein, starch, egg white, alginate, etc. to promote cohesion among the protein molecules and thereby to improve the texture and flavor of the finished product. For chopping the frozen blocks, it is ideal to use a vacuum mixer, which helps to disintegrate the mince and make the proteins available for binding with the ingredients. A vacuum mixer also removes any air that could be introduced in the chopped product, which can result in uneven heating during cooking. The chopped paste is extruded as a flat sheet (approximately 1–2 mm thick) molded into desired shapes and set by placing on a cooking belt where it is heated. Heating is done at 90–93°C for 30–100 sec on a stainless-steel belt, drum. Final texture is developed during thermal pasteurization, which is performed after bundling, cutting, and packaging.[32] The pasteurization step eliminates bacterial pathogens that might grow during the storage of the product. Generally, *surimi* seafood should be cooled from 60 to 21.1°C or below within 2 h and to 4.4°C or below within the next 4 h and should be held at 4.4°C or below at all times during storage and distribution. Figure 8.5 shows some of the commercial seafood analogs prepared from *surimi*. A patented process for production of shrimp

TABLE 8.3
A Typical Formulation of Shrimp Analog

Ingredient	Amount (g)
Surimi	1000
Salt (NaCl)	25
Ice water	150
Sugar (sucrose)	50
Potato starch	70
Shrimp extract	20
Monosodium glutamate	5
Shrimp flavor	2
Crab flavor	2
Glycine	1
Sodium succinate	0.3
Color	As needed

Surimi
↓
Mixing of ingredients
(*surimi* base, water, wheat starch, vegetable oil,
egg white, flavors, natural colorants) to a smooth paste
↓
Pumping, extrusion and cooking
↓
Drying
↓
Rolling and coloration
↓
Film wrapping
↓
Cutting
↓
Vacuum packaging
↓
Pasteurization
↓
Chilling
↓
Boxing
↓
Storage at 0/+4°C/−18°C
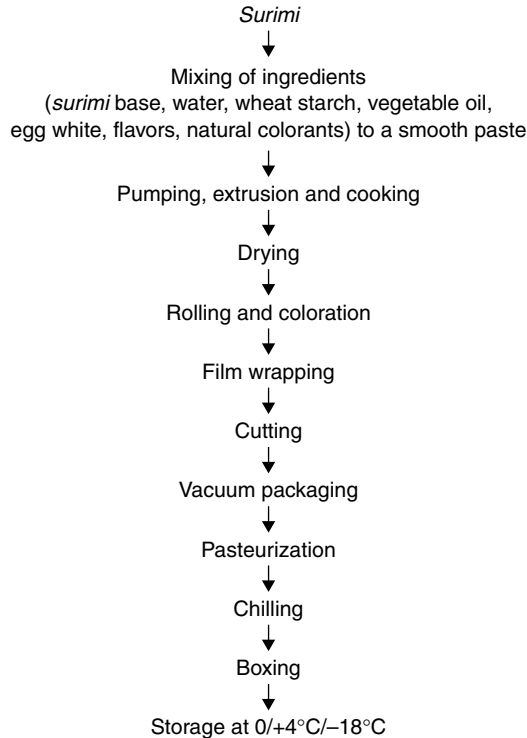
FIGURE 8.4 Flowchart for *surimi* crab leg production (Courtesy: *VICIUNAI*, Senamiescio a.7, LT_5640, Plunge, Lithuania)

analogs consists of mixing *surimi* with glucomannan, carrageenan, protein, and starch, extruding the mixture and cutting the extruded material to suitable sizes, and heating the pieces in hot water bath followed by chilling.[78,79] A typical *surimi* factory is shown in Figure 8.6.

The various ingredients allow modification of the extent of gelation, and hence the texture of the analog products. Thus, the compressive force (cohesiveness) and penetration or shear force (firmness) of molded (shrimp type) and fiberized (crab leg type) products decreased with increasing concentration of cellulose in the *surimi*. It also provided a better texture in products after freeze-thaw cycles.[80] The gel strength and sensory acceptability of seafood analogs also depend upon the storage temperature as well as duration of storage. In a study, crab leg and scallop adductor analogs were frozen and stored at various temperatures (−10 to −35°C) for 2 months. The quality of the sample was evaluated in terms of jelly strength, free drip, and sensory analysis. The jelly strength and the content of the free drip increased with the storage time at each storage temperature, ranging from −35 to −10°C; the adverse effects being more at higher temperature. The products had

FIGURE 8.5 Seafood analogs from *surimi* (Courtesy: *VICIUNAI*, Senamiescio a.7, LT_5640, Plunge, Lithuania)

a shelf life of one month at $-20°C$.[81] Sensory evaluation showed that baked and microwave oven heated imitation crab legs were softer than unheated controls.[82]

Instead of conventional steaming, alternate heating methods such as ohmic and microwave heating have been experimented with to improve the quality of seafood analogs. The advantages of these methods are that they are rapid and uniform, and avoid the use of hot transfer surfaces, thus allowing accurate temperature control of the product. Ohmic heating easily denatures proteinases before myosin degradation occurs, protecting both the myosin and the actin in the *surimi* gels to give better product quality. Ohmic heating of Pacific whiting *surimi* gel gave shear stress and

FIGURE 8.6 *Surimi* processing factory (Courtesy: *VICIUNAI*, Senamiescio a.7, LT_5640, Plunge, Lithuania)

shear strain that were more than twofold of those of conventionally heated gels.[68] A new method for cooking gel has been developed, which uses a patented microwave reactor that assures a uniform energy distribution during cooking. This helps better prediction of performance of a *surimi* batch on-line and evaluation of ingredients in influencing *surimi* quality.[83] The microwave heating, similar to ohmic heating, allows rapid inactivation of proteolytic activity to improve the textural properties of arrowtooth flounder fillets.[68] Ohmic heating influenced electrical conductivity of Pacific whiting (*Merluccius productus*) *surimi* having varying moisture contents.[84]

While thermal pasteurization of the packaged products is the conventional process, recently, like other processed-food industries, the *surimi* industry has also shown interest in postprocess nonthermal pasteurization technologies that would ensure microbial food safety without compromising the rheological and nutritional properties.[85] Electron beam pasteurization at a dose of 5 to 8 kGy has been reported recently for the pasteurization of *surimi*. The treatment resulted in an increase in the shear stress of *surimi* gel; however at higher radiation doses, the gel strength decreased. There were some changes in the myosin heavy chain in both uncooked and cooked *surimi* gels, and such changes were lower when frozen *surimi* was treated. Protein analysis suggested that the firmness of *surimi* gel could be enhanced by hydrophobic interaction during electron beam irradiation.[86] An entire *surimi* plant capable of processing 3000 pounds *surimi* into imitation crab meat, lobster tail or imitation shrimp per h line can cost up to US$ 1 million.[79,87] To enhance the safety of the product, application of the Hazard Analysis Critical Control Points (HACCP) protocol with respect to *surimi* manufacture has been advocated.[40]

8.3.7 Other Uses of *Surimi*

Several kneaded jelly-type products such as kamaboko from *surimi* are highly popular in Japan. These products are prepared by grinding the washed mince in the presence of salt, sugar, and possibly glutamate, alginate, and potato and corn starch for 30–40 min. The paste is then spread on a wooden plate made usually from pine or cedar wood. It is then steamed for 20–90 min to attain an inner core temperature of 75°C. The treated mince undergoes setting at an ambient temperature, resulting in a translucent product that maintains its shape. Whereas traditional kamaboko is processed by steaming, broiled, and fried *surimi* products are called chikuwa and tempura, respectively. Kamaboko accounts for over 60% of the fish paste market in Japan. *Surimi* produced from croaker, shark, or threadfin bream enjoys good demand for the manufacture of best quality kamaboko. Products may also be shaped into fish balls and related products, which are set by soaking in water prior to cooking in order to prevent their shape changing in the air. Kamaboko is generally prepared from marine fish since the products from freshwater species have poor acceptability.[49] Ice storage and washing of the mince prior to processing kamaboko influence the yield and sensory quality of the product. By washing the sardine mince three times decreased the yield from 27 to 21%, although the treatment removed 80% of the lipid and improved the texture and color of the prepared kamaboko.[88]

Surimi can be also integrated into poultry and meat products for replacement of fat, and also acts as an emulsifier and binder. The emulsifying and binding properties of *surimi* help to develop novel products from meat, having lesser fat, fewer calories, and less sodium, while the protein content of the product will be comparable with the natural meat products. These products range from 100% *surimi*-based "meat block," to *surimi*-meat blends. Ingredients in these products include natural beef flavor, hydrolyzed soy protein, starches, carrageenan, sodium caseinate, and other additives. A popular product, "Spicy Bites," a battered and breaded cured pork and *surimi* nugget contains 15% Alaska pollock *surimi*. Usually, *surimi* from the Alaska pollock, silver and red hake, and Atlantic cod are used in these products. Other items include *surimi*-incorporated poultry products, beef steaks, and frankfurters, in addition to *surimi*-incorporated pastas and protein drinks.[36] A luncheon meat containing *surimi* and beef flavoring, a smoked product made from *surimi* and lean pork, and summer sausage containing *surimi* and deboned chicken have been produced.[89]

Another application of *surimi* is to prepare functionally active powder from fish like threadfin bream. Such powders have been prepared by drying *surimi* from lizardfish, threadfin bream, and purple spotted big eye fish. The resulting powders contained 73% protein and 17% carbohydrate (from the cryoprotectant added during *surimi* preparation). Functional properties such as solubility, gelation, water holding capacity, emulsification and foaming properties, and color varied depending upon the fish used.[90] The disadvantage of the presence of significant amounts of carbohydrate in the powders could be avoided and a preparation having more than 90% protein could be developed by modification of the *surimi*

process. In this process, the gelation of the washed fish muscle was effected by mild acidification using either acetic or lactic acid. The acid-induced gel in dilute solution is spray dried or concentrated by other means (see Chapter 13). *Surimi* has been employed in extrusion cooked products (see Section 8.6).

Recently *surimi* has been employed as a carrier for nutritionally important omega-3 fatty acids. *Surimi* from cod (*Gadus morhua*) containing the fatty aids was prepared by the addition of 500 mg of oil as oil-in-water emulsion to 85 g of *surimi*. Addition of the emulsion had no effect on gel strength, although in some case, changes in *surimi* color due to the presence of carotenoids in the oil has been noticed. Presence of sodium tripolyphosphate, which was added as cryoprotectant in the *surimi*, retarded development of rancidity of the fatty-acid-enriched *surimi* during frozen storage. The results suggested that *surimi* containing nutritionally beneficial polyunsaturated acids could be developed with good stability and gel strength.[91]

8.3.8 Market for *Surimi*

The world *surimi* market is centered in Japan, the biggest consumers of *surimi* in the world. However, during the 1990s, *surimi* has made great inroads into international markets, including those of Europe and USA. It has been estimated that at present about 10% of U.S. households purchase *surimi* seafood. The largest *surimi* producers in 2002 were the United States (220,000 mt), Thailand (140,000 mt), and Japan (100,000 mt); but other producers in recent years include Argentina, India, and China. In Europe, 60,000 mt of *surimi* were used for further processing in 2001. The *surimi* market appears to be stable at about 40,000 mt in North America and 80,000 mt in South Korea.[54] One way to further expand the worldwide *surimi* consumption is to tap the markets in East Asia and Southeast Asia, where *surimi* is used in their traditional foods such as fish balls. World market for *surimi*-based products comes from crabsticks and fish balls, where lower grades of *surimi* are used. While the *surimi*-based products were earlier viewed as imitation seafood, there is a change of image. These products are now being considered as fresh, ready-to-use items available at an affordable price. *Surimi* seafood is now promoted as suitable for meals as a center-of-plate item, or mixed in salads. Thinly sliced *surimi* seafood is also used like meat in sandwiches and other quick meals.[54] In recent times, however, due to economic reasons, there has been a slump in the *surimi* market particularly in Japan. Figure 8.7 shows markets for *surimi* in Korea, other parts of Asia, Europe, and North America during 1991 and 2001.

8.3.9 Alternate Methods of Making *Surimi*

While the conventional *surimi* process is widely practiced, novel methods have been explored. A new process for making *surimi* based on the selective precipitation of desired proteins has been developed. In this process, the mince homogenate in water is subjected to either acid (pH 2.5–3.5) or alkali (pH 10.8–11.5). The proteins remain soluble under these conditions. It is then
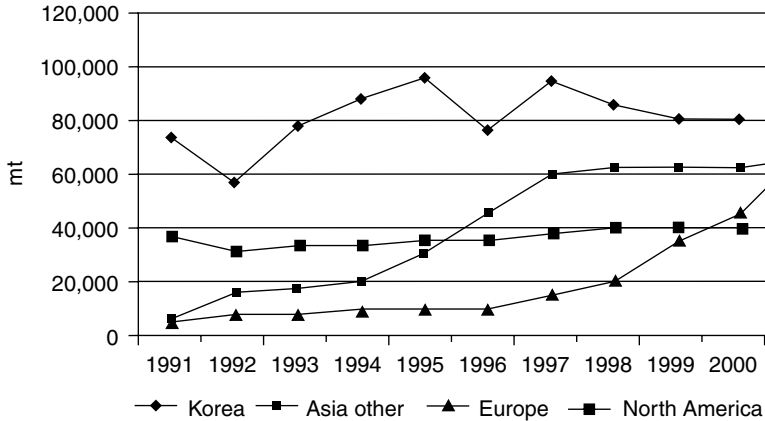
FIGURE 8.7 Important markets for *surimi* during 1991–2000 (Reprinted from Moller, A.B. Studies on seafood value addition. FAO/GLOBEFISH, Special Market Study, Rome, Food and Agriculture Organization of the United Nations, Rome, Italy, 2003. With permission)

centrifuged to remove insoluble impurities such as bone, skin, oil, and membranes. The pH of the aqueous suspension is then adjusted to its isoelectric point (between pH 5.2–6.0). This results in a precipitation of the myofibrillar proteins, which can be collected by centrifugation in a decanter centrifuge. The total yield of the collected proteins is more than 90%. The pH of the isolated proteins can be adjusted to any desirable pH, for example, 7.0–7.4. It is then mixed with cryoprotectants and frozen. The technique gives better yield and lower lipids in the product.[28]

Another development is onboard processing of *surimi*. Modern trawlers are capable of processing *surimi* in order to avoid the delay in transportation of the raw material and other disadvantages associated with processing in coastal factories.[25] Comparison of the quality of sea-processed Alaska pollock *surimi* with that of onboard produced *surimi* has shown superior quality of the former.[25] The lowest grade of *surimi* was observed for the product prepared from 7 to 10 days old fish transported in dry refrigerated holds of fishing boats and processed on shore.[36]

Conventional *surimi* contains salt that is added to dissolve the proteins and thereby enhance gelation. In view of recent interests in low-sodium food products, alternative techniques of gelation have been developed. One of the methods makes use of mild-acid induced gelation of fish meat. Elasmobranchs, consisting of sharks, rays, and skates do not fetch much consumer value because of their peculiar characteristics such as odor and appearance. Meat from these species is unique in that it can form a thick gel in the presence of mild acids such as acetic or lactic acid. For this, shark (*Scoliodon laticaudus*) meat is washed similar to the conventional *surimi* process. However, because of the high content of collagen and difficulties in mechanical deboning, the meat pieces are washed soaking overnight in excess of cold (<10°C) water to remove urea, blood, and pigments. After one more washing

in cold water for 1 h, the washed pieces are homogenized in an equal amount of cold water. To the slurry a few drops of glacial acetic acid are added, while gently stirring the slurry. Reduction of the pH from 6.5 to 3.5 results in the gelation of the proteins as indicated by the thickening of the slurry. Holding the acidified slurry for 2 h at ambient temperature (27–28°C) completes the gelation process. Since the presence of salts is detrimental to mild acid-induced gelation, salts are incorporated only at a later stage during the development of the food product using the gel. To make restructured steaks, the gel is filled into molds of desired shape and exposed to open steam for 15 min. After cooling, the restructured products are deacidified and salted to taste by dipping in a solution containing 4–5% each of NaCl and baking soda. After the salting step, the product can be breaded and battered by conventional methods.[92–94] An added advantage of mild acid-induced gelation is the facility to prepare microbiologically stable gel products due to low pH. Employing this technique *surimi* has been prepared from Alaska pollock. An addition of 2% acetic acid significantly increased the firmness of the gel. There was no peak in storage modulus with the addition of acid in the temperature range of 32–43°C, suggesting that the acid-induced gelation did not require setting, unlike conventional *surimi*. KCl, NaCl, or starch weakened the firmness of the gel.[95]

Another method of gelation makes use of the enzyme transglutaminase. The enzymes from various sources including microorganisms can induce crosslinkages in myofibrillar proteins to allow texture modification in washed fish meat (see Chapter 14). Transglutaminase mediated *surimi* has been prepared from several fish species. Optimum treatment conditions for processing *surimi* from Chilean jack mackerel have been reported.[96] A physical method to induce gelation in fish proteins is subjecting the washed meat to high hydrostatic pressures. This aspect has been discussed in Chapter 11.

8.4 SAUSAGE

Sausages including Vienna sausages, frankfurters, and bologna are complex mixtures of muscle tissue, solubilized proteins, binders, spices, salt, and water. Myosin and actomyosin are the major proteins that significantly influence the structure and functional properties of sausages. The products are stabilized through an emulsion of protein film around fat globules. Fish sausage is a product in which fish flesh is mixed with additives, stuffed into suitable casings and heat processed. Mince from both freshwater and marine species has been shown to be useful in sausage making.[97] In Japan, where interest in fish sausages was initiated with the developments in kamaboko type products, the principal raw material for preparation is frozen red flesh from fish such as tuna, marlin, and shark, as well as whale meat.

Surimi, because of its high functionality, is an ideal raw material for sausage manufacture. Usually animal or vegetable fat is mixed with fish mince or *surimi*. The mixing of ingredients are mixed with the fish by using a silent cutter.

TABLE 8.4
A Typical Recipe for Fish Sausage

Material	Proportion (%)
Minced fish meat	70.0
Salt	2.5
Sugar	1.5
Polyphosphate	0.2
Monosodium glutamate	0.2
Spice mixture (Pepper, coriander, ginger, garlic, etc.)	To taste
Color solution	0.1
Corn starch	9.0
Crushed ice	10.0
Fat (vegetable, hydrogenated)	5.0

The duration of the mixing operation is completed in 10–12 min and the temperature of the fish paste is maintained at 15–16°C during mixing by addition of crushed ice. The fish paste is stuffed into krehalon or some other casing and sealed with aluminum wire using a ringing machine. The product is heat processed at $90 \pm 2°C$ in a constant temperature water bath and then cooled in chilled water. The sausage is further heated at 98–100°C for 30 sec to remove wrinkles on the surface. The heat processed products are stored at chilled temperature $(6 \pm 2°C)$. Smoking can also be employed to enhance flavor. A typical recipe for fish sausage is given in Table 8.4. The sausage has moisture content of 68%, protein 17%, and fat 5.5% with a gel strength of 250 g cm and expressible water above 6% as shown in Table 8.4.[98]

Surimi from underutilized species of trevelly, yellow striped trevelly, and unicorn leatherjacket have been used for the preparation of burger. The *surimi* from these fish species was prepared by leaching the muscle twice in dilute saline to remove blood, pigments, and also fat. The leached muscle is blended with 2.5% each of sucrose and sorbitol, and 0.2% of sodium tripolyphosphate, as cryoprotectants, and stored frozen until used. For burger preparation, the frozen *surimi* is cut into smaller pieces, blended with ingredients. The composition for burger consisted of 63% *surimi*, 16% vegetable mixture, and other ingredients including vegetable oil, fish flavor, salt, garlic powder, pepper, and cinnamon. The smooth paste obtained is then formed, blast frozen to −20°C, battered and breaded, and stored under frozen conditions.[99]

Packaging has a significant effect on the product shelf life. Because fish contains more unsaturated lipids than red meats, the packaging material for fish sausages must be more impermeable to light and oxygen in order to control development of off-flavor through lipid oxidation. At present, polyvinylidene chloride (PVDC) casings, which are impermeable to oxygen and water, are generally used for packaging sausages in Japan.[100] Fish sausages containing 100% sardine meat or 76% *surimi* and 24% sardine meat were more stable against oxidation when

packaged in polyethylene terphthalate (PET), aluminum foil, or polypropylene (PP) casings when stored at 30°C than in PVDC alone. As expected, rancidity was more in the 100% sardine-product than in its *surimi*-based counterpart.[100] Curing with sodium nitrite can inhibit oxidation in sausages.

The heat treatment usually given to fish sausage is not enough to make the product sterile. This necessitates addition of antimicrobial food preservatives to control microorganisms. Chemical preservatives used in the formulation include nitrofurylamide, nitrofurazone, and sorbic acid. In Japan, the development of the fish sausage industry was rapid from 1950 to 1975, as the Japanese government was liberal in permitting chemical preservatives in the products. Developments in the production of synthetic casing materials also helped the sausage industry. However, the industry in Japan received a set back with the ban of nitrofurylamide, a chemical preservative which was used in fish sausage preparation. This has led to search for alternate preservatives. Nisin, produced by lactic acid bacteria, *Lactobacillus lactis*, has been examined as an antimicrobial in fish sausage. Fish sausage treated with 50 ppm of nisin enhanced the storage life of sausage from 2 to 22 days, while at refrigerated temperature, the enhancement was from 30 to 150 days. There was a slow decrease in the nisin content with respect to an increase in storage period.[98]

Freshwater fish has been examined for sausage production. Process development for fish sausage from hybrid Clarias catfish meat was conducted using ratios of catfish meat to Alaska *surimi* at varying concentrations, along with pork fat. A fish meat to *surimi* ratio of 40:60 with 10% pork fat had maximum acceptability. Incorporation of 3% fish oil or 0.5% calcium phosphate or calcium lactate had additional advantage in sensory acceptability.[101] Another product that could be developed is a pate. Three types of pates from flesh of different quantities of tuna (*Thunnus thynnus*) liver and mackerel (*S. scombrus)* were developed. Their nutritional values were comparable with commercial fish pates from large-scaled scorpion fish, salmon, and anchovy.[102] Table 8.5 gives proximate composition and quality characteristics of fresh fish sausage.

8.5 EXTRUSION COOKING

Food extrusion has been practiced for over 50 years, particularly for development of cereal-based breakfast items. Food extruders are considered as high-temperature short-time (HTST) reactors that can transform a variety of raw ingredients into modified intermediate and finished products. Advantages of this technology are its versatility, high productivity, quality, possibilities for product design, and absence of effluents during processing.[61,103] The process involves forcing a mixture of starch and other ingredients, at low moisture content (15 to 45%), through a barrel under variable conditions of temperature and pressure. This results in the melting and gelation of starch that binds itself together with the other ingredients. The movement of the material through the barrel can be through single, twin, or multiple screw conveyors, which provides high or low shear on the product, depending upon the equipment used. The screw configuration has a significant influence on the residence time of the product in the barrel and energy input, which

TABLE 8.5
Proximate Composition and Quality Characteristics of Fresh Fish Sausage

Parameters	Proportion
Moisture, %	68.64
Protein ($N \times 6.25$), %	16.76
Fat, %	5.64
Ash, %	2.67
pH	6.80
Gel strength, g cm	245.00
Expressible water content, g	6.27
Total volatile basic nitrogen, TVBN (mg N per 100 g)	6.93
Peroxide value (milli equivalents of oxygen per kg)	12.26
Free fatty acids (% oleic acid in fat)	2.20
Total plate count (cfu/g)	300
Aerobic spore formers (cfu/g)	6.10

Source: From Raju, C.V., Shamasundar, B.A., and Udupa, K.S., *Int. J. Food Sci. Technol.*, 38, 171, 2003. With permission from Blackwell Scientific Publishers, London.

have direct bearing on the quality of the extruded product. When the product emerges out of the extruder, it expands due to the sudden drop in the pressure. A suitable die at the end of the barrel can help in giving suitable shapes to the emerging product. Twin-screw extruders have better mixing, a uniform shear rate, good heat transfer, and can operate at higher moisture contents compared with their single-screw counterparts. Table 8.6 shows variables at different stages in extrusion cooking that influence product quality. These variables facilitate the design of the processing parameters to obtain the required product quality. Because of this, twin-screw extruders are finding increased applications for chemical modification of food ingredients to create tailor-made products.

Application of extrusion technology for protein rich products is of comparatively recent origin with success realized only in the case of soybean products.[104] Unlike starch, proteins show poor melting and expandability when subjected to extrusion cooking.[5] Ideal conditions for protein extrusion include mechanical mixing, formation of a homogenous suspension, and alignment of the protein in the flow direction.[104] Extrusion processing of fish muscle started in the 1980s.[105] The aim was to use underutilized species, bycatch and muscle recovered from byproducts of filleting, and *surimi* operations. Attempts have been made to develop dry, expanded snack food products from low-moisture blends of fish muscle and starchy ingredients using single and twin-screw extruders.[105–109] Texturization of sardine meat through extrusion cooking has been reported. A mixture of the fish meat and defatted soy flour in a ratio 7:3 having a 50% moisture content was extruded in a twin-screw extruder equipped with a long cooling die. The

TABLE 8.6
**Variables at Different Levels in Extrusion Cooking
that Influence Product Quality**

Levels	Variables	Effects
Machine	Screw configuration	Residence time
	Screw speed	Barrel pressure
	L/D ratio	Flow behavior
	Die design	Thermal energy
Process	Throughput	Thermal energy
	Barrel temperature	Rheology
	Die temperature	Phase change
		Component interactions
		Fragmentation of components
		Microbial inactivation
Product	Fish mince	Protein denaturation
	Feed composition	Starch gelatinization
	Moisture content of feed	Nutritional value
		Sensory quality
		Microbial quality

Source: Adapted from Harper, J.M., *Extrusion Cooking of Foods*,
Vols. 1 and 2. CRC Press, Boca Raton, 1981; and Areas, J.A.G., *Crit.
Rev. Food Sci. Nutr.*, 32, 365, 1992.

extruded product had the texture of animal meat but differed from fish products.
The effect of the extrusion process variables such as ratio of solids of fish to
wheat flour, length/diameter (L/D) ratio of the extruder barrel, and temperature of
extrusion were examined. The L/D ratio affected the rheological behavior of the
dough during extrusion.[109,110] Generally, incorporation of fish proteins reduced
extrudate expansion and increased hardness. The problem could be solved by the
incorporation of the mixing elements in the screw profile or by the proteolytic
degradation of fish proteins prior to extrusion.[105,111] Arrowroot flounder, which
has high inherent proteolytic activity, has been found amenable to extrusion cook-
ing. The fish was subjected to autolysis followed by drying. The autolyzed fish
along with 1.5% of rice flour was subjected to extrusion cooking in a twin-screw
extruder at 105°C. The product had lower apparent density and breaking strength,
but enhanced extrudate porosity and expansion ratio as compared with samples
incorporating fish muscle not subjected to autolysis.[105]

Twin-screw extrusion at high moisture levels offers possibilities for processing
protein-based products. Texturization of *surimi* using a twin-screw extruder has
been reported. The conditions used were a screw speed of 150 rev/min, barrel
temperature of 160 to 180°C, a feed rate of 30 kg/h and a die temperature of about
10°C. The treatment resulted in a complete liquefaction of *surimi* in the barrel
and texturization started in the screw outlet before it was transported to the die.

The texture of the products compared well with that of lobster, crab, and squid. Stickiness of the product in the barrel could be reduced by the incorporation of phosphate, which also prevented pressure fluctuations in the barrel.[112]

Extrusion cooking of *surimi* may induce chemical changes in the proteins. Frozen *surimi* of walleye pollock (*Theragra chacogramma*) was ground with 2.5% NaCl and cooked in a twin-screw extruder at a barrel temperature of 160–170°C. Analysis of the proteins showed that not only myosin heavy chain but also other components such as actin, tropomyosin, troponin, and myosin light chains were all involved to form a high molecular weight product during extrusion cooking.[113] Development of products incorporating proteins from soybean and *surimi* has been attempted. Thus, extrusion at moisture contents up to 80% facilitates emulsification, gelation, restructuring, microcoagulation, and fiberization of specific protein constituents. Nonetheless, the expansion characteristics of products may not be improved. The adverse reactions between proteins and lipids may be controlled by addition of oxidizing and reducing agents depending on the raw material used. Fibrous gelled structures were obtained when mixes containing 70–80% *surimi* and 30–20% soy concentrate or gluten were used. These were treated at moisture contents of 60–70% at a barrel temperature of about 140°C that facilitated protein plastification. The equipment required long dies with cooling, which helped to partially solidify the material. *Surimi* from Alaska pollock, sardine, and salmon were used in these processes. An extruded crab analog prepared from Alaska pollock *surimi* is already in commercial production in Japan.[114]

The enormous potential of extrusion cooking to produce texturized protein from seafood, particularly underutilized species is yet to be exploited. The process, under appropriate processing conditions, can improve functional characteristics of proteins without losing nutritional quality. A versatile pilot high moisture (70–80%) twin-screw extrusion system has been set up in France. The equipment produces not only fibrous nonmeat products (such as vegetarian patties, sandwich fillings, and pizza toppings) from soy, wheat, cereals, and legumes but also for the production of fibrous value-added products from trimmings of meat processing. The system is capable to make products in a wide range of shapes (ropes, flakes, cubes, and patties) with different physical properties (e.g., smooth, rough, shiny, or marbled surface appearance with light to dark coloration). Additives such as flavorings, preservatives, colorants, oils, and vitamins can be added. There is potential for scaling up of the process to capacities of 200–600 kg/h.[115]

Development of spun fibers is another method of texturization for proteins that makes use of gel formation of either the protein or matrices in which the proteins are resuspended. This brings about a change in the chemical environment of the molecules that facilitates the making of continuous fibers. In one process, a suspension of the protein in sodium alginate or pectate sol is passed through spinnerets into a coagulating bath of acidified calcium acetate, where calcium-induced gelation of alginate or pectate helps formation of fiber in which the protein is entrapped. Alternatively, dissolving protein in alkali and then pumping it into an acid bath can form fibers. Further treatment is necessary to obtain stable fibers.[116]

8.6 TRADITIONAL PRODUCTS

Fish mince serves as a major source of raw material for the preparation of various traditional products. These include patties, balls, wafers, loaves, burgers, fish fingers, fish fritters, dehydrated salt minces, breaded patties, cutlet, and pickled products.[2,3,5,117] Table 8.7 presents some fish mince-based products that have been developed from tropical fish. Preparation of fish fingers from croaker (*Sciaenid* spp.) and pink perch (*Nemipterus* spp.) consisted of extruding the mince along with food additives, dipping the pieces in batter, rolling in a breading mix, followed by frozen storage of the packaged products.[118] Development of salted and dried minces from threadfin bream and oil sardine has also been described.[119]

Processing of smoked, dried, and powdered sardine into instant soups has been discussed.[120] Precooked frozen burgers from sardine meat has been prepared by chopping the fish mince with 14% emulsion curd, 8% bread crumbs, 3% soybean protein, 1.5% salt, 2% sugar, and small amounts of bicarbonate, polyphosphates, and spices. The seasoned sardine meat was fried in soybean oil at 165°C for 3 min.[121] Mince from whole sprat gave acceptable fish balls, the quality of which was improved by prewashing the mince and incorporation of food additives. Development of an instant fish soup mix from Alaska pollock consisted of initially preparing kamaboko, seasoning, casing, slicing, and then freeze-drying. The product had good rehydration capacity.[122]

Fish ball is a popular and nutritious fish jelly product in Malaysia. It is made from fish meat ground with salt to a smooth sticky paste. Other ingredients, namely, 2% whey protein concentrate and 0.5% carrageenan are added to enhance the texture and flavor of the paste that is then shaped and cooked. Mince of fresh threadfin bream (*Nemipterus* spp.) is a common raw material. Production of fish balls in Thailand has grown to an industrial scale requiring approximately 35 tons of raw material per day.[123] Another popular product in Malaysia is fish crackers. It is made from fish species such as sardine and jewfish along with tapioca and sago flours and has been reported to possess the highest linear expansion. The type of fish used does not influence sensory characteristics.[124] Spiced minced fish cakes that are ready to fry or can be used in stews were produced from trawler bycatch. The cakes were found to be organoleptically acceptable to Nigerian consumers for a minimum period of three months at a frozen storage temperature of −20°C.[125]

Cooked fish pastes having varied consistencies have been prepared. A viscous fish-flesh sol, denoted as shio-*surimi*, is prepared by cooking Alaska pollock *surimi*, water, sodium chloride, and pregelatinized starch. The most viscous type of paste with a consistency of mayonnaise or tomato ketchup can be obtained from the shio-*surimi* containing 4.5 to 9% pregelatinized tapioca or potato starch and 150 to 300% water. The viscosity of the paste may be increased when salad oil or butter is further added to the shio-*surimi*. Furthermore, a type of paste with the consistency of French dressing can also be obtained by grinding the resulting paste with rice vinegar and salad oil or butter. Cooked fish pastes prepared in this way may be utilized as a new form of marine food.[126] Development of a creamy fish protein by controlled hydrolysis of Alaska pollock has been reported.[127]

TABLE 8.7
Some Fish Mince-Based Products from Tropical Fish

Raw material	Products
Anchovies	Minced muscle blocks
Bombay duck	Dried laminates
Barracuda	*Surimi*
Cat fish	Dehydrated salt mince, patties, sausage
Croaker	Sausage, *surimi*, fish fingers
Flying fish	*Surimi*
Jew fish	Cutlet
Lizardfish	Cutlet, *surimi*, dehydrated salt mince
Miscellaneous fish	Creamy fish bites, salt dried minces, fish fingers, fish balls
Perch	Fish fingers
Ribbon fish	Fish balls, sausage, *surimi*
Sardine	*Surimi*, marinbeef, salted dried mince
Shark	*Surimi*
Silver belly	Sauce
Sole	Minced muscle blocks
Threadfin bream	Cutlet, flour, hydrolysate, *surimi*, spray dried protein powder, salted dried mince
Mixed bycatch (containing pelagic and demersal fish)	Fish ball

Source: From Grantham. G.J., Minced Fish Technology: A Review. Fisheries Technical Paper 216, Food and Agriculture Organization of the United Nations, Rome, Italy, 1981; Jantawat, P. and Yamprayoon, J., *Asean Food J.*, 5, 108, 1990; Lee, C.M., in *Seafoods: Chemistry, Processing Technology and Quality*, Shahidi, F. and Botta, J.R., Eds., Blackie Academic and Scientific Publisher, Glasgow, U. K., 1994, p. 13; Putro, S., *Technical Handbook. 2. Processing of Surimi and Fish Jelly Products*. Infofish, Malaysia, 1989; Young, R.H., Strategy for shrimp by-catch utilization. FAO Fisheries Circular 745, Food and Agriculture Organization of the United Nations, Rome, Italy, 1982; Reddy, L., Shetty, T.M.R., and Dorai, K.C., *Fishery Technol.*, 27, 133, 1990; Sudhakaran, R. and Sudhakara, N.S., Studies on the preparation of salted and dried minces from threadfin bream and Indian oil sardine. FAO Fisheries Report, 317, Food and Agriculture Organization, Rome, Italy, 1985, p. 338.

Fermented items, fish protein concentrates (FPCs), fish protein hydrolysates (FDHs), and seafood flavors are prominent among other traditional products. Fermented fishery products are highly popular in Southeast Asia, which are being used to add variety to the diet and contribute to general nutrition. Fermented products from fish may be classified broadly into two types: fish and salt formulations and formulations containing fish, salt, and carbohydrates. In the former category, fermentation results from the autolytic enzymes present in the tissue while high levels of salt (>20%) prevent microbial deterioration of the meat. Fish sauce is produced by this method. The long fermentation time for fish sauce (5–12

months) may be reduced by addition of exogenous proteases. Aerobic fermentation results in a higher volatile fatty acids content compared with anaerobic fermentation. The latter imparts a little sweet and less acidic and rancid aroma to the product.[128] Lactic acid fermentation of mixtures of fish or carbohydrates (cooked rice, cassava among others) in the presence of small amounts of salt (6–10%) provides possibilities for developing a number of products from the underutilized fish species. The organisms used for fermentation generally belong to the species *Lactobacillus*, *Streptococcus*, *Pediococcus*, or *Leuconostoc*.[129] Fermentation of mullet sausage by *P. acidilactici* helped to provide safety from microbial pathogens.[130] Solid state fermentation of bonito is another example.[131] Some of the popular Asian mold-fermented foods such as shoyu, miso, katsuobushi, and tempeh are comparable to fermented items such as cheese and meat products.[132]

A new technology for producing paste-like fish products using fermentation by suitable lactic acid bacteria *Lactobacillus plantarum*, *L. lactis*, *L. helveticus*, *Pediococcus pentosaceus* L or *P. pentosaceus* S has been reported. Ground mackerel mince was mixed with 4% sucrose, 1% glucose, 2% NaCl and various amounts of water depending upon the type of products. The mixture was fermented for 48 h at 37°C. Rapid growth of LAB resulted in a decline in pH, suppression of main microflora and increases in whiteness of the product. Depending upon the water content of the mince, the products had qualities comparable with butter, pudding, or custard.[133]

During the 1960s and 1970s, there was a lot of interest in the development of FPC for use as a protein supplement in cereal-based foods. The products were colorless and odorless powders having negligible amounts of fat prepared by solvent extraction followed by dehyration of fish mince.[5] However, poor functionality and residual solvent in the product were responsible for poor commercial acceptability of the product. Alternate methods have been developed to prepare protein concentrates without the disadvantages of conventional FPC. A popular product from fish mince developed in Japan is known as marinbeef. The product is prepared by kneading and extruding fish mince containing salt into alcohol to obtain grains of denatured protein. The product is off-white in color, has no fishy odor, and has better rehydration capacity than FPC. Spray dried protein powders have been prepared making use of mild-acid induced gelation of washed fish muscle (see Chapter 13).

Unlike FPC, FPHs have better acceptability, although, commercial production of FPH has not been explored fully. FPH is prepared by digestion of fish or fish mince using proteolytic enzymes at the optimal temperature and pH required by the enzymes. Liquefaction of the protein takes place within a few hours and the hydrolysate is decanted and centrifuged to remove scales and bones. The solubilized proteins are concentrated, usually by spray drying or by isoelectric precipitation. Lean fish species are an ideal raw material for FPH. Washed mince or *surimi* can also give good products.[134–137] The production of FPH has been described by several scientists.[135–138] Enzymes such as papain, ficin, trypsin, pancreatin, pronase, or proteases from fish itself may be used to produce a wide range of hydrolysates. Uncontrolled hydrolysis may produce short-chain peptides, which lack the
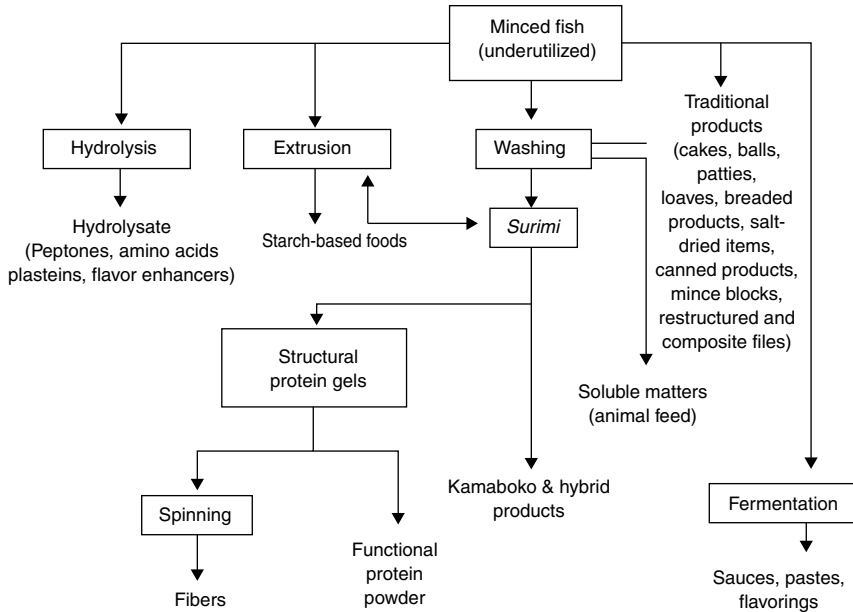
FIGURE 8.8   General scheme for preparation of products from fish mince (Reprinted from Venugopal, V. and Shahidi, F., *Crit. Rev. Food Sci. Nutr.*, 35, 431, 1995. With permission)

functional properties of the native proteins. Therefore, the degree of protein hydrolysis is important in optimizing the process parameters for product development. Furthermore, bitterness is a common problem in the FPH due to the formation of peptides containing bulky hydrophobic groups toward their C-terminal. The bitterness may be masked by exoprotease treatment (referred as plastein reaction, a process that reverses hydrolysis) and/or incorporation of certain additives such as glutamic acid or glutamyl-rich peptides, polyphosphates, gelatin, or glycine into the products. Protein hydrolysates are generally used for modification of functional properties of foods and in dietetic foods as a source of small peptides and amino acids and also as flavoring. Several novel applications of FPH have also been pointed out recently. These include its use as a microbiological growth media, as a fertilizer to increase crop growth and yield, and as a stimulator for expression of many commercially valuable phytochemicals. These phytochemicals can help production of a number of neutraceuticals that can have applications in food products.[137] Application of FPHs as antioxidants has been reported. Freeze-dried FPH from herring containing 77 to 87% proteins with a degree of hydrolysis ranging from 10 to 18% was reported to have a significant antioxidant activity.[139] A factory in Japan makes a product called "bio-fish flour" by enzymatic digestion of sardines and it is marketed as a milk substitute.[140] Figure 8.8 gives a general scheme for the utilization of fish mince for the development various products.

8.7 IDENTIFICATION OF FISH SPECIES IN PRODUCTS

In the commercialization of further processed fishery products it is important to identify the fish species used in the products. Most of these products consist of muscle tissue from fish and invertebrates. Differences in the molecular structure and metabolism of muscles can be utilized to characterize and identify seafood. Raw products may be also analyzed by isoelectric focusing of sarcoplasmic proteins. Two types of sarcoplasmic calcium-binding proteins, para-albumins of fish and soluble calcium-binding proteins of invertebrates are especially useful for species identification. Due to their thermal stability, these proteins give species-specific patterns for cooked products too. Two other plausible techniques are urea gel isoelectric focusing and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). These methods are suitable for the identification of products where the sarcoplasmic proteins had been removed by washing steps such as imitation crabmeat made from *surimi*. The myosin light chains give protein patterns that are characteristic for many species. Paramyosin, which is absent from vertebrate muscle, indicates the presence of mollusk muscle.[141] Creatine and arginine, which are richly found in crustacea, can be used for differentiation between imitation crab or shrimp meat and real crustacean meat. Presence of the enzyme arginine kinase activity can also enable differentiation between raw fish muscle and invertebrate muscles. Octopine serves as an indicator for the meat of cephalopods and mussels (see also Chapter 5).

8.8 INDUSTRIAL PROSPECTS

The foregoing summarizes varied possibilities for use of mince, particularly from underutilized fish species, in product development. The bulk of the current mince production amounting to more than 600,000 t, is from commercial underutilized, pelagic and bycatch species, while commercially, further processing of fish is mostly restricted to coated products.[142] *Surimi* industry has expanded not only in Japan, but also in several western countries during the last few years. Clarification of issues such as labeling, uniform grading systems, and improvements in the texture of analogs would help in further development of the *surimi* industry. The potential of extrusion cooking technology of low cost fish meat has yet to be tapped. It is noteworthy that depending upon the fish, the individual minced product may require suitable modifications. Sometimes, mince collection itself poses problems due to the inherent structural properties of the fish. The various problems associated with the utilization of different fish species have been identified.[37] Factors to be considered by the industry include identification of reliable and defined resources, use of effective harvesting systems, preparation of acceptable products using suitable processing techniques, and a proper distribution system in an identified market. The decision to process seafood into a secondary product is often complex and requires a sound understanding of the interrelationship of the substrate, the equipment that will be performing the transformation, the added ingredient(s), and regulatory considerations. Further processing of seafood

TABLE 8.8
Consumption of Value-Added Products from Fish in South America

Country	Raw material	Material source	Product
Equador	Frozen mince	Shark, small pelagics	Fish sticks
	D/S mince	Shark	Cake, sweet and salty cookies, Hamburgers, ham, meat extenders
Peru	Mince	Small pelagics	Hamburgers, "beef steak"
	Marinbeef	Sardine, horse mackerel	Meat extender/replacer
	Functional powder	Mackerel, sardine	Protein supplement in foods
Chile	Mince	Hake, mackerel	Sausage
Brazil	FPC/A	Sardine	Protein supplement in foods

Number of beneficiaries of institutional feeding programs (1000s)

Product	Equador	Peru	Chile	Brazil	Total
School lunches	740	3,000	824	25,000	29,564
Mother and child programs	300	93	1,094	5,000	6,487
Factory and other canteens	—	40	20	3,000	3,060
Total	1,040	3,133	1,938	33,000	39,111

Source: From UNIDO, Sectoral Studies Branch, *Food Rev. Int.*, 6, 1, 1990. With permission from Marcel Dekker.

encompasses the transformation of a raw aquatic commodity into a product that is designed to meet a consumer need. Factors such as taste, intended method of storage, preparation for the table, cost, resource availability, and marketability certainly play obvious and important roles in this endeavor.

A number of products and market opportunities are available particularly for developing countries, for value-added fishery products.[143,144] Many of the products can find applications in nutritional schemes for school children, families, and factory workers, as indicated in studies conducted in South America, as shown in Table 8.8. Production data, processing methods, and shelf life of seafood produced and marketed in Southeast Asia included boiled, canned, comminuted, cured, dried, fermented, frozen, powdered, flaked, smoked items, fish meal, and crackers as summarized by the Southeast Asian Fisheries Development Centre.[145] High priced high quality fish balls are being made from wolf herring, coral fish, Spanish mackerel, and eel.[22] Development of minced fish and mince-based products provides dual opportunities for utilization of low value fish for human consumption as well as diversification of fish processing industries for international trade in value-added products.[146] During the last few decades, minced fish has established itself as a new item of commerce, which resulted in preparation of a draft Code of Practice by the Codex Alimentarius Commission.[147]

The lack of desired progress in the utilization of fish species employing these processes is due to the fact that much of the technical innovations at the product and process levels have been technology-driven rather than market led. Therefore, market strategies, including efforts to increase consumer acceptability are required.[1] Market expansion for mince-based products depends on design of appealing products, careful process development, identification of consumer preferences varying in considerable cultural backgrounds, and adherence to strict quality standards. Developing countries may benefit the most from better management of the low cost fishery resources because of advantages such as good resources and cheap labor.[148]

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9 Coated Products

9.1 INTRODUCTION

Breading and battering has been extensively employed for value addition of food items including muscle foods, particularly poultry items. The battered, prefried and then frozen products constitute an extensive sector in the ready-meals market.[1] Because of their convenience and taste these items are liked by most consumers, as indicated by the volume of global trade in such products. In view of the increasing consumer demands, the technology has made several advances during the past few years. While many developments originated from United States and Europe, the Japanese have also made substantial contributions to take the industry to the present level of sophistication. Today, several varieties of batters and breading in different colors and mesh size are readily available and are being used. The entire operation of processing of coated products starting from portioning/forming to packing of finished products in appropriate packaging materials are being carried out using highly sophisticated and automatic machinery of varying capacities.[1–6]

9.2 FUNCTIONAL ADVANTAGES OF COATING

In essence, a coated food product is one that is coated with another foodstuff. Coating by battering and breading enhances a food product's characteristics such as appearance, flavor, and texture. The process protects the natural juices of foods from the effects of freezing or reheating, thereby ensuring a final product that is tender and juicy on the inside and at the same time crisp on the outside. Moisture loss due to dehydration is a common problem during storage of frozen food products (see Chapter 4). Functionally, coating provides a moisture barrier to the product, helping in reduction of weight loss during frozen storage and also while reheating before consumption. These advantages offer consumer appeal for the product through improving sensory value of the processed items.[2] Coating also provides an opportunity to increase the nutritional value of a product through incorporation of nutrients in the coating. The advantages of coating are summarized in Table 9.1.

9.3 THE COATING PROCESS

The unit operations in development of coated products are portioning/forming, predusting, battering, breading, flash frying, freezing, packaging, and storage. The portioning/forming aspects with respect to fishery products have been discussed

TABLE 9.1

Advantages of Coating Fishery Products

Provides a crispy texture and appealing color and flavor

Enhances the nutritional quality through incorporation of nutrients

Acts as a moisture barrier minimizing moisture losses during frozen
  storage/microwave reheating

Acts as food sealant by preventing natural juices from flowing out

Provides structural reinforcement to the substrate

Increases the bulk of the substrate thus reducing the cost of the finished product

Source: Adapted from Joseph, A.C., in *Seafood Safety*, Surendran, P.K. et al.,
Eds. Society of Fisheries Technologists (India), 2003. With permission.

later (see Section 9.6). This section will discuss general operations in developing
a coated product.

9.3.1 Predusting

Predust usually is a very fine, dry, raw flour material that is sprinkled on the moist
surface of the frozen- or fresh-food substrate before any other coating is applied.
It improves the adhesion of the batter, because it absorbs part of the water on the
surface of the food. If the batter is applied to a surface that is too moist, it can slip,
leaving some areas uncovered. Also, the use of predust tends to increase pickup.
The most commonly used predusts are wheat flour, gums, and proteins, alone or
in combination. These can also absorb moisture and help to form a structure.[6]
The protein materials that produce crusts with better adhesion than starch or gums
include dried egg albumen and wheat gluten. These can produce better results
on the basis of yield and visual scores. Soy concentrate produces good adhesion
although not as high as albumen or gluten. In many processes, predusts are used
to include flavorings such as spices and seasonings.

9.3.2 Batter

Batters are of two types, adhesive and tempura. The traditional adhesive batter is
a fluid, basically consisting of flour and water, into which the product is dipped
before it is cooked or fried. A bond between the product and the coating is formed.
The proportion of batter and water is usually in the ratio of 1:2. Higher amount
of water might affect the functional need of fixing the crumbs onto the batter and
also will necessitate longer time to freeze the batter.[2] The desired viscosity and
pick up decide the ratio of components in the batter mix.

   The ingredients that constitute the batter include starch, salt, seasonings, gums,
egg, and many other items.[7] The batter also usually incorporates a leavening agent
to favor expansion of the product during frying. The functional roles of individual
ingredients in the batter have been discussed recently.[8] Each ingredient performs
its functions to contribute to the unique characteristics and functionality of the

TABLE 9.2
Major Ingredients and Their Functions in Coated Products

Class of ingredients	Components	Function in the product
Polysaccharides	Wheat flour, corn flour, starch/modified starch, and gums	Improves viscosity, emulsifying and foaming capacity, texture, and shelf life
Proteins	Milk powder, milk protein fraction, egg albumin, seed proteins, and single-cell proteins	Improve water absorption capacity of the flour and thus increase the viscosity of the system
Fat/hydrogenated oils	Triglycerides, fatty acids	Texture, flavor
Seasonings	Sugar, salt, spices	Enhance plasticizing effect, flavor, and impart antioxidant and antibacterial properties
Leavening agents	Sodium bicarbonate, tartaric acid	Release carbon dioxide in tempura batters
Gums	Xanthan, gum Arabic, etc.	Viscosity and water holding capacity
Water	—	Gelatinization of starch, hydration of proteins, improves batter viscosity

Source: Adapted from Fiszman, S.M. and Salvador, A., *Trends Food Sci. Technol.*, 14, 399, 2003 and Joseph, A.C., in *Seafood Safety*, Surendran, P.K. et al., Eds. Society of Fisheries Technologists (India), 2003, p. 1. With permission from Elsevier.

coatings, as shown in Table 9.2. The commonly used ingredients may be grouped under five categories, namely, polysaccharides, proteins, fat/hydrogenated oils, seasonings, and water. Polysaccharides (wheat flour, corn flour, starch/modified starch, and gums) provide viscosity, suspension-characteristics, emulsifying capacity, besides controlling the forms, texture, and shelf life of the coated products through their interaction with proteins/lipids. Proteins (milk powder, milk protein fractions, egg albumin, cereal flours, seed proteins, and single-cell proteins) improve water absorption capacity of the flour and thus increase the viscosity of the system. Fat/hydrogenated oils contribute to food flavor. Seasonings are sugar, salt, and spices. Sugar provides plasticising effect, flavor, and is involved in browning reactions; salt improves the taste and texture, and spices enhance the flavor and impart antioxidant and antibacterial properties. Sodium bicarbonate and tartaric acids are some of the leavening acids used to release carbon dioxide. Gums such as xanthan serve in viscosity control and water-holding capacity. Small quantities of egg albumin, milk, whey, soy flour, and color may be added to provide specific functional effects such as gel or film formation, improvement of texture, etc. Water serves as a medium for the ingredients and is involved in gelatinization of starch, hydration of flour proteins, and batter viscosity. Generally wheat flour is added at 40%; corn flour, 30%; rice flour, <5%; leavening agent, <3%; and gums, <1%.

Modifications of composition to suit widely different products have resulted in development of sophisticated, complex coating systems.

Apart from the adhesive batter, the tempura, developed in Japan, is the puff-type specialty batter. Corn flour is important in tempura batters. Tempura-type batters form a crisp, continuous, uniform layer over the food, constituting its final coating. They also provide crust coatings of exceptionally high volume, which are also light in texture. The tempuras are used at very high viscosity levels and always contain raising/leavening agents. Because of the leavening action of these batters, they cannot be pumped without substantial loss of the leavening gas (carbon dioxide).[4] The wider use of tempura batter in conjunction with coarse crumbs represents a new coating process for modern processing.

The batter is mixed by agitation; however, care must be taken to incorporate a lot of air in it. Small air bubbles may agglomerate and coalesce into a larger bubble on the surface of the product (fish) and may be blown off during subsequent stages exposing uncovered areas. The material to be coated is carried between two conveyor belts through a bath of batter ensuring that the fish is completely immersed. The gap between the upper and the lower belts is adjustable to accommodate products of different thickness.[2]

9.3.2.1 Rheological properties of the batter

The characteristic property of the batter is its viscosity, which determines its performance during frying and quality of the finished product. The viscosity affects the pickup and quality of the adhering batter, the handling properties of the battered product, its appearance, and final texture.[9] Rheology of batter has been studied in detail with a view to improve its functional properties.[10,11] The rheological properties are influenced by the composition and proportion of the ingredients, the solids–water relationship and temperature, shear rate, shear time, previous thermal, and shear histories. The batters generally exhibit shear-thinning behavior, time-dependence, and thixotropy. Incorporation of thickeners or proteins makes the flow behavior complex. This suggests that batters require a steady flow behavior over a range of shear stresses, in order to optimise the mixing, pumping, and coating operations.[8,12,13] While conventional viscosity measurements have limitations, the dynamic rheological measurements provide information on real-time behavior of the batter during coating and gelation-dependent structural changes that take place while cooking of the coated product.[8,14] A thin, not very viscous, batter is capable of releasing a large quantity of water and produces a porous coating that absorbs a lot of oil. A thick-batter layer can lead to an incompletely cooked final product, lack of crispness, and a generally hard, lumpy appearance, apart from having adverse influence on oil uptake during flash frying.[15]

9.3.2.2 Temperature

When the batter dough is reconstituted in water for the battering step, the temperature of the water used is important, since it determines the viscosity. Once

reconstituted, it should be kept at a temperature low enough to maintain the viscosity and also to control the growth of microorganisms. However, very low temperature should be avoided to prevent freezing of the batter on the conveyor. The ideal water temperature has been suggested to be between 10 and 15°C. At temperatures below 10°C the viscosity of the batter could become too high impeding proper handling and at temperature above 18°C, the viscosity could become too low.[8]

9.3.3 Breading

"Breading" is a cereal-based coating, often of breadcrumbs. The main ingredients are almost the same as for batters, and consist of flours, starch, and seasonings. Breading is coarse in nature and applied to a moist or battered food product prior to cooking. Texture, mesh size, porosity, and absorption are the major factors contributing to the texture of the breading. Mesh size may be coarse, medium, or fine, and is important in the formation of an attractive and economical coating system. Coarse particles are desirable to achieve textural targets; however, its excess use on a small surface area may cause its falling off during handling and transportation. Hence a balance among different mesh sizes is desirable. Coarse, dense crumbs may be acceptable when the food is oven heated and nonoily appearance is desired. Dense crumbs absorb less oil when prefried; however, they may yield an unacceptable hard texture when fully fried. It is appropriate to select a coating with medium particle size.

The major functional characteristics of breading are its volume to unit area, browning rate, moisture absorption, oil absorption, color, and texture.[2] A high volume, for example, as obtainable in the case of fish fingers, permits good coverage with breading. However, it will be difficult to apply coatings on cube-shaped products. Browning rate depends on the proportion of reducing sugars in the breading. Fast browning rates necessitate use of high processing speeds, reduced frying time, and lower frying temperature. Moisture absorption of breading is a function of its particle size, porosity, and gelation. Porosity and mesh size determines the texture of the coated food. Oil absorption and rate of heat transfer are higher in porous than in dense granules. Oil absorption and exchange of oil for moisture during frying are important factors in the development of texture. Color in the final product can be due to reducing sugars and also to added color.

A wide variety of breading materials is available in different sizes, colors, flavors, shapes, and sizes. They have varying properties depending on their particle size and constituents. Flour breaders are general purpose breaders and constitute mainly wheat/corn flour, starches, gums, coloring, and seasoning. They can be used alone or in combination with other types of crumbs. Cracker meal/traditional breading is widely used in fish products. Unless other ingredients are added, cracker meal does not develop color as quickly as other breading. Cracker-meal breaders, which are used to develop a cracker type, relatively hard texture, consist of unleavened flour. The particle size of flour may vary from coarse (60–140 mesh size), medium (20–60 mesh), to fine (4–6 mesh).

Home-style breadcrumbs are more porous than cracker meal and tend to absorb more oil and moisture. They cannot tolerate long frying times and tend to darken more quickly. Japanese style crumbs, also called "Oriental style or Panko crumb," has a characteristic flake-like elongated structure and excellent visual appeal, and provides a unique surface-texture when fried. Because of its lightness, it is possible to produce the crumb in large sizes without the sensation of hard particles. Special care is required in applying the crumb because of its fragility. Conventional breading machines damage the structure and the selection of an appropriate machine is essential. Extruded crumbs are light and tend to float in oil, may turn black in the fryer and deteriorate the oil quality. Because of these fryers are usually provided with a system to remove the floating particles.[2] Flavors help transform traditional seafood dishes. A wide range of flavors and coatings are being developed for this purpose.[16] Crumb coatings are usually colored with natural vegetable extracts to give a golden-brown appearance. These colors include paprika, annatto, turmeric, or caramel.

9.3.4 Frying

Fat is the frying medium. Besides being the heat transfer medium, it is also a food ingredient that will influence the eating quality. Some fats may have specific flavor which may be carried over to the product.[2] Usually bleached and refined vegetable oils are used for frying. At the high temperature of frying, some fat may undergo changes such as oxidation and polymerization. It is therefore important to use an oil of good quality for frying. The quality is determined in terms of content of free fatty acids, smoke point, peroxide value, as well as color as per prescribed standards. According to normal manufacturing process, prefrying in oil is carried out at 180 to 200°C for about 30 sec followed by freezing the product. By keeping the coated product in the fryer for a relatively short time of 30 sec, actual heat transfer to the heated product is restricted to the coating surface, while the core of the product, such as fish, remains frozen. Little quality is lost since the product is frozen immediately after frying.[17] In commercial process, the overuse of oil has some disadvantages. Keeping the oil hot for long periods of time in the presence of air and moisture leads to oxidative, thermal, and hydrolytic reactions resulting in formation of undesirable rancid flavors, aldehydes and ketones, and other poducts.[18] These can have adverse effect on the product quality.

9.3.5 Packaging

Conventional packaging materials like flexible plastic films alone are not suitable for packaging, since they provide little mechanical protection to the products. As a result, the products get damaged or broken during handling and transportation. The packaging may be a paperboard carton or a poly-lined paper bag or poly-bag, which is heat-sealed. The bags are designated to give support to a larger quantity of items such as fish fingers.[19] In recent times, thermo-formed containers are commonly used for packaging coated products. These trays produced from food-grade

materials are suitable for packaging breaded items both for domestic as well as export markets. Trays made of polyvinylidene chloride, high impact polystyrene (HIP) and high-density polyethylene (HDPE) are unaffected by subzero temperatures and provide protection to the contents against desiccation and oxidation during prolonged storage. The packaged products are usually stored at $-10°C$ or lower. The development of sophisticated "value-added" products has coincided with the increase in domestic microwave ovens. This has placed pressure on the packaging industry to provide suitable packaging. Such packaging must be capable of withstanding the high temperature encountered in a conventional oven and yet to be able to cope with the relatively rapid reheating in microwave ovens. Cooking instruction for such packs must be carefully designed to ensure that the consumer is in no doubt as to how to achieve the best results.

9.4 PRODUCTION OF COATED PRODUCTS

The process of production of coated food products is shown in Figure 9.1. The unit operations involved are portioning or forming, predusting, application of the batter, application of the breading, prefrying or flash frying, freezing, and packing. As mentioned earlier, predusting helps uniform batter adhesion in frozen or even greasy food surfaces. After predusting the product is subjected to batter coating,

Portioning

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Predusting

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Application of batter

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Application of breading

↓

Flash frying

↓

Freezing

↓

Packaging

↓

Cold storage
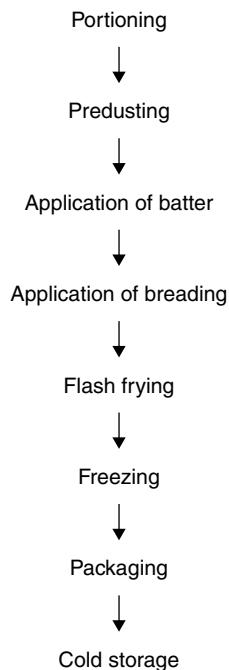
FIGURE 9.1   Process flowchart for the production of coated products

which is applied using a batter applicator. The low viscosity batter can be applied in an overflow configuration; however, batter with medium viscosity requires a total submersion system depending upon the product requirements. The batter may be applied using a pour-on application system in addition to the submersion method. Irregular shaped products should be placed on the line with the concave surface downward to prevent air pockets and inhibiting batter pickup. The speed of the applicator may be adjusted to ensure proper batter pickup. Any excess batter is blown off in an air current using an air blower kept as close to the product as possible to prevent the excess batter forming lumps and causing blockage in the breading machine.[2]

Breading is applied to the battered products using breading applicators. Specially designed breading machines are used to ensure uniform particle size distribution or granulation on both top and bottom of the product with minimum crumb breakdown. For soft products the crumb depth is maintained as thin as possible to avoid product damage in the machine. A deep bed of crumbs is necessary for frozen or hard products. Pressure rollers are used to apply sufficient force to press the crumbs over the product. Any excess crumb is blown off, since such particles carried to the fryer will appear as black specks on the product. The coated products are often flash fried at 180–200°C for 20–30 sec in order to set the coating on the fish portion. The coated product is passed through the frying medium over a conveyor for the required time. The flash-fried product will have the surface partially thawed whereas the center will still be in the frozen stage. During the frying stage, important changes take place within the coating, namely, starch gelatinization, protein coagulation, browning reactions, fat absorption, leavening release, moisture reduction, and flavor development. The main problem to the technologist is optimization of the process parameters for retention of coating on the product. The flash-fried product is cooled in a current of air using fans. Cooling allows the coating to recover from the frying shock and also to stabilize itself. The product is then subjected to freezing by passing through a conveyor belt and is frozen till the core temperature is around −10°C. The freezing step maintains the quality of the product, which is packaged to prevent dehydration, desiccation, and rancidity development.

9.5 COATED FISHERY PRODUCTS

Seafoods have historically been subjected to further processing through coating. Battering and breading techniques have contributed significantly to value addition of fish fillets, shellfish, and mollusks. The first commercially successful coated fish product was termed "fish finger" or "fish stick." Later several other products came into the market with the advent of the fast-food trade. The different popular coated finfish items include cod, haddock, pollock, perch, and catfish among others, while crustacean products consist of shrimp, crab cakes, and crawfish. Coated mollusks are clams, oysters, scallops, and squid. Some of the fancy items are butterfly shrimp, squid ring, stuffed squid rings, fish cutlets, and fish burgers. The coating technology has shown further impetus with the development of *surimi* industry and fabricated fishery products. Figure 9.2 depicts some of the coated fishery products.

FIGURE 9.2 Coated fishery products (Courtesy *VICIUNAI*, Senamiescio a.7, LT_5640, Plunge, Lithuania)

9.5.1 Fish Finger or Fish Portion

Frozen skinless and boneless, lean fish fillets generally prepared onboard fishing vessels are the raw material. Species commonly used are Alaska pollock, saithe, hake, and hoki. These fillets are salted in dilute brine to improve the color, taste, and texture. The fillets are then molded into blocks of standard size and weight and kept frozen till used for coating. If fillets are smaller in size they could be used for making composite fillets. Addition of edible adhesive materials could be used for better binding among the fillets. Other binding agents are soy protein, starch, or carrageenan. The fillets are placed in a large fillet-shaped mold and compressed with a low-pressure ram.

For production of coated products, the frozen fillet is cut by a series of band saws and automated cutting machines to the desired shape and size. The band saws determine the portion thickness, while the chopper cuts the portion into the desired width and shape. A loss of material of the order 5 to 10% usually occurs in the process. Microwave technology, used commercially to temper frozen blocks of fish, eases cutting operations for fish stick and portion production. There are forming/molding machines available for products of specific shapes. The common shapes are square, rectangular, wedge, and "French cut." The portions are predusted, battered, and breaded. The coated product is then flash fried at about 180°C for 20 sec, frozen, and packed. The product prepared from composite fillets is nearly indistinguishable from fillets obtained from large fish. The fish contents in these product is above 70%.

As compared with the rectangular portions, slightly irregular, natural looking portions are becoming popular. The fish processing industry has been using specialized machinery to shape frozen block into irregular portions for the last 15 years. The shaping machines work on the principle of applying pressure to frozen fish flesh, which will then become sufficiently fluid to fill the contours of the three-dimensional molds into which the frozen portions have been placed. The temperature remains at or near 10°C. The portions are then ejected from the moulds, which are then used for coating. The molds may also be shaped like fillets, tails, loins, nuggets, etc. The reasons for the success in this area are capacity for accurate portion control, high yield, diverse shapes, and acceptable texture.[20]

Battered and breaded products such as fish fingers, fish cakes, etc. provide a method for value addition of currently increasing production of freshwater fish such as catfish and tilapia. The reason for strong consumer demand for these freshwater fish is their white, easy-to-prepare fillets. For example, in Thailand where catfish contributes to more than 15% of the total freshwater fish production the potential of coating technology has been pointed out.[21] In the United States, catfish is now the fifth most consumed fish with the domestic production reaching 280,000 t in 2000. A wide variety of block-based frozen products is marketed in Europe including products that are completely coated with breadcrumbs, battered or topped with sances[20] An amount of 82,240 t of coated fish fillets were traded internationally according to the Food and Agriculture Organization (see Chapter 1).

9.5.2 Coated Fish Mince Products

Fish mince from marine as well as freshwater fish can be used for processing into a variety of coated products. Foaming machines are used for reshaping large sections of mince blocks, which are initially cut into smaller size and pressed into single or multiple shapes in a shaping cavity. Keeping the temperature of the process <3 to 5°C, helps to preserve the desired flaky texture. The portions can be used for breading and coating. For fish cutlet, cooked fish mince is mixed with cooked potato, fried onion, spices, and other optional ingredients. The mass is then formed into desired shapes having an average weight of about 40 g. The formed cutlets are battered, breaded, and flash fried before packing and freezing.

Fish patties (burgers) are similar to fish cutlets. Burgers are made using mince of lean white-meat fish and are only mildly flavored. Patties are battered, breaded, and flash fried before packing and freezing. *Sushi* bars are becoming increasingly popular in both developed and developing countries.

9.5.3 Shrimp

A number of battered and breaded products can be prepared from both wild as well as cultured shrimp. The shellfish in different forms, such as peeled and deveined, butterfly, round tail-on, cooked, and peeled, are used. The preparation involves three processing steps, predusting, application of batter, and breading. If the raw material is frozen, it has to be completely thawed and its surface must be free of moisture, for better adhesion of the coating. Presence of moisture results in poor sticking of the coating, which blows off during frying to give a poor quality product. Polyphosphate treated products with a heavy bound water content too can create such a situation. The problem can be alleviated to some extent by proper predusting. Starch-based predust is generally used. Gums (guar or xanthan gum) improve batter adhesion to the product through thermal gelation. Other ingredients, which have parallel or complementary effects, are methylcellulose, hydroxypropyl methylcellulose, carboxymethylcellulose, alginates, and carrageenan. Popular batters that can be used without breading are tempura or fish and chip batters, which is flour and starch based with a leavening agent such as bicarbonate. The thickness of the coating of batter/breading may be as high as 40 to 60% by weight of the product. As the batter/breading mix is relatively cheaper than seafood the products can be marketed at a competitive price. Shrimp are coated with batter and bread crumbs and flash fried for 20 sec at 180°C in refined vegetable oil. They are then frozen and packed in individually quick-frozen (IQF) form, preferably in thermoformed containers.[5,22] In northern Ireland, prawns (*Nephrops norvegicus*) caught by boats sailing from Northern Ireland are generally "headed" at sea and the tails blast frozen on landing. Once peeled the larger tails are refrozen, dipped in water to provide a glaze, and then packed for sale. Small tails are reformed then breaded and battered to become "scampi." Both of these products are stored and sold frozen.[23] Three categories of raw breaded shrimp are recognized based on the percent shrimp meat present, namely, lightly breaded (65% minimum shrimp), breaded (50% minimum shrimp), and imitation products (50% minimum shrimp).[24]

9.5.3.1 Coated stretched shrimp (*Nobashi*)

Increasing the length of peeled and deveined shrimp, minimizing its curling by making parallel cuttings at the bottom, and applying pressure using simple mechanical devices is a new technique. Increasing the length by about 1–2 cm depending on the size of the shrimp is possible by this method (Chapter 4). The stretched shrimp will have better appearance compared to conventional peeled and deveined shrimp. It also fetches higher price. The stretched shrimp, because of its increased surface area, will have more pickup of coating during battering and breading and

also good appearance. Coated stretched shrimps are packed in thermoformed trays under vacuum and frozen at −40°C.[22] A wide range of minced shrimp products are becoming popular globally, such as shrimp balls, shrimp nuggets, shrimp cakes, etc.

Scampi is targeted at the economy retail and catering markets. The small scampi are beheaded at sea and peeled on shore by means of a water jet inserted between shell and tail meat. The meat is mixed with polyphosphate or starch solution and extruded via a machine such as the Koppens former directly onto a continuous steel-band-belt freezer.[25] Frozen, tail-on scampi may be coated with tempura batter or bread crumbs.

9.5.4 Squid

Squid rings and stuffed squid are the popular coated products. Cleaned squid tubes are cut in the form of rings of uniform size, cooked in 3% boiling brine for 1–2 min followed by cooking, breading, and battering. The coated rings are flash fried (at 175–180°C for 20 sec), cooled, frozen, and packed.[22] The influence of corn flour, wheat, and leavening agent on the sensory quality of coated, fried squid rings has been examined recently.[26] The crispness of fried squid ring can be increased by incorporation of dextrins in batter formulation.[27] Stuffed squid is prepared from small squid, which are not generally processed for export. The cleaned tubes from small squids are filled with a stuffing mixture prepared using cooked squid tentacles, potato, fried onion, spices, etc. The stuffed squid are then battered, breaded, and flash fried.[22] Coated squid meat shaped in the form of frozen fish fingers has been marketed in Europe recently.[20]

9.5.5 Bivalves

Bivalves such as clams, oyster, mussels, etc. can be converted into coated products by the procedure discussed earlier. For clam products, the meat is shucked from depurated live clams and blanched in boiling brine. The meat is then battered,

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TABLE 9.3

Some Factors that Need to be Considered in Developing a Coated Seafood Product

Organoleptic qualities	Customer handling
Species	Nutritional properties
Seafood availability	Cost of production
Coating ingredients	Marketability
Seafood/coating content	Defects level
Plant and machinery	Supplier specifications
Packaging	Consumer abuse testing
Shelf life	

breaded, flash fried, and packed. Other bivalves such as oyster, mussels, etc. can also be converted into coated products by the same method. Table 9.3 indicates factors that need to be considered in developing coated seafood.

9.6 RECENT PROCESS INNOVATIONS

Several innovations are occurring in the coating industry in order to attract increasing consumer interests in the products, many of them being in the area of fishery products. These are discussed below.

9.6.1 Reduction of Oil Update During Frying

Present consumers are concerned about health hazards such as obesity and heart diseases, which are linked with consumption of excess of oil. These concerns can have a negative effect on the marketability of coated products, which can absorb cooking oil as much as 15 to 30% of its weight during the flash-frying process. Furthermore, repeated use of oil for frying and its overheating can produce a wide variety of lipid-degradation compounds, which can have adverse influence on consumer health. The content of oil in a fried product depends on a number of factors such as nature of the oil, frying time, temperature, shape, porosity, composition, and the surface area of the food.[9,28] Judicious control of these parameters can help reduce oil uptake in the fried products. Since composition of the batter is a major factor that influences oil uptake, attempts have been made to improve the batter composition with a view to reduce oil in the fried, coated products. Recent research has shown that a small amount of hydrocolloids (usually 1% of the formulated dry weight of the batter) can reduce oil absorption. The gelling ability of hydrocolloids together with their hydrophilic nature enable them to absorb less oil during the frying process.[8,29] The hydrocolloids most commonly used in this respect are methylcellulose and hydroxypropyl methylcellulose.[30,31] Powdered cellulose with particular fiber length (>100 μm) is also capable of reducing oil absorption.[30] Other dietary fibers useful in this respect are oat, soy, or pea. In addition, these substances develop a greater mechanical resistance than conventional batters, making it easier for the coagulated product to stay intact during handling. Further, they improve product appearance because of their ability to give the coating an even golden color after frying.[30]

9.6.2 Elimination of the Frying Step

A process for the manufacture of frozen, battered food products that altogether eliminates the troublesome flash-frying step has been developed.[8] In this process, an improved batter incorporating methylcellulose is used. Further, instead of frying in oil, the coated product is immersed in hot (70°C) water. The hot water dip makes the batter coagulate as a result of gelation of the cellulose. After the heating step, the product is further heated in a microwave oven for short period cooled, frozen, and packaged for storage at a temperature of −18°C or below. The second heating

FIGURE 9.3   Squid rings

blocks the structure formed by thermogelation of the methylcellulose. The product is consumed after frying. A key point in this method is the control of temperature during reconstitution of the batter dough and also during the battering step. A water temperature of 10 to 15°C has been suggested for reconstituting the batter. In this temperature range the methylcellulose becomes completely hydrated and is capable of forming a strong gel when the battered product is immersed in a hot water bath. At temperatures below 10°C or above 18°C the viscosity of the batter is not suitable for convenient use.[8]

There are a number of advantages in the improved process. Since the oil-frying step is eliminated, the product is free from oil and associated concerns regarding the presence of oil in the product. Elimination of this step also avoids the necessity for industrial fryers thereby resulting in considerable energy saving and cost reductions. Interestingly, the final product has texture, flavor, and color properties very similar to those of a traditionally manufactured product. While a conventionally prepared seafood product has about 35% oil content, the same product prepared by the innovative method contained about 16% oil.[8] Figure 9.3 shows squid rings and Figure 9.4 shows improved process for production of coated squid rings.

9.6.3 Microwave Cooking

Batter composition has also been modified to suit microwave cooking of the coated product, which is the currently popular heating method prior to consumption. It may be pointed out that the most appreciated sensory parameter of fried, coated products is their crispness. A lack of crispness may lead to chewy toughness or a mushy softness. Ideally, the coating should exhibit a structure that sufficiently resists the initial bite but then should disappear with a quick melt-away in the mouth.[32] However, prefried products cooked in microwave ovens tend to be undesirably soft and soggy because during microwave heating water is released from inside of the food to the outside. Improvement in the batter composition

has helped better felt crispiness of the microwave oven-cooked products.[8] In the improved process, which eliminates the frying step as discussed earlier, microwave heating of the product, prior to consumption, leads to further cross-linking of the cellulose, so that the coating formed does not soften when it is cooled. A dry mix that contains 60 to 70% of high amylose corn flour to provide crispy coating after microwave cooking has been developed.[33] Another method involved cooking the product before the battering and prefrying steps, which enhanced the sensory attributes of the product.[34]

9.6.4 Other Process Improvements

The sensory acceptability of breaded fishery products could be improved by innovations in the coating process with a view to modify the texture. This can be done either through increasing the number of coatings or by addition of ingredients to the fish before enrobing with batter and crumb. Such products, such as the "Kiev" style products, which consist of a savory filling surrounded by fish flesh and coated
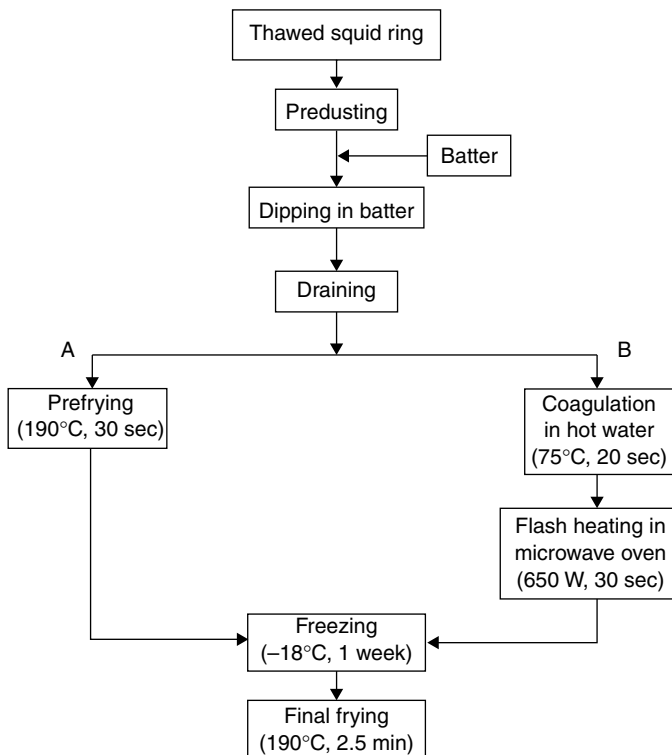
FIGURE 9.4 Improved process for production of coated squid rings. (A) Traditional process and (B) innovative process (Reprinted from Fiszman, S.M. and Salvador, A., *Trends Food Sci. Technol.*, 14, 399, 2003. With permission from Elsevier)

with breaded crumbs, can command much higher price. The packaging requirements for these products are similar to those for the simpler breaded products.[19] Another method, which avoids traditional coating and frying methods, uses a dry mix of seasoning and binder, designed as *Remora* to hold fish together during processing, cooking, and serving fish. *Remora* also contributes toward texture and flavor. The product is able to maintain its shape without the use of batter or crumb. It can be formed into a variety of different shapes, added to sauce, or enhanced with a glaze of novel coating. This unique blend of ingredients can add value to any fish variety.[35]

9.6.5 Grilling and Sauce Coating

While microwave oven heating is the current usual practice at home, in commercial food services, grilling of the product is becoming popular. Apart from regular breaded and battered fishery products, grill marked and sauce coated fillets are attracting consumer interests. The latter products appeared in the market in the 1990s. In order to get a high quality grilled product, it is important to optimize the temperature parameters during the grilling process. This involves mapping the temperature distribution in grill heating surface before cooking to minimize temperature variations. A study was therefore made on the heating rate and temperature distribution profiles of a clam-shell grill under commercial cooking conditions. It was found that the grill surface temperature dropped to 113°C from a preset 177°C after frozen patties were placed on the grill surface. The temperature of the patties recovered to only 129.4 to 144.5°C at six locations at the end of the cooking process with a maximum temperature difference of 15°C. The contact heat transfer coefficient between the grill and patties varied with heating temperature and time during cooking.[36]

A grilled fish fillet having less fat and flavored with sauce has been developed. In this process, the fillet was applied with olive oil and then shed to the cooking tray in a grill. This significantly reduced the fat content of the cooked fish. The use of a flavored sauce over the grill-marked fish gave flavor to the product. The sauce generally is composed of water (40–60%), vegetable oil (10–50%), seasoning (5–25%), and gum thickener (0.2–1%). Popular sauce flavors include lemon-pepper, Polynesian (pineapple sauce sprinkled with toasted coconut), smoked barbecue, and tomato. The sauce is applied to the seafood using conventional batter recirculating equipment. The sauce is deposited on the grill-marked fish. The sauced fish are individually packaged in pouches using skin-sealing trays or placing each portion in a compartmentalized tray that keeps the pieces with its sauce coating physically separated from an adjacent piece. A popular fish-in-sauce product in Europe, particularly Germany, is "*schlemmer filet*," which consists of a rectangular portion cut of whitefish fillet block, often cod or pollock, topped with a layer of sauce. Individual boil-in-bag fish-in-sauce products comprise portions out from fish-fillet blocks with sauce variants such as butter, mushroom, and parsley. Boil-in-bag process lines tend to be highly automated using pouch-packaging system.[20] A pilot-plant demonstration unit has been installed for applying freezing layers of

sauces and gravies on to IQF-foods. The IQF product is fed into a chamber when the sauce and liquid nitrogen are sequentially injected resulting in the formation of frozen layers of sauce on the food, uniformly coating each piece of the food product. The coating can be as high as 50%. The system can also use liquid carbon dioxide with a slightly different injection system.[37]

9.7 EQUIPMENT AND INGREDIENTS

Progress in coating technology has been synonymous with development in machinery and equipment. Following the first breading machine developed in 1931, several sophisticated equipments have come into the market. Modern predusting machines handle products on a continuous basis, to give a uniform coating, while avoiding transfer of dust to the environment. Products enter the machine onto a bed of predust and pass below a shower of predust, with controls to minimize excess dusting. For battering, liquid enrobers are available, for pumping thick batters and also for tempura batters. Conventional pumpable batters are normally recirculated within the applicator or pumped continuously between a batter make-up system and applicator. Tempura batters, which contain raising agents, should not be pumped since continuous pumping removes the carbon dioxide and inhibits the characteristic "rise" of the batter. The tempura application machine takes care of these requirements. Machine for application of bread crumb consists of a hopper with pneumatic vibrating exist systems that ensures an equal covering of the large and small particle crumb onto the product. Sophisticated frying systems have also been developed to ensure high quality oil.[3] Commonly used equipment for fishery products are graders, band saw, forming machines, kneading machines, predusters, battering and breading machines, cookers and fryers, and packaging machinery. Other machinery includes meat–bone separators, shrimp peeling and deveining machinery, cryogenic freezers, and spiral-belt freezers. A variety of fully automated and computerized battering and breading machines of varying capacities are available.

A wide array of ingredients is used for the various processes in the coating operations. Suppliers produce a range of coatings and batters such as varieties of breadcrumbs and Japanese style coatings, batter mixes, and flavored coatings, breading, batters and stuffing, low fat breading, and Japanese crumbs. There are also suppliers of confectionery crumbs and batters, dry savory flavors, bakeable caramel, and other ingredients for meat, fish, poultry, and vegetables. Suppliers of some equipment and breading, flavoring, and other ingredients have been indicated in the Appendix.

9.8 QUALITY CONTROL

Quality control of coated products is important for standardization of the process. The quality control is important not only at the levels of the raw material, batters, and packaging but also to determine the acceptable storage life under frozen conditions. One of the chief quality parameters of coated products is the amount of coating and fish flesh content. Fish flesh content is determined using the formula,

$(W_a/W_b) \times 100$, where W_b is the weight of battered and breaded test sample, W_a is the fish meat devoid of the coating. Fish content varies between 30 and 70% in these products.[20] The Food Advisory Committee of the U.K. government has recommended a minimum fish content of 55% for battered, and 60% for the fingers coated with breaded crumbs. In the United States, the Department of Commerce has specifications for flesh contents of battered products. A minimum of flesh content of 75% has been specified for raw, breaded fish portions, and 65% for pre-cooked portions. The flesh requirement for raw and precooked scallop products is 50%. The values for other products are fish sticks, 72%; precooked product, 65%; lightly breaded shrimp, 65%; and raw breaded shrimp, 50%. No specifications for flesh contents have been prescribed for finfish and oyster products.[17]

Length of storage is also important in determining the quality of the product. While the acceptable shelf life of the product is greatly dictated by the nature of the raw material, the process also has an influence. Long-term frozen storage of battered/breaded products could lead to moisture migration to the coating/breading. It has been reported the coated fillets of croaker (*Sciaenid* spp.) and perch *Nemipterus* spp. were acceptable up to 22 weeks of storage at $-20°C$.[2,38] Packaging is another important component that determines the shelf life of the product. Fish fingers packed in low- or high-density polypropylene; nylon pouches remained in satisfactory condition up to 32 weeks at $-20°C$.[39]

Although instrumental methods have been used to evaluate texture of fried, coated products, parameters such as crispness or crunchiness, fragility, tenderness, etc. are hard to quantify using empirical mechanical methods.[8] Measurement of puncture with a plunger is the technique that is mostly used.[10] Other parameters such as greasiness, juiciness, oiliness, and mealiness of coatings have been assessed with trained panelists.[11] Analysis was done on the microstructure of battered squid rings produced by the modified process. In the improved process (Figure 9.3), the prefried products had a structure that interconnected the batter and the squid muscle, whereas, in the fried product, the two layers separated.[8]

9.9 CONSUMER ACCEPTABILITY

Breaded fishery products are generally well accepted by the consumers throughout the world. This is essentially due to their appealing crispness and flavor. Whereas, development of unpleasant and characteristic odor and toughening of the meat are known to be associated with prolonged frozen storage of fishery products, these factors may not be predominant in coated products and, therefore, they do not seriously influence consumer acceptance of these products. A survey involving 350 home economic students showed that the acceptability of cod products, which included breaded fillet fish fingers, minced fish fingers, and also water-glazed fillets remained essentially unchanged even after one year of storage of the products at $-18°C$. While a trained panel detected significant increases in cold storage odors and toughening, the consumer panels did not seem to be influenced by these to any great extent. Rather, they were more influenced by the form in which the fish was presented, preferring the fillet codfish fingers most and the minced codfish fingers

least. The overall acceptability of glazed fillet deteriorated with time, there being little difference between minced finger and glazed fillet after 12 months.[40]

9.10 MARKETABILITY

Marketing of coated fish products is different from that for traditional seafood products. Attractive appearance, specific packaging, and appealing display are important factors that can help successful marketing of any coated product. Packaging requirements change with product form, target group, market area, species used, and other factors. A wide variety of block-based frozen, coated products is marketed by large and popular retail chains of Europe. The most common product in terms of total quantity and overall distribution in Europe seems to be fish fingers.[20] Whereas fish finger and other coated finfish products traditionally enjoy domestic markets in several Western countries, several shrimp-based products unique to Asia have entered major international markets in recent years. Their popularity could be attributed to a growing migrant population and the influence of travel and exposure of a majority of consumers to exotic taste and food habits.[24] Table 9.4 presents volumes of international trade in some value added fishing products. Several monetary reasons for exporting the value-added further processed shrimp products have been identified. These include potentials for higher profitability and also less sensitivity of such products to fluctuations in price and consumer acceptability, as compared with raw commodities. Nevertheless some issues remain that need to be resolved before third world shrimp processors can confidently enter these new markets. Access to international markets is often difficult, and in some instances, impossible without joint ventures or, at least, contract manufacture. The regulatory and markets requirement for product composition and presentation vary considerably from country to country. In addition, processing and ingredient technology necessary for the manufacture of further processed shrimp

TABLE 9.4
Some Value-Added Product Traded Internationally During 2000–2002

Product	2000	2001	2002
Fish fillets, coated in batter, cooked or not	75,071	77,784	82,240
Fish, spiced, marinated	8,339	7,254	7,809
Fish, meat, balls, cakes, sausages, etc.	8,760	7,749	6,570
Imitation crab sticks	27,148	22,745	20,102

Volumes are given in tons.

Source: Adapted from *FAO Yearbook: Fishery Statistics*, *Commodities*, Vol. 95. Food and Agriculture Organization of the United Nations, Rome, Italy, 2002. With permission.

may not be readily available in third world countries. Efforts are required to address these problems for global expansion of the coating industry.[41,42]

In conclusion, the increasing interests in ready-to-prepare and ready-to-eat products can be the driving forces for success of the coated seafood product industry. Apart from the popular species, the under-utilized fish can be an easy choice as raw material for coated products. Besides, the upcoming aquaculture industry can provide considerable amounts of raw material for value addition through this process. The coating process can be a boon to these species, because of the scope of the process to enhance flavor and acceptability of these species. Parallel progresses in the coating and *surimi* technology has immense potential for value addition of fishery products.

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10 Radiation Processing

10.1 INTRODUCTION

Food irradiation is a process that has significant potential to enhance the shelf life and microbial safety of fishery products and other muscle foods. Research in food irradiation started a few years after the discovery of x-ray by the German physicist Roentgen in 1895. During the early years, studies on irradiation of food items were pioneered by Massachusetts Institute of Technology and the Natick Laboratories in the United States, which led to recognition of the advantages of the technique. Efforts of these institutions and also of other laboratories worldwide led to detailed understanding of the chemistry and microbiology of food irradiation, process development, and recognition of commercial benefits of the technology.[1–8] The major advantages of low-dose irradiation are control of spoilage of perishable food commodities, enhancement of their hygienic quality and safety, and use of radiation treatment for inactivation of food-borne insects, as an alternative to chemical fumigants. The two major problems that confront the current commercial fisheries are high perishability and possible presence of pathogenic microorganisms, which pose problems in their marketing (Chapter 2). This chapter discusses the scope of irradiation of seafood to alleviate these problems.

10.2 RADIATION TREATMENT OF FOOD

10.2.1 Radiation Sources

Generally, ionizing radiations emitted by radioisotopes, ^{60}Cobalt and ^{137}Cesium, are used for food preservation. ^{60}Co isotope (half-life, 5.27 years) emits two gamma rays of 1.17 and 1.33 million electron volts (MeV), whereas, ^{137}Cs (half-life, 30.2 years) emits one gamma ray of 0.66 MeV. ^{60}Co is made by neutron bombardment of ^{59}Co, which stabilizes by emitting radiations and forming nickel, as follows:

$$^{59}_{27}\text{Co} + ^{1}_{0}\text{n} \rightarrow ^{60}_{28}\text{Ni} + \beta + 2\gamma$$
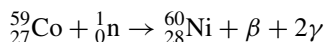
On the other hand, $^{137}_{55}$Cs, a fission by-product, emits β-particles and a γ ray to stabilize $^{137}_{56}$Ba. From a practical point of view, ^{60}Co is preferable to ^{137}Cs since,

the latter apart from having weaker gamma ray, is also water soluble, thus posing problem of environmental contamination.

The major radiation source for treatment of food products has been ^{60}Co because of higher penetration power of the gamma rays and relatively more uniform dose distribution. However, increasing uses of food irradiation, shortage in the supply of ^{60}Co, and public concerns using the isotope are encouraging the use of other sources, particularly, electron beams (EB) and X-rays. EBs are produced by Van de Graaff generators or linear accelerators and therefore, can be turned off and on. EBs can be converted into more penetrating X-rays using sophisticiated equipment. For food irradiation EBs at energy levels up to 10 MeV and X-rays at energy levels up to 5 MeV are permitted. Even though electrons are less penetrating than gamma rays, they can be very useful for irradiating large volumes of free-flowing food items, such as grains, thin fish fillets, or food packages (not more than 8 to 10 cm thickness) with a density of 1 g/cm^3. However, the effects of gamma rays and EBs on food are comparable. X-rays have similar penetrating power as gamma rays. However, X-rays are not used in food irradiation due to poor conversion of accelerated electrons to X-rays. Selection of radiation source is made based on the type of the product being irradiated, radiation dose required, and the availability and economics of the source. In the past few years there has been increasing interest in using EBs instead of radioisotopes. Accelerators are being developed to meet the demands of emerging commercial applications. The electrically driven accelerators can eliminate some of the public concerns associated with the transportation, installation, and operation and waste disposal of radioisotopes. Table 10.1 gives comparison of gamma rays, EBs, and X-rays with respect to their applications.

TABLE 10.1
Comparison of Ionizing Radiations from Different Sources

Type of source	Advantages	Disadvantages
Gamma rays from Cobalt-60, Caesium-137	Good penetration	Low energy and intensity
	Reliable	Continuous emission
	Proven technology	Licensing tightly regulated
		Characterized as nuclear and hence consumer concerns
		Limited suppliers
Electron beam (EB)	High efficiency and intensity	Limited penetration (0.5 cm/MeV)
	Possibility for on and off operation	Machine parameters not easy to monitor
		Potential for interruption
		Limited range of operation
X-ray	Hybrid of EB and gamma rays	Low conversion efficiency
	Good penetration	Higher cost than EB
	Small radiation area	High heat in converter plate
	Simple conveyors	

10.2.2   Interaction of Radiation with Food

The electromagnetic radiations, gamma and X-rays having lower wavelength (<300 nm) and higher energy than visible light can cause ionization by removing electrons from the outer shells of atoms. These radiations interact with foods in three ways, namely, Compton effect, photoelectric effect, and pair production.[1] In Compton effect, an incident photon interacts with the atoms and transfers its energy, causing ejection of electrons. The ejected electrons, referred as Compton electrons, cause further excitation and ionization in the food material. The incident photon continues after collision in a changed direction with less than its original energy. Since gamma rays and X-rays produce Compton electrons on interaction with foods and because these electrons react the same way as accelerated beam electrons, the changes produced in the irradiated material are the same regardless of the type of radiation used. The photoelectric effect is caused by photons with energy less than 0.1 MeV, whereas pair production occurs with photons of energies above 10 MeV. These effects are generally unimportant mechanisms of energy transfer in irradiated foods.[1,2]

The radiation-induced ionization in atoms and molecules giving rise to free radicals leads to subtle changes in the chemical structure of treated biological materials. (Formation of free radicals is not unique during gamma irradiation, since they are also formed during metabolic processes in human body, during heat pasteurization, cooking, baking, broiling or frying, or exposure of foods to infrared or microwaves.) The main primary reaction during irradiation of foods is the radiolysis of water which gives rise to various active species:

$$H_2O \rightarrow {}^{\bullet}OH\,(2.7) + e_{aq}^{-}\,(2.7) + {}^{\bullet}H\,(0.55) + H_2O_2\,(0.45) + H_3O^{+} + H_2$$
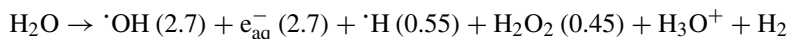
The figures in parentheses indicate the relative amounts, expressed as G values, of each species formed as a result of absorption of 100 ev (1 J/kg) of absorbed energy. Among primary radiolytical products of water, the hydroxyl radical is a powerful oxidizing agent and is most reactive toward unsaturated compounds, especially with conjugated polyene chains. Free radicals (${}^{\bullet}R$), electrons, and cations produced by primary effects of ionizing radiations are very reactive, which can interact among themselves or with food constituents. Such secondary effects include combination of free radicals, dimerization, and capture of electrons by cations. The hydrated electrons are also reactive and react mostly with aromatic compounds, carboxylic acids, ketones, aldehydes, and thiols. Hydrogen atoms react by abstracting hydrogen from C—H bonds or they can add to olefinic compounds. The reactivity of free radicals depends on their diffusibility in the medium, which is dependent upon the availability of free water in the product. In dry solids or frozen foods, diffusion is restricted and hence the changes induced by free radicals are also less.[1,2]

10.2.3   Absorbed Dose and Dose Rate

The quantity of energy absorbed by the food during irradiation is called "absorbed dose." The unit (SI) for irradiation dose is the Gray (Gy), which is equal to

the absorption of energy equivalent to 1 J/kg of absorbing material. One Gray is equivalent to 100 rad, or "radiation absorbed dose." The dose rate of gamma rays from commercial ^{60}Co sources is 1–100 Gy/min, while those of EBs from accelerators are 10^3–10^6 Gy/sec. When an EB penetrates in an aqueous medium, the dose somewhat below the surface is higher than at the surface. This is due to the formation of secondary electrons, which because of their lower energy, are more effectively absorbed than the primary electrons. Thus, a 10 MeV EB giving a dose of 10 kGy at the surface will deposit about 12.5 kGy at 2 cm below the surface. The absorbed dose decreases with increasing depth and at about 5 cm the limit of penetration is reached. In contrast, the absorbed dose decreases continuously with gamma and X-rays. Since uniform absorption of dose by the material is not attained, the ratio of highest dose (D_{max}) to lowest dose (D_{min}) is taken as dose uniformity ratio or overdose ratio. The measurement of D_{max} and D_{min} in the product is basic to any consideration of the effectiveness of the process, the legal acceptability of the treatment, and the design and economics of the irradiation plant. Average dose (D_{av}) is the value obtained by dividing the total dose measured by the number of measurements. The minimum absorbed dose should be sufficient to achieve the technological purpose and the maximum absorbed dose should be less than those, which would compromise consumer safety, wholesomeness, or would adversely affect the properties of the food.

As per "good irradiation practice," the dose range should be as narrow as technically feasible. The ratio of maximum to minimum doses in radiation-processed foods is usually in the range of 2 to 3. A maximum dose of 10 kGy is currently applied depending on the nature of the food and purpose of treatment. This is in accordance with the conclusion of the Food and Agriculture Organization (FAO)/ International Atomic Energy Agency (IAEA)/WHO Joint Expert Committee on Food Irradiation (JECFI) that irradiation of any food up to an overall average dose of 10 kGy causes no toxicological hazard and introduces no nutritional or microbiological problems.[1,9] On the basis of the findings of the JECFI, the Codex Alimentarius Commission (CAC) developed General Standards for Irradiated Foods and a Recommended International Code of Practice for the Operation of Radiation Facilities Used for Treatment of Foods.[10] According to the Code, the limiting maximum dose was defined to allow less than 2.5% of the product to receive a dose above 15 kGy (which is 50% above the "overall average" dose of 10 kGy).

10.2.4 Conditions of Irradiation

Food irradiation is essentially a cold process because the treatment does not cause a significant rise in temperature. Treatment of fresh fish to a dose of 10 kGy results in a temperature rise of only 2.3°C. However, the temperature of the product being irradiated has an influence on radiation-induced changes. Movement of free radicals increases with the product temperature, affecting the overall rate of radiolysis. Treatment at lower temperature reduces the production of volatiles in food products, which are known to affect the sensory quality of irradiated foods.[11] Such changes are at a minimum in frozen products.

The presence of oxygen during irradiation and subsequent storage has an important influence on the radiolytic changes occurring in the food system.[1,2] Irradiation in presence of oxygen can lead to formation of hydroperoxy radicals ($^{\cdot}HOO$) through the reaction of hydrogen atoms formed during radiolysis of water with oxygen. Superoxide can be formed by the reaction of solvated electron with oxygen ($^{\cdot}O^{-}$). Both hydroperoxyl and superoxide radicals can give rise to hydrogen peroxide as:

$$2^{\cdot}HO_2 \rightarrow H_2O_2 + O_2$$

$$O_2^{-} + {}^{\cdot}HO_2 + H^{+} \rightarrow H_2O_2 + O_2$$
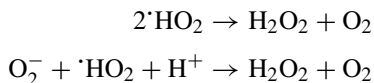
Upon irradiation, oxygen can also form ozone (O_3), a very powerful oxidizing agent. During gamma irradiation, oxygen from the atmosphere can diffuse into the system, than is depleted in the foods by reactions, as mentioned earlier. In EB irradiation, which is usually at higher dose rate than gamma irradiation, oxygen in the system is depleted at a rate greater than it is replaced by diffusion of the atmospheric oxygen. Oxygen facilitates indirect reactions through the formation of active oxygen groups including superoxide anion radical and hydroxyl radicals at high dose rates, which can influence the end result of irradiations. At high dose rates, free radicals can be formed in such high concentrations, in which recombination of radicals rather than reaction of radicals with food components is favored.[12]

Apart from the environment and temperature, other factors that influence the radiation-induced changes are absorbed radiation dose, viscosity, and composition of the food. These can be usually controlled in order to avoid the formation of less-desirable end products. It has been estimated that a dose of 10 kGy may yield up to 1 millimole of radiolytic products for 1 kg of irradiated food. It has also been generally agreed that irradiation of food does not cause formation of unique radiolytic products in amounts that can cause any hazard to the consumer.[7]

10.3 EFFECT OF IRRADIATION ON FISH MUSCLE COMPONENTS

10.3.1 Proteins and Amino Acids

Extensive data on radiation chemistry of amino acids, proteins, and other food components are available.[11,13,14] Free radicals formed by radiolysis of water, namely, $^{\cdot}OH$, $^{\cdot}H$, e_{aq}^{-} react with amino acids leading to abstraction of proton and reductive deamination. The produced radicals will react further by disproportionation reactions, which are followed by decarboxylations and deaminations, giving rise to ammonia and pyruvic acid, for example, in the case of alanine. In the presence of oxygen, oxidative deamination occurs instead of reductive deamination. Cystine, cysteine, and methionine act as scavengers and react more readily with free radicals than the aliphatic amino acids. The aromatic amino acids, phenylalanine and tyrosine react readily with the transient species formed during radiolysis

of water. Hydroxylation of phenylalanine to form tyrosine isomers is one of the principal reactions. Further hydroxylation converts these isomers to dihydroxy phenylalanine (DOPA). Subsequent oxidation of DOPA and polymerization can produce melanin-type pigments ("blackspot"), as observed in the case of shrimp (Chapter 2).

When proteins are irradiated in the presence of water, all the reactions that are possible with amino acids are also possible with the macromolecules. However, as compared with amino acids, proteins are more stable to irradiation, due to their more rigid molecular structure. A large proportion of radiation energy deposited in an irradiated protein apparently goes into protein denaturation, although much less as compared with heating. There can be structural changes in food proteins consequent on radiolytic reactions. Such reactions may cause changes in functional properties such as viscosity.[1] The extent of radiolytic reactions is too small to cause appreciable changes in the amino-acid content of food proteins.[15]

10.3.2 Color

Color is an important parameter in determining the quality of fishery products.[16] Radiolytic products can cause oxidation of myoglobin leading to discoloration, off-odor, or off-flavor compounds. Significant color changes have been reported when myoglobins isolated from fishery products are irradiated. Thus, the pigments purified from Black pomfret (*Coryphaena hippurus*) and Indian salmon (*Eleutheronema tetradactylum*) showed quantitative differences in terms of dose required for conversion of metmyoglobin to oxymyoglobin.[17] In fillets exposed to radiation doses of 1–3 kGy, packed aerobically or anaerobically at 0–2°C, it was observed that the extractability of myoglobin reduced progressively as a function of storage period, although there was no decrease in visual color and organoleptic acceptability.[17] The pigment in Indian mackerel was more resistant when irradiated *in vivo*, as compared to *in vitro* irradiation.[18] This has been suggested to be due to presence of antioxidants in the meat, which protected the pigment. Presence of CO_2 favored formation of metmyoglobin from oxymyoglobin in the case of catfish fillets. Low-dose irradiation in presence of CO_2 further enhanced the formation of metmyoglobin and made the fish fillet slightly pinkish.[19]

10.3.3 Lipids

The direct effect of radiation on lipids is the production of cation radicals and molecules in the excited state, as shown below:

$$R-CH_2-O-CO-(CH_2)_nCH_3 \rightarrow R-CH_2-O-(CH_2)_nCH_3^+ + e^- \text{ (ionization)}$$

$$R-CH_2-O-CO-(CH_2)_nCH_3^* \text{ (excitation)}$$
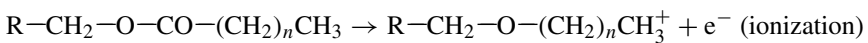
The main initial reaction of cation radicals is deprotonation, followed by dimerization or disproportionation. Excited triglycerides can also undergo a wide variety

of reactions leading to a large number of products.[1,20] The general mechanism of the radiolysis of fats is thought to involve primary ionization, followed by migration of positive charges toward the carboxy groups and the double bonds, and cleavage at preferential positions near the carbonyl group. The resulting free radicals engage in various reactions leading to the formation of stable radiolytic products, which have been classified as primary, recombination, and secondary products according to the mode of their formation. In the case of unsaturated fatty acids, dimerization reactions may take place. The course of radiolysis in fats is significantly influenced by temperature. Fatty acids, when exposed to high-energy radiation, undergo preferential cleavage in the ester-carbonyl region giving rise to certain radiolytic compounds, typical for each fatty acid. Of these, two hydrocarbons are produced in relatively large quantities. One has a carbon atom less than the parent fatty acid and results from cleavage at the carbon–carbon bond near to the carbonyl group; the other has two carbon atoms less and one extra double bond, and results from cleavage at β to the carbonyl. These hydrocarbons have been detected in the radiolytic products of fish oil.[20] The free radicals formed by radiolysis of water may also react with lipids to give various products. Depending upon the site of cleavage of the triglycerides, various primary and recombination products including esters, ketones, hydrocarbons, and diglycerides are formed.

Fish lipids, being more unsaturated, are sensitive to oxidation compared with those of red meat.[6,20–23] It is generally assumed that irradiation in the presence of oxygen leads to autooxidation, and that the pathways are the same as in the light-induced or metal-catalyzed autooxidation. In presence of air, higher extent of lipid oxidation has been reported.[18] During prolonged storage the peroxides may react with other constituents. Myoglobins present in the muscle of several finfish species may also enhance radiation-induced lipid oxidation.[17] Since fishery products contain comparatively higher concentrations of unsaturated lipids, the influence of irradiation on lipid oxidation on sensory quality of fishery products has been examined in detail. However, it was observed that rancidity was not significant in air-packaged irradiated Indian mackerel.[18] Eventhough there was initial increase in 2-thiobarbituric acid (TBA) value, an index of rancidity in irradiated fish, the TBA values decrease during storage. Irradiation at 0.5 kGy had no significant effect on TBA value of both air and CO_2/air atmosphere-packaged catfish fillets, having 3 to 11% fat.[19,24]

The influence of gamma irradiation up to 5 kGy on fat oxidation in skin and flesh fractions of Indian mackerel, white pomfret, and seer during ice storage was examined.[21] TBA values increased in both control and irradiated fish particularly in mackerel and seer meat, indicating development of rancidity. In mackerel, however, the TBA values declined toward later part of storage. Since lipids are significantly present in the skin, the influence of irradiation on fish skin was examined. The skin of pomfret, but not that of mackerel and seer showed radiation-induced oxidation, which increased further during ice storage.

Influence of irradiation on fish triglycerides and fatty acids has been examined. In the case of mackerel, there was progressive decrease in the initial content of triglycerides (85 g/100 g of lipids) with increase in free fatty acids during

refrigerated storage. This was true both in unirradiated and irradiated fish.[6] Irradiation of grass prawns at 10 kGy caused 16% decrease in linoleic acid contents, while no significant decrease was noted in linolenic acid.[25] Irradiation of tilapia at 1.5 to 10 kGy caused a decrease in $C_{14:0}$, $C_{16:0}$, and $C_{16:1}$ fatty acids. In the case of Spanish mackerel, $C_{16:0}$ and $C_{16:1}$ fatty acids were decreased by irradiation at 1.5 to 10 kGy.[26] Concentrations of some fatty acids, including $C_{18:0}$ and $C_{18:1}$, however, increased due to irradiation. There were no changes in fatty acid compositions of two species of Australian marine fish irradiated at doses up to 6.0 kGy. These results generally agreed that irradiation of fish had only marginal effects on essential fatty acids.[26,27]

10.3.4 Vitamins

Many authors have studied the influence of irradiation on the stability of vitamins in foods including fishery products.[22,28,29] These studies in general, have concluded that irradiation-dependent losses of vitamins in finfish products were not significant except perhaps in the case of thiamine.[6] Sensitivity of thiamin to irradiation has been observed in grass prawns.[25] In tilapia and Spanish mackerel, the loss of thiamin was significant only at radiation doses of 4.5 kGy and above.[30] The losses of riboflavin by irradiation, heating, and a combination of irradiation and heating were 6, 9, and 16%, suggesting that nicotinic acid and riboflavin were more resistant.[30] Nevertheless, some loss of riboflavin during storage of irradiated cod and dogfish fillets and meat products has been reported.[16] Irradiation of shrimps at 2.5 kGy caused 15% loss of riboflavin in air, 8% in vacuum, 20% in nitrogen.[1] With increase in dosage, tocopherols in tilapia and Spanish mackerel decreased, with further loss during storage.[26,31]

Loss of folic acid in shrimp was 18% when irradiated in air at 2.5 kGy.[22] When ground fish fillets were irradiated with a dose of 1 kGy at a temperature of 0°C, mackerel and cod lost 13 and 16% pyridoxine, respectively; at 10 kGy, the losses in corresponding fish items were 13 and 46%, respectively.[27] The effect of irradiation up to 6 kGy on fatty acid composition and Vitamin E content of black bream and redfish, two major Australian marine fish was evaluated. There were no significant changes in the above parameters.[27] There was no loss in vitamin B_{12} in clam meat following heat processing after irradiation at a dose of 45 kGy.[1] Losses of vitamins are generally higher in thermally processed foods as compared with low-dose irradiated items.

10.3.5 Enzymes

Low-dose irradiation may have some effects on the fish muscle enzymes. Lysosomes are cellular organelles, which contain high concentrations of hydrolytic enzymes including proteolytic enzymes such as cathepsins. The distribution of lysosomal hydrolases in the muscle and skin has been ascertained in many fish varieties.[32,33] Gamma irradiation is known to damage lipoprotein membrane and release hydrolytic enzymes from lysosomes, which are present in skeletal muscle

and skin. It was observed that the levels of lysosomal *N*-acetyl-β-glucosaminidase and acid phosphatase increased considerably following irradiation of pomfret, shrimp, tilapia, and Bombay duck at 2.5 kGy.[32,33] Irradiation is beneficial in the control of Ca^{+2}-mediated proteolysis and softening of the muscle.[34] In irradiated shrimp muscle cells, calcium ions in the sarcoplasmic reticulum and nuclei were not released to the myofibrils, and remained *in situ* during 8 days of postirradiation (5 kGy) storage at 4°C. In contrast, the ions in the myofibrils of unirradiated muscle cells increased after the same storage period and caused muscle softening. Irradiation, however, can enhance blackening of shellfish such as shrimp. The treatment activates a pro-phenoloxidase from shrimp and lobster, the activated enzyme catalyzes the oxidation of phenolic compounds causing melanosis or "blackspot" in the shellfish (Chapter 2).[6,23] Low-dose gamma irradiation has also been shown to enhance the sensitivity of sarcoplasmic proteins of fish muscle to hydrolysis by trypsin.[18]

10.3.6 Texture

Irradiation can have influence on textural attributes of fish and shellfish muscle.[35–37] In Bombay duck, irradiation enhanced drip formation in the fish to a value as high as 20%. The problem however could be reduced to 7–8% by preirradiation dipping of the fish in 10% sodium tripolyphosphate and sodium chloride.[35] Treatment at a dose of 0.66 and 1.31 kGy caused a decrease in gel strength of mince of hake (*Urophycis chuss*).[36] However, irradiation at 1.5 kGy did not affect the solubility and viscosity characteristics of structural proteins of Indian mackerel.[38] The degree of textural changes in precooked lobster by irradiation at 1 kGy was comparable to that developed after storage for 3–4 months.[37] These changes could be minimized by reducing the temperature during irradiation.[39]

10.4 Effects on Biological Contaminants in Fishery Products

10.4.1 Microorganisms

Since one of the major benefits of low-dose irradiation of seafood is inactivation of contaminant microorganisms, this aspect has been studied in detail by many authors.[40–44] The inactivation of living cells by irradiation is due to scission of single or double strands of DNA, which is essentially caused by the ˙OH radical formed by radiolysis of water. The damage caused to DNA is enough to cause inactivation of a large fraction of the microbial cells. In addition to DNA damage, ionizing radiation has also been shown to cause damage in the membrane and other structures causing sublethal injury. A dose of 0.1 kGy can damage up to 2.8% of the DNA in bacterial cells, 0.14% of the enzymes, and 0.005% of amino acids.[45] A large volume of information is available on the sensitivity of microorganisms to radiation treatment. Radiation sensitivity of microorganisms differs with genera

and even species, all the extrinsic conditions (media, temperature, oxygen, pH, water activity, etc.) being equal.[2,40,43,46,47] Gram-negative bacteria, which are more responsible for spoilage of fishery products, are generally more sensitive than gram-positive bacteria. Vegetative cells are highly sensitive as compared with bacterial spores, although some bacteria such as *Dinococcus* (formerly *Micrococcus*) *radiodurans* and members of *Moraxella–Acinetobacter* require comparatively higher doses for their inactivation. Irradiation at low (up to 1 kGy) to medium (up to 10 kGy) doses essentially brought about suppression of spoilage-causing gram-negative organisms, such as *Pseudomonas*, *Proteus*, *Aeromonas*, etc. leading to a predominance of biochemically less active gram-positive bacteria. The radiation sensitivity of pathogens including *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Salmonella typhimurium* when irradiated in fish medium was not affected by the fat content of the fish, which ranged from 0.39 to 32.5%.[48]

The radiation survival profiles of gram-negative microorganisms, namely, *Aeromonas hydrophila*, *Proteus vulgaris*, a *Pseudomonas* spp., and an *Achromobacter* spp. isolated from spoiled fish have been examined.[14,40,43] The survival curves of gram-positive organisms are generally characterized by an initial shoulder indicating resistance to radiation, which is many times higher than those of the gram-negative organisms, as shown in Figure 10.1. The sensitivity
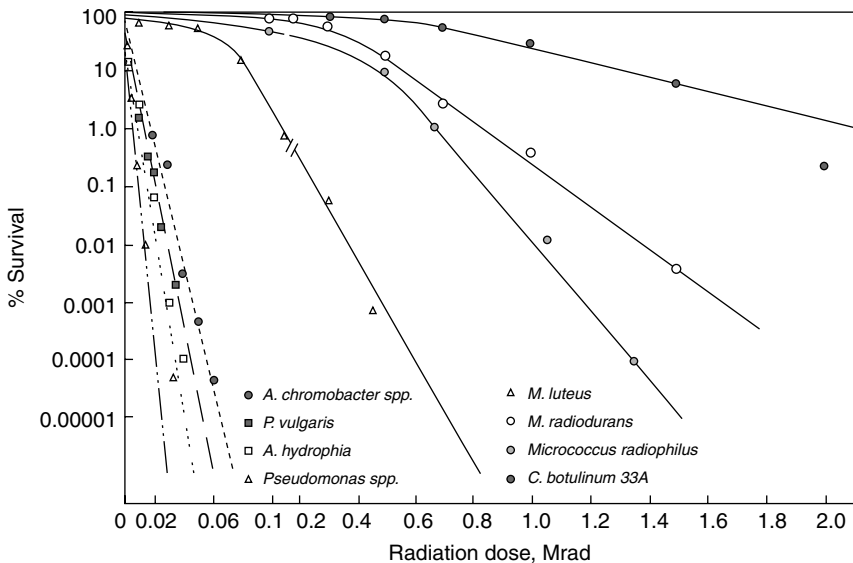
FIGURE 10.1   Radiation sensitivities of some bacteria. Percent survival was obtained after exposing buffered cell suspension to gamma radiation at a dose rate of 0.11 kGy/min (Cited from Venugopal, V., Doke, S.N., and Thomas, P., *Crit. Rev. Food Sci. Nutr.*, 39, 391, 1999. With permission)

to radiation, defined as the dose required for killing 90% of microbial population, is expressed as D_{10} value. D_{10} value is influenced by the type of organism as well as irradiation conditions. Gram-negative organisms had lower D_{10} values (0.04–0.09 kGy) as compared with gram-positive organisms. The D_{10} values of typical spoilage-causing organisms such as *Pseudomonas* spp., *Aeromonas* spp., and *Proteus* spp. range from 0.045 to 0.07 kGy. The microbial flora of fresh fillets of Atlantic cod caught in the summer months composed of mesophilic gram-positive bacteria, with a predominance of micrococci, bacilli, and coryneforms bacteria. The D_{10} values (kGy) of these organisms showed variations, as observed in the case of *Flavobacterium* spp. (0.47–1.06), *Micrococcus* spp. (0.13–0.83), *Staphylococcus* spp. (0.1–1.12), *Aeromonas* spp. (0.09–0.11), *Bacillus* spp. (0.88–1.11), *Streptococcus* spp. (0.16–0.36), and Enterobacteriaceae (0.09–0.11).[49] Resistance to irradiation by certain gram-positive microorganisms such as *Micrococcus radiodurans* and *Micrococcus radiophilus* has been attributed to their unusual ability to repair large numbers of breaks in DNA caused by irradiation.[45–47] The D_{10} values of spores of *Clostridium botulinum* A-33, *C. botulinum* B-53, and *C. botulinum* E have been reported to be as high as 3.3, 3.3, and 1.4 kGy, respectively.[1] The radiation treatment also inactivates microorganisms responsible for histamine formation in some species of fish.[50]

Molds and yeasts have lower resistance as compared with bacterial spores. A dose level of 3 to 5 kGy may be required to control mold growth in dried fish. When compared with bacteria, viruses require higher radiation doses for inactivtion.[51] Gamma irradiation of the hard-shelled clam, *Mercenaria mercenaria*, and the oyster, *Crassostrea virginica* significantly reduced virus carriage numbers without unduly affecting desirable sensory qualities. Hepatitis A virus and rotavirus SA11 in the shellfish were reduced with D_{10} values of 2 and 2.4 kGy, respsectively.[51] It was suggested that ionizing radiation was capable of providing an extra, highly effective safeguard of shellfish sanitary quality when combined with traditional depuration treatment.[52] Table 10.2 gives D_{10} values of some microorganisms determined in fish/shellfish media under different treatment conditions.

10.4.2 Insects

Considerable differences exist between the species in radiation doses required for their sterilization. Radiation sensitivity differs greatly in the different metamorphic stages of eggs, larvae, pupae, or adult. Females are generally more sensitive than males. A minimum dose of 0.15 kGy can prevent development of insects in dried fish at a moisture level below 20%. Larvae continue to live but feeding rates are reduced. At a dose of 0.25 kGy, larvae and adult insects become inactive and feeding ceases. The doses required to kill various insect pests in dried (moisture level, below 40%) fishery products are in the range of 0.25 to 1 kGy.[53–55]

TABLE 10.2

D_{10} **Values of Some Microorganisms in Fish/Shellfish Media**

Pathogen	Medium	Temperature (°C)	Atmosphere	D_{10} value (kGy)
S. aureus	Surface of prawns	-10 ± 2	Air	0.29
V. cholerae	Surface of prawns	-10 ± 2	Air	0.11
V. flavialis	Shrimp paste	-20	Vacuum	0.44
V. mimucus	Shrimp paste	-20	Vacuum	0.75
V. parahaemolyticus	Shrimp homogenate, 1% NaCl	-20	Vacuum/air	0.44/0.07
V. parahaemolyticus	Fish paste	Ambient	Air	0.03/0.06
V. vulnificus	Shrimp paste	-20	Vacuum/air	0.30/0.35
V. alginolyticus	Shrimp paste	-20	Vacuum	0.19
A. hydrophila	Fish homogenate	0	Air	0.14
A. hydrophila	Shrimp paste	0	Air	0.10
S. flexneri	Shrimp paste	Frozen	—	0.22
S. paratyphi A	Oyster paste	5	—	0.75
S. paratyphi B	Oyster paste	5	—	0.85
S. typhimurium	Shrimp or fish paste	0–2	Air	0.10–0.15
S. typhi	Crab meat	—	—	0.87
S. faecalis	Shrimp paste	—	—	5.0–7.5
B. cereus	Shrimp or fish paste	0–2	Air	0.2–0.3
L. monocytogenes	Shrimp or fish paste	0–2	Air	0.15–0.25
Y. enterocoliticus	Shrimp or fish paste	0–2	Air	0.10–0.15
Hepatitis A virus	Clams and oysters	—	Air	2.02

Source: Summarized from Monk, J.D., Beuchat, L.R., and Doyle, M.P., *J. Food Prot.*, 58, 197, 1995; Radomyski, T. et al., *J. Food Prot.*, 57, 73, 1994; Lewis, N.F., Alur, M.D., and Kumta, U.S., *Indian J. Exp. Biol.*, 9, 45, 1971; Lewis, N.F., *J. Gen. Microbiol.*, 66, 29, 1971; Nerkar, D.P. and Bandekar, J.R., *J. Food Safety*, 10, 75, 1990; Bandekar, J.R., Chander, R., and Nerkar, D.P., *J. Food Safety*, 8, 83, 1987.

10.4.3 Parasites

A number of parasitic protozoa and helminths occur in fish and other muscle foods. Irradiation has the potential to control foodborne parasitic diseases such as trichinosis, toxoplasmosis, teniasis, and opisthorchiasis that are prevalent in a number of developing countries. Hamsters, rabbits, and cats can be susceptible to infection by the fluke when they are fed with metacercariae from infected fish. In most cases, a dose as low as 1 kGy can generally render these parasites non-infective without causing significant change in the physicochemical or sensory properties of the treated food.[56–58] Tapeworms (such as *Diphyllobothrium latum*, *D. yonagoense*, and *D. pacificum*) and other parasites (*Anisakis simplex*) can be inactivated by irradiation. The minimum effective dose (MED) to inhibit maturation of the liver fluke is 100 Gy, as determined by the feeding studies on hamsters.

Parasites belonging to *Anisakis* spp. require a higher dose ranging between 2 to 7 kGy for inactivation. A dose of 250 Gy inactivated viable cysts of *Entamoeba histolytica*, while 300 Gy is sufficient to eliminate *Toxoplasma gondii*.[1] The food-borne zoonosis, clonorchiasis, is prevalent among Chinese and other southeast Asian populations, as a result of eating raw or half-cooked freshwater fishes or shrimp. Opisthorchiasis and paragonimiasis are caused by *Opisthorchis viverrini*, *O. felineus*, and *Paragonimus westermani*, which may be present in farmed fish and half-cooked crustacea that are infected with.[58] A dose of 0.15 kGy is enough to inactivate *Clonorchis sinensis*, the causative parasite.[57] Comparable radiation dose can also control *O. viverrini*, *O. felineus*, and *P. westermani*.

10.5 RADIATION PROCESSES FOR FISHERY PRODUCTS

10.5.1 Radurization (Pasteurization)

Radurization (synonymous with pasteurization) is the irradiation process for extension of shelf life of fresh fishery products in ice or under refrigeration. The extension in shelf life due to irradiation depends on several factors, including initial quality of the fish, temperature during treatment, irradiation dose, packaging conditions, and storage temperature. In determining the optimum dose for radurization, two factors are most significant. These are the qualitative and quantitative changes in the microbial growth and the radiation dose responses of tissue constituents that govern the organoleptic attributes of fishery products.[59,60] The optimum dose is selected to give an extended shelf life to a product in ice in such a way that it should have a terminal spoilage pattern not considerably different from that of unirradiated samples. In radurized fish, the microbial population and composition are significantly altered depending upon the dose of irradiation, packaging conditions, storage temperature, and fish species.[6,17,22,23] Radurization is done in the dose range of 1–3 kGy which is sufficient to reduce initial load of spoilage-causing organisms by about 1–3 log cycles. The treated product has a shelf life 2–3 times that of unirradiated samples. The treatment is effective for extension of shelf life of most marine and freshwater fish species. Initial quality of the fish is important in obtaining maximum extension in shelf life. While fish iced immediately after catch is ideal, fish stored in ice for 2–3 days after harvest can also be treated to get appreciable extension in shelf life. A longer delay can adversely influence postirradiation shelf life.[59–61] Radurization processes have been developed for a variety of fishery products including marine fish, freshwater fish, and shellfish.[6,17,22,32,62,63] Aerobic packaging has been found satisfactory for several low and medium fatty fish species, since vacuum packaging of fresh fish has several disadvantages, such as oozing of drip and possibility of outgrowth of *C. botulinum* spores under adverse storage conditions.

Research done at the Bhabha Atomic Research Center, India, has shown the feasibility of radurization for a number of low and medium fatty tropical fish and shellfish. The optimum dose and the extended shelf life over that of controls at 0–2°C obtained for these fishery items are: shrimp (dose, 1.5 kGy; 18–20 days);

Bombay duck (1 kGy, 18–20 days), white pomfret (vacuum packaged to prevent oxidative rancidity) (1 kGy, 35 days); black pomfret (1 kGy, 25 days); seer steaks (1 kGy, 23 days); Indian mackerel (1.5 kGy, 25 days); and Indian salmon (1 kGy, 25 days).[6,17,18,60,64–68] Lean fish species show the least irradiation-induced rancidity, while fatty fish can be treated only if adverse sensory changes as a result of irradiation are insignificant. Indian mackerel (*Rastrelliger kanagurta*) has been examined in detail to understand the irradiation effects on microbial, sensory, physicochemical and related aspects.[66] The shelf life of the aerobically packaged Indian mackerel was enhanced from 10–12 days to 25 days at a dose of 1.5 kGy without development of rancidity and were acceptable to a taste panel. Vacuum packaging was found to adversely affect the appearance of the irradiated product by drawing out muscle fluids during storage. Preirradiation ice storage beyond 5 days significantly reduced postirradiation storage life of the fish. A number of under-utilized fish species have been examined for shelf life extension by irradiation. A minimum of two-fold extension in chilled shelf life of these low-cost fish such as catfish (*Tachysurus* spp.), croaker (*Johnius dussumieri*), ribbon fish (*Trichiurus* spp.), anchovies (*Anchoviella* spp.), sole (*Cynoglossus* spp.), lactarius (*Lactarius* spp.), silver bar (*Chirocentrus dorab*), Bombay duck (*Harpodon nehereus*), and horse mackerel (*Meglaspis cordyla*) helps in their convenient utilization for product development.[68]

Radiation process is also amenable for shelf life extension of subtropical fish and shellfish species.[69] The effects of low-dose irradiation on microbiological and sensory qualities of fish species including sweet lip, red emperor, mackerel, whiting, mullet, barramundi, sand crab, Moreton Bay prawns, and king prawns were studied. A dose of 1 kGy resulted in 1.5 to 4 log reductions in total bacterial numbers and extended the shelf lives without adversely affecting the sensory qualities. No adverse changes were noticed in these products when irradiated up to 3 kGy.[62] The other fish species examined included hoki and blue mackerel.[70]

Radiation preservation of freshwater fish species, including gwyniad, trout, and carp have been reported.[62,71] While unirradiated carp could be stored for 15 days, a dose of 5 kGy preserved the fish up to 35 days. Trout required a lower dose of 0.5 kGy for maximum shelf life extension of 21 days without affecting flavor attributes, while gwyniad required an optimum dose of 1 kGy for shelf life extension up to 23 days. Irradiation at 1–5 kGy of carp and trout, reduced the psychrotrophic bacteria by 2–4 log cycles and mesophilic bacteria by 1.6–3 log cycles, depending upon radiation dose. Fresh, unirradiated whole and steaks of rohu (*Labeo rohita*) were acceptable up to 14 and 20 days when stored under ice. Irradiation at 1 kGy enhanced the chilled shelf life of whole and steaks of the fish to 28 and 32 days, respectively. Irradiation at a dose of 2 kGy extended the shelf life of Nagli fish (*Sillago sihama*) at 0–2°C from 7–19 days. Even though evisceration is generally considered to be advantageous, dressing prior to irradiation had no additional benefit in extending the shelf life of the fish. Total bacterial count, total volatile basic nitrogen, trimethylamine, and sensory evaluation data revealed no significant differences between whole and dressed Nagli fish. Irradiation also inactivated *V. parahaemolyticus*, *S. aureus*, and also *Listeria* and *Yersinia* spp.[65]

Iced headless shrimps, though it is better keeping them with shells rather than deshelled, develop black spots due to melanosis. Since irradiation activated polyphenoloxidase and favored formation of melanin pigment, blanching prior to irradiation has been used to prevent melanosis by inactivating the enzyme. The heat treatment eliminated heat-sensitive spoilage organisms, while irradiation at 1 kGy along with heat had a synergistic effect in extension of the shelf life of the shellfish. The spoilage pattern of unirradiated and irradiated shrimp differed greatly. While unirradiated shrimps spoiled with the development of sliminess and musty odors, the irradiated samples were free from such changes. The refrigerated (0 to 2°C) shelf lives for fresh, blanched (steaming for 4 min), and irradiated (1.5 kGy) peeled and deveined shrimp were 2, 7, and 18 weeks, respectively.[17,64,72] Table 10.3 summarizes the influence of gamma radiation on the shelf life of some marine fish and shellfish, while Table 10.4 shows shelf lives of some irradiated freshwater fish species.

Some efforts have been made to scale up the above laboratory processes for semicommercial conditions. Packaging of some dressed pelagic fish such as sardine, mackerel, etc. invariably leads to poor appearance of the packaged fish due to drip and oozing of blood during chilled storage. In addition, packaging of large amounts of the catch at the landing centers may not be viable in addition to likely growth of *C. botulinum* in the pouches under reduced oxygen levels. Therefore, storage of irradiated fish under nonpackaged condition is desired. Eviscerated, washed, and nonpackaged Indian mackerel held under ice in perforated boxes (1 mm) $(30 \times 13 \times 20 \text{ cm}^3)$ was irradiated at 1.5 kGy. An outer aluminum box carrying the perforated fish container allowed collection of melt water from ice during irradiation. After the treatment, the boxes were held in a cold room at 0–2°C. Ice was replenished and the melt water was removed during storage. It was observed that nonpackaged irradiated fish had a shelf life of 20 days, while the nonpackaged unirradiated fish had a shelf life of 14 days, as judged by sensory evaluation. The extension in shelf life was achieved as a result of radiation-dependent reduction of contaminant bacteria coupled with the washing action of melt water from ice on the surviving organisms. The melt water also removed blood and drip from the fish giving a better appearance as compared with packaged fish.[73] The system was also examined for bulk preservation of anchovy (*Stolephorus commersonii*), a small pelagic fish abundantly available in India. Nonpackaged whole anchovy in lots of 20 kg were subjected to gamma irradiation at 2 kGy. The treated fish were stored under ice in insulted boxes, which were held at 12–13°C, to enable slow melting of the ice and continuous washing of the fish by the melt water during storage. Periodic quality evaluation of the product showed that the nonpackaged irradiated fish had better quality attributes and was acceptable up to 17 days in comparison to a storage life of 13 days for their unirradiated counterpart. Eventhough packaged fish had a longer shelf life of 20 days, packaging caused drip accumulation and poor appearance of the fish.[74]

Some fishery items may show some adverse reaction consequent to radiation processing.[6] White pomfret (*Stromateus cinereus*), for example, shows yellowing of the skin as a result of interaction between radiation-induced malonaldehyde with nitrogenous constituents of the skin. Preirradiation vacuum packaging of the

TABLE 10.3
Effect of Gamma Radiation on the Shelf Life of Some Marine Fish and Shellfish

Item	Radiation dose (kGy)	Storage temperature (°C)	Shelf life (days)
Atlantic mackerel	2.5	0–1	30 (8–10)
Atlantic halibut steaks	3.0	0–1	30 (6–8)
Cod fillets	1.5	0–1	28–35 (7–9)
Sole, grey fillets	1–2	0–2	29 (10)
Sole, English fillets	2–3	0–1	28–35 (10)
Haddock fillets	1.5–2.5	0–1	18 (12)
Ocean perch fillets	1.5–2.5	0–1	28 (12–14)
Yellow perch fillets	3	0–1	28
Channel catfish	1–2	0	20 (4)
Mackerel, Indian	1.5	0–2	25 (12)
Pomfret, white	1–2	0–2	29 (10)
Ribbon fish	1.0	0–1	28 (8)
Salmon, Indian	1–2	0–1	25 (10)
Shrimp, white/pink/brown	1.5–2	0–1	21–30 (14)
Shrimp, European brown	1.5	1–2	23 (9–16)
Shrimp, deep sea	2	0–1	34 (blanched 2 min and irradiated)
Shrimp, tropical	1.5–2	2–3	42 (unblanched) 130 (blanched)
Clam, baby	1–4.5	0–2	28
Crabs	2	2–3	28 (7) (cooked and vacuum packed)
	0.75		
Lobster, Norwegian	2–3	0–1	35–42 (5–6) (blanched)

Note: Numbers in parenthesis indicate shelf life of unirradiated samples.

Source: Summarized from Kumta, U.S. et al., in *Radiation Preservation of Food (Bombay)*. Proceedings Symposium, International Atomic Energy Agency, Vienna, 1973, p. 403; Rodrick, G.E. and Dixton, D., *Code of Practice for the Irradiation of Seafoods*. International Atomic Energy Agency, Vienna, 1994; Nickerson, J.T.R., Licciardello, J.J., and Ronsivalli, L.J., in *Preservation of Food by Ionizing Radiation*, Vol. 3, Josephson, E.S. and Paterson, M.S., Eds. CRC Press, Boca Raton, 1983, p. 13.

fish can suppress the yellowing phenomenon.[17] Low-dose irradiation has shown to cause some bleaching of color of salmon fillets, possibly due to the bleaching of asthaxanthin. Bombay duck (*Harpodon nehereus*) shows excessive drip formation during postirradiation chilled storage. However, the problem could be controlled by dip treatment in sodium chloride and sodium tripolyphosphate before irradiation.[35]

TABLE 10.4

Effect of Gamma Radiation on the Shelf Life of Some Freshwater Fish and Shellfish

Item	Radiation dose (kGy)	Storage temperature (°C)	Shelf life (days)
Carp	5	0–2	35 (15) (vacuum packed)
Rohu, whole	1	0–2	28 (14)
Rohu, whole	2	0–2	35 (14)
Rohu, steaks	1	0–2	32 (20)
Salmon	1–1.5	2.2–2.6	20
Trout	1	0–2	28 (vacuum packed)
Freshwater prawn	1.45	0–1	28
Mussels	1.5–2.5	2–3	42 (21)
Scallops	0.75	0–1	28, raw (13–17)
			43 cooked (13–17)
Lobster meat, cooked	1.5	0–1	28
Oysters	2	0–1	21–28

Note: Numbers in parentheses indicate shelf life of unirradiated samples. Samples were aerobically packaged unless otherwise specified.

Source: Adapted from Kumta, U.S. et al., in *Radiation Preservation of Food (Bombay)*. Proceedings Symposium, International Atomic Energy Agency, Vienna, 1973, p. 403; Rodrick, G.E. and Dixton, D., *Code of Practice for the Irradiation of Seafoods*. International Atomic Energy Agency, Vienna, 1994; Nickerson, J.T.R., Licciardello, J.J., and Ronsivalli, L.J., in *Preservation of Food by Ionizing Radiation*, Vol. 3, Josephson, E.S. and Paterson, M.S., Eds. CRC Press, Boca Raton, 1983, p. 13; Panchavarnam, S. et al., *Fish. Technol.*, 40, 36, 2001.

10.5.2 Radicidation (Sanitization)

Radicidation denotes sanitization of frozen fishery products by the elimination of pathogenic microorganisms by irradiation.[62,75–79] Frozen shrimp, fish fillets, minced fish blocks, and other items exported from several Asian exporters are often contaminated with pathogenic organisms belonging to *Salmonella* spp., *Vibrio* spp., and others. Since most of the nonspore forming pathogens have comparable lower resistance to ionizing radiation, as shown in Table 10.2, exposure of the products to low doses of gamma radiation enables sanitization of the products. Similar to radurization, radicidation doses also depend upon the nature of the product and handling conditions. A dose of 2 kGy has been found adequate to significantly eliminate different pathogens, including *Shigella* spp., *S. typhimurium*, and *S. aureus* from frozen shrimp.[80] A radiation dose of 4 kGy was found to completely eliminate these organisms from frozen prepackaged shrimp artificially inoculated with 10^8 cells of *Salmonella* spp.[80] *V. parahaemolyticus*

could be effectively reduced at a dose of 1 kGy.[75–78,81,82] *L. monocytogenes* is another important pathogen sensitive to radiation.[83]

Twelve imported shrimp items were screened for their microbial quality. It was observed that the total number of aerobic bacteria ranged between 2×10^4 and 6×10^6 cells/g. A total of 66 isolates of vibrios were obtained in eight samples, while, *L. monocytogenes* was isolated in two samples. A dose of 3 kGy reduced the vibrio species by 3 log cycles, whereas comparable reduction in *L. monocytogenes* and *S. typhimurium* required a dose of 3.5 kGy.[82]

The role of radiation in sanitizing shellfish has been established by other studies also.[84] Cobalt-60 irradiation of the hard-shell clam and oyster significantly reduced virus carriage numbers without unduly affecting the sensory qualities. A D_{10} value of 2 kGy was found to inactivate hepatitis A virus in clams and oysters. It was concluded that radiation in conjunction with depuration could improve the safety of the shellfish. A WHO Task Force Meeting on the use of irradiation to ensure hygienic quality of food held in Vienna emphasized the advantage of irradiation as compared to other methods in controlling food-borne illness caused by pathogenic microorganisms and parasites including *Salmonella*, *Campylobacter*, *Trichinella*, and *Toxoplasma*. Further, the treatment can also inactivate *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, which have also been responsible for a number of food-borne disease outbreaks.[76,79] It was recognized that irradiation could be a critical control point in ensuring the microbiological safety of foods.[76] However, the radiation treatment should not be seen as the sole control mechanism for hygienization of food products. Proper sanitation and good manufacturing practices must be followed to ensure a clean as well as safe product for the consumer.[76] This is important in view of certain limitations of irradiation treatment, which include radiation resistance of some microorganisms such as viruses, spores, or their toxins. Microbial toxins such as *Staphylococcus* enterotoxin and aflatoxin cannot be inactivated by irradiation.

10.5.3 Disinfestation of Dried Fishery Products

Dried fish forms a major source of protein in a number of equatorial countries. Dried fishery products frequently suffer severe losses due to infestation by flesh flies (*Sarcophagidae*), beetles (*Dermestes*, *Cornestes*, and *Necrobia* spp.), and mites (*Lardoglyphus* and *Lyrophagus* spp.).[6,84–86] These infestations are heavy in dried products containing 7–21% salt and stored under tropical conditions, at 20–32°C and air humidity of 73–87%. Based on a conservative estimate, the loss represents about 2.75 mt of dry fish for a year.[84–86] A large quantity of dried fish is lost in Bangladesh due to infestation by earwig, hide beetles, and copra beetles. The most destructive pest is the hide beetle, *Dermestes maculatus Deg*. The infestation problems during storage of dried fish have prompted many processors to illegally apply household insecticides while processing. Application of fumigants such as ethylene dibromide (EDB), methyl bromide, ethylene oxide, phosphine, and sulphur oxide has been practiced for the control of insects and nematodes in food items for several decades. In recent times, these chemicals

are being phased out for health, environmental, or occupational safety reasons.[1] The U.S. Environmental Protection Agency, for example, has banned EDB. Further, the import of any food treated with EDB is also prohibited for sale in the United States.[3]

Irradiation has distinct advantages over other commercial methods of pest control. Apart from being less energy consuming than fumigation, it does not leave toxic residues in the product. It has been observed that a dose of 0.5 kGy can effectively disinfest dried fish and fishery products at a moisture level of below 40%. It is important that the material has to be packaged prior to irradiation to prevent reinfestation by insects. Disinfestation studies on dried mackerel showed that eggs, larval, and pupal stages of hide beetles *Dermestes maculatus Deg* could be inactivated at a dose of 0.2 kGy. Fungal organisms, which are not inactivated at this dose, could be controlled by reducing the final moisture content of the product to about 13% and use of proper packaging.[87,88] Results of studies on dried fish in Bangladesh, Philippines, and Indonesia have shown that irradiation of packaged, salted, dried fish products at doses in the range of 0.1–1.0 kGy prevented development of beetle larvae and adults.[87,88] Commercially dried marine fish such as mackerel, shark, tuna, and saram (*Scomberomorus commersoni*) contain 36–50% moisture and 10–12% salt. The products often harbor molds such as *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. Giving a dip in potassium sorbate solution prior to dehydration, packaging in 0.1-mm polyethylene pouches and irradiation at 0.5–0.75 kGy could enhance the acceptability of the product up to 6 months at ambient temperature. Precautionary measures that need to be taken for successful disinfestation of dried fishery products include good sanitation practices to prevent deposition of insect eggs on fish products and use of suitable packaging to prevent reinfestation after the radiation treatment. Table 10.5 shows the shelf life of some dehydrated fishery products treated by gamma irradiation for disinfestation.

Sensory attributes are of paramount importance for acceptability of irradiated fishery products. Fishery products should be treated at a threshold dose of radiation, above which organoleptic changes may occur adversely affecting the taste, smell, texture, and hence overall acceptability of the treated products. Optimization of the treatment conditions can also alleviate specific problems relating to certain fish species. These include radiation-induced oxidation of lipids in the case of fatty species, fading of the red color of the gills and appearance of a greenish yellow color in fish such as pomfret, salmon, and tuna, textural changes leading to drip formation in Bombay duck and odor changes in catfish. Changes in sensory quality are negligible in products such as shrimp irradiated under frozen conditions for hygienization purposes. Off-odors associated with irradiated products packaged in plastic pouches often include rancid-like aromas. These aromas have been recognizable at specific irradiation thresholds, which are different for each product and packaging material. It is therefore essential to limit the dosage to an optimal level required to accomplish the desired task. The doses standardized for different fishery products (Tables 10.3 and 10.4) have taken into consideration the sensory properties of irradiated products apart from other aspects. Irradiation

TABLE 10.5
Effect of Irradiation on Disinfestation and Shelf Life of Dried Fish

Fish	Irradiation dose (kGy)	Insects	Postirradiation shelf life	Remarks
Bombay duck	0.25	Not identified	1 year	Packaging in high density polyethylene
Croaker	0.25	Not identified	1 year	Packaging in high density polyethylene
Mackerel	0.30	Not identified	9 months	Packaging in high density polyethylene
Mackerel	0.3	*D. maculatus*	5 months	Heavy damage if not irradiated
Mackerel, salted, and dried	0.5–0.75	*Necrobia* spp.	6 months	Product contains 40% moisture and 12% salt
Milk fish, smoked	4	Mold growth	5–15 weeks	Sorbate (0.1%) dip before irradiation
Shark	0.5–0.75	*Necrobia* spp.	6 months	Product contains 40% moisture and 12% salt
Rohu (freshwater fish)	0.25–1		6 months	Dipping in 2% sorbate before irradiation Packaging in 0.1mm polyethylene film

may not be beneficial for some smoked fish. Irradiation decreases the intensity of specific flavor and aroma of smoked salmon. Modified atmosphere packaging may not be advantageous for fish items having significant amount of myoglobin such as catfish. Table 10.6 presents the ideal treatment conditions and benefits of individual process for the major radiation processes for seafood, namely, radurization (pasteurization), sanitization (radicidation), and disinfestation.

10.6 COMBINATION PROCESSES INVOLVING IRRADIATION

The effects of ionizing radiations on food can often be advantageously combined with other processing treatments. The resulting "combination treatments" may involve synergistic or cumulative action of the combination partners, leading to a decreased treatment requirement for one or both the agents. This in turn may result in savings in cost and energy and may bring about improvement in the sensory properties and bacteriological quality of the food thus treated. Preservative effect of a combination of treatments in controlling microbial growth and resulting spoilage is based on "hurdle technology," and involves creation of a series of hurdles in the foods for microbial growth. Such hurdles include heat, irradiation,

TABLE 10.6
Important Radiation Processes for Seafoods, their Treatment, and Storage Conditions

Treatment and storage temperature	Radiation process	Benefits
−1 to +3°C (Postirradiation storage: under ice)	*Radurization* (Radiation pasteurization) Dose: 1 to 3 kGy Reduction of initial microbial content by 1–2 log cycles Specific reduction of spoilage causing organisms	Extends chilled shelf life of marine and freshwater fishery products 2–3 times Additional benefit includes reduction of nonspore forming pathogens
−10 to −20°C Packaged, frozen, ready-to-export fish can be treated before shipment. Frozen storage	*Radicidation* (Radiation hygienization) Dose required: 4–6 kGy Elimination of nonspore forming pathogens such as *Salmonella*, *Vibrio*, *Listeria*, etc.	Improvement of hygienic quality of frozen, materials for export such as frozen shrimp, cuttle fish, squid, finfish, fillets, and IQF items
15 to 30°C Ambient storage	*Radiation disinfestation* Dose required <1 kGy Elimination of eggs and larvae of insects	Dry products free from spoilage due to insects. From dried fishery products including fish meal and feed for aquaculture Inactivation of *Salmonella* spp. and other pathogens

low temperature, water activity, pH, redox potential, chemical preservative, etc.[89] (see Chapter 6).

Heating and irradiation work synergistically in extending the shelf life of irradiated foods. Heating before irradiation enhances the antimicrobial effects of irradiation. Thus, gamma irradiation of organisms such as *S. typhimurium* with sublethal doses increases their sensitivity to heat treatment, the sensitivity increasing with the irradiation dose.[89] Steaming followed by irradiation at 1–5 kGy has been shown to extend the shelf life of fishery products including shrimp.[6] The use of preservatives such as sorbic acid in combination with irradiation has potential to control off-flavor development during irradiation, besides providing safety against outgrowth of *C. botulinum* spores. A combination of sorbic acid treatment and irradiation at 1 kGy synergistically extended the keeping quality of iced cod fillets.[89] A combination process involving dip in 0.5% sorbic acid, blanching, dehydration to a moisture level of 40%, and irradiation at 2.5 kGy has been developed to prepare shelf stable dehydrated shrimp.[6] Shark and ray belonging to Elasmobranchs stored at 0–2°C rapidly lose their acceptability due to the formation of ammonia. Combination treatment involving steaming for 5 min and gamma irradiation at 1 kGy could extend the refrigerated shelf life of these fish up to 30 days.[6]

A method of onboard processing shrimp in the Netherlands involves boiling the shellfish for about 10 min in either seawater or water containing added salt. The cooked shrimp containing 1–3% salts are cooled in seawater, which leads to reinfection with the marine microflora. Incorporation of 0.4% benzoic acid in the cooking medium controlled the microorganisms. The product has a shelf life of 8 days at 4°C. A dose of 1 kGy could further extend the shelf life of the treated shellfish up to 16 days.[90] Common salt is known to exert bacteriostatic effect in addition to its ability to improve texture and flavor of food products. Therefore, the effect of salting of fresh fish before irradiation was examined on postirradiation shelf life. Gamma irradiation at 2 kGy of fresh eviscerated threadfin bream (*Nemipterus japonicus*) gave an extension of chilled shelf life from 8 to 22 days. However, preirradiation salting of the fresh fish in 10% brine for 1 h did not result in further extension in shelf life. Nevertheless, salting prevented drip formation in the fish packages during the course of storage, resulting in a better appearance for the product.[91] Irradiation at 1–3 kGy of salted and vacuum-packaged seabream gave a shelf life of 27–28 days at refrigerated temperatures, as compared with 14–15 days for the nonirradiated, salted samples.[92] Combination treatments involving radiation have also been found beneficial for preservation of low-acid foods.[93] Potential also exists for combination of low-dose irradiation with conventional cook-chill processes in order to improve the hygienic quality of the prepared products (see Chapter 5).

Radappertization or radiation sterilization involves blanching the fish at 70–80°C to inactivate the autolytic enzymes, hermetically sealing them under vacuum either in metal cans, flexible pouches, or metal or plastic trays, freezing the product and irradiation in the frozen condition at 45–65 kGy. The radiation dose is based on the analogy with the 12D concept in thermal processing for inactivation of 10^{12} microbial cells. The products can be stored without refrigeration for long periods, the limiting factor being the integrity of the primary packaging material.[1,6]

10.7   OTHER MISCELLANEOUS APPLICATIONS

Individually quick frozen (IQF) shrimp forms one of the most popular items of international seafood trade. As discussed in Chapter 2, these products invariably harbor pathogenic microorganisms, which have become a problem in their international trade. Scope exists for hygienization of the product using EB radiation.[94] Frozen shovel-nosed lobsters (*Ibacus peronii*) often exhibit a garlic-like off-odor, which is caused by traces of *bis*(methylthio) methane. This compound can be destroyed and the off-odor can be removed by irradiation at a dose of 25 kGy.[6] Traditional methods of long flushing and depuration (self-purging) of shellfish in clean waters has not proven effective in alleviating the danger of contaminating pathogenic microorganisms. Irradiation can either eliminate or reduce the number of these organisms including Hepatitis A virus to a level where the risk of consumption is considerably reduced.[6]

Biohazards with respect to farmed fish and shellfish have become a serious problem in the globally expanding aquaculture operations. These hazards can

TABLE 10.7
Potential Applications of Ionizing Radiations in Seafood Processing

Extension of shelf life of fresh fish under chilled condition
Elimination of pathogens in fresh and frozen seafood
Hygienization of individually quick frozen fishery products
Reduction of pathogens, including viruses from oysters
Removal of off-odors from some fish and shellfish species
Reduction in fecal coliforms in live hard-shell clams
Hygienization of aquafeed/poultry feed
Hygienization of fish meal
Development of shelf-stable products using combination treatments

occur through biological and chemical contamination from both freshwater and coastal ecosystems. The biological hazards in cultured fish include presence of parasites, insects, pathogenic microorganisms, and viruses. The safety issues associated with aquaculture products have been the subject of a recent international survey.[95] Irradiation of farmed fish can immensely enhance the hygienic quality and protect the consumer, especially when these fish items are consumed as raw or minimally prepared.[96] It has been recognized that often the bacterial contamination of aquacultured fish arises due to poor hygienic quality of fishmeal used as protein source in aquafeeds. Low-dose irradiation can be used to enhance the hygienic quality of animal feeds, including aquafeeds. Irradiation at 5 kGy is more effective than fumigation for hygienization of dry fish powders.[6] The treatment can also enhance hygienic quality of food ingredients required for seafood-based products. Table 10.7 summarizes the potential applications of ionizing radiation in seafood processing.

10.8 WHOLESOMENESS OF IRRADIATED FISHERY PRODUCTS

The wholesomeness of irradiated foods is determined in terms of four criteria, namely, (i) absence of induced radioactivity, (ii) absence of pathogens and their toxins, (iii) nutritive value, and (iv) presence of toxic, mutagenic, or carcinogenic radiolytic products.[1,7,9] It is now well established that irradiation employing isotopes of ^{60}Co or ^{137}Cs is unable to induce radioactivity in the treated food items including fishery products. Also radioactivity cannot be induced using electron or X-rays at the maximum permitted energy levels of 10 and 5 MeV, respectively.[1] The FAO/IAEA/WHO Joint Expert Committee on the Wholesomeness of Irradiated Food has therefore recommended a dose of 5 MeV for X-rays and 10 MeV for electrons as the maximum permissible energy for radiation treatment of food. The U.S. Food and Drug Administration, the U.K. Advisory Committee on Irradiated and Novel Foods, and the Codex Alimentarius Commission have also adopted the 10- and 5-MeV limits for electrons and X-rays, respectively.[6]

The wholesomeness aspects of irradiated foods including seafood has been investigated in detail.[1,7,9] Wholesomeness testing studies involving multigeneration animal feeding studies are traditionally the most widely used techniques for evaluation of toxicological safety and nutritional adequacy of irradiated foods. Parameters examined in these studies are body weight changes, food consumption, reproductive performance, longevity, pathology, liver function tests, and tests for teratogenicity and mutagenicity. In a comprehensive review paper, 1223 studies on the wholesomeness aspects of 278 irradiated food items have been summarized.[97] Among the food items, the fishery products included sardine, salmon, Indian mackerel, shrimp, ocean perch, flounder, white fish, and also fish meal. The survey led to a general conclusion on the safety of irradiated foods including fishery products. Animal feeding experiments are costly, time consuming, and cumbersome. Therefore, less expensive, short-term methods have been developed for screening wholesomeness of foods. These tests evolved with the understanding of genetic material and its response to disturbances. Such tests include chemical changes in DNA, induction of DNA repair, differential sensitivity of normal and repair of deficient organisms, activation of viruses by DNA damages, and chromosomal and nuclear abnormalities. The detection of mutation *per se* is the aim of a number of tests such as Ames test, in which sensitivity to mutation in *Salmonella typhimurium* is examined with respect to its growth requirements. Many studies on irradiated food have also employed *in vivo* mammalian short-term tests. Prominent among these are dominant lethal, cytogenetic, micronucleus, and host-mediated assays. In general, these studies have also not shown any adverse effects of consumption of irradiated foods including fish.[1,7,9]

There have been concerns relating to microbiological safety of foods subjected to nonsterilizing doses of radiation. Such concerns included influence of selective changes in microflora on survival of pathogens, potential hazards due to mutational changes in pathogens that can make them more virulent, and development of radiation-resistant strains. Detailed investigations on these aspects led to the conclusion that microbiological safety of irradiated food is comparable with that of foods preserved by other acceptable preservation methods. However, it was pointed out that the gain in microbiological or keeping quality attained by food irradiation has to be safeguarded by proper control in the food irradiation facilities and by proper care of the product before and after processing.[1,7,76]

10.9   PACKAGING ASPECTS

The major requirements of packaging of fishery products, namely, insulation properties, control of dehydration, oxidation, prevention of odor permeation, protection from bacterial and chemical spoilage, mechanical damage, and enhancement of economic viability, as discussed in Chapters 2 and 4 are also essential for packaging of irradiated fish. Proper packaging is also required to prevent microbial contamination of the irradiated fishery product.[6] Sensitivity to radiation is an important parameter in selecting the packaging material for fish. Radiation-induced changes

depend on the chemical structure of the polymer, the composition and processing history of the plastic, and the irradiation conditions. Certain gaseous radiolysis products including CO_2, CO, hydrocarbons, alcohols, ketones, carboxylic acids, etc. are formed due to irradiation. This may also lead to deterioration of mechanical properties. Irradiation does not generally affect permeability of packaging material. Changes in terms of UV and visible spectral characteristics are minimum at doses used for treatment of food. Flexible films or laminates to be selected for radurized fish must have a minimum thickness of 0.025 mm primarily to act as a barrier to bacterial penetration. Flexible materials such as Nylon 11, Saron coated nylon, polyolefine-coated polyester, laminated paper/aluminum/polyethylene, and polyethylene films (0.165 to 0.76-mm thickness) are suitable for packaging of radurized fish. Polyethylene film of about 0.1 mm thickness can control insect penetration, bacterial permeability, water-vapor transfer and weight loss in dried fish during storage.[98]

10.10 DETECTION OF IRRADIATED FISH

Increased commercialization and possibilities in international trade of irradiated foods, differing regulations among countries, and consumer demand for information on processing details have highlighted a need for developing tests for foods treated with radiation. During the past few years, several techniques have been developed for identifying irradiated foods including fishery items, which rely on irradiation-dependent subtle changes in the treated products. The different criteria required for a detection method include specificity, sensitivity, dose dependence, simplicity of application, calibration, and low cost. Since the radiation-induced changes occurring in the foods are minute and depend upon the material, a universal test is not available for the purpose. Several techniques have been developed during the recent years for detection of radiation treatment depending upon the nature of the food and the dose employed. These include tests based on electron spin resonance, thermoluminescence, generation of chemical species such as hydrocarbons and cyclobutanones, or evaluation of biological, microbiological, viscometric, or impedance properties of irradiated foods.[99–101]

Electron spin resonance (ESR) spectroscopy is used to detect unpaired electrons in free radicals. Electron spin is a consequence of the magnetic dipole of the unpaired electron of the free radical. Generally, the free radicals formed during irradiation of food are so short-lived that they cannot be detected. Nevertheless, if they are trapped in a relatively hard component of the food such as bone in a fish fillet, shell of the shellfish, their presence can be confirmed by ESR spectroscopy. The test can be used for irradiated scallop, trout bones, and sardine scales. Thermoluminescence-based method depends upon the production of glow by contaminating minerals during heating. A limitation in the use of ESR or thermoluminescence spectroscopy is that the irradiated fish should contain bone, shell, or intestinal grit in order to trap radiation-induced free radicals or electrons. Further, ESR technique requires a relatively sophisticated equipment, which is time consuming and costly.

TABLE 10.8
Methods for Identification of Irradiated Fishery Products

Identification technique	Product	Dose range (kGy)
Electron spin resonance (ESR)	Pink shrimp, tiger prawn, king prawn, and Mediterranean trevette	1–5
ESR	Whiting	1–5
ESR	Scampi tail	1–5
ESR	Norway lobster	1–5
ESR	Shrimp	1–5
ESR	Crustacea	1–6
Thermoluminescence	Shellfish	1–5
Evolution of hydrocarbons	Muscle foods	2
Evolution of H_2 and CO	Shrimps	5
o-tyrosine	Shrimp, fish, mussels, frog legs	5
Cyclobutanone	Prawns, Norway lobster	5

Source: Summarized from Furuta, M. et al., *J. Agri. Food Chem.*, 45, 3928, 1996; Delincee, H., *Rad. Phys. Chem.*, 48, 378, 1996; Haire, D.L. et al., *Food Res. Int.*, 30, 249, 1997.

Detection of chemicals formed from irradiated lipid component of fish or shellfish has been shown to be applicable to clam, shrimp, and oyster. Detection of 2-alkylcyclobutanones (where the alkyl group is attached to the second position of the ring), formed as a result of irradiation-induced ring closure of fatty acids has been found applicable to lipid containing fishery items. Detection of trapped gases such as hydrogen and carbon monoxide generated by radiolysis is another method, which has been examined in foods including shrimp and other seafood.[99] A few biological methods have also been proposed for identification of irradiated muscle foods. Certain other methods make use of changes in DNA, electrical impedance, immunochemical, viscosity, and differential scanning calorimetry and other parameters.[6] While validity of many of these methods needs further confirmation, those based on ESR and 2-alkyl cyclobutanone have indicated promise with respect to identification of irradiated crustacea and fatty fish. Table 10.8 summarizes the various methods for detection of irradiated fishery products.

10.11 INFRASTRUCTURE AND ECONOMICS OF FISH IRRADIATION

The techno-economic advantages of food irradiation are: (i) higher shelf life and quality of treated foods, (ii) ability to ensure hygienic quality, (iii) its

use as alternative to fumigation, (iv) energy conservation, and (v) cost effectiveness.[102–104] Radiation facilities using ^{60}Co require the construction and operation of source storage (a water pool), source handling (generally using electrical power and gravity), and massive shielding to protect workers and the environment. The facility can be graded to provide different levels of product throughput by designing and installing an adequate load of the radioisotope along with ancillary product-storage and handling provisions. Presently, ^{60}Co sources of capacities ranging from kilocuries (30,000 to 100,000 kCi) to several megacuries are operating worldwide for radiation sterilization of medical products.[2] EB facilities may not require heavy shielding as the ^{60}Co-based food irradiators. (Table 10.1). EB irradiators may be mobile and adaptable to existing food-processing facilities giving unique opportunities to combine technologies and reduce cost.[2] Dosimetry is an important aspect of process control to measure dose distribution, set product parameters, control compliance with dose limits, and verify control of the process. Irradiation treatment requires slightly higher capital costs and demands a critical minimum capacity for economic operation. However, unlike most other processes, irradiation has a lower operating cost, especially with regard to the energy requirement.

The seafood irradiation plant may be located at a central point near the landing centers or aquaculture farms where sufficient amounts of catch would be available for processing. International acceptance and trade in irradiated food products including seafood demand, not only implementation of good manufacturing practice, but also proper process control during irradiation. Code of practice for the irradiation of seafoods has been provided.[10,61] Table 10.9 summarizes the process parameters to be considered for irradiation of fishery products. Cost estimates for pasteurization of frozen shrimp using 10 MeV electron accelerators range between Can \$32 and 100/ton.[94] Commercial scale irradiation of oysters to eliminate vibrios has been calculated to cost US 5 to 8 cents/lb in 1993.[104] According to another estimate, irradiation of 175 million lb of seafood at 3 kGy for elimination of *Vibrio* spp. would be at the rate of US 1 cent/lb.[105] Table 10.10 indicates future research needs in development of radiation processes for food products including seafood.

10.12 CONSUMER ATTITUDE TOWARDS IRRADIATED FOODS

Consumer acceptance is a major parameter for any food processing operation to become commercially viable. A few decades ago, consumer organizations were concerned about irradiation of foods. Their main concerns were possibility of foods becoming radioactive, possible replacement of good sanitary practices, and safety of workers as well as environmental safety. However, educational programs undertaken during the last several years have shown that these fears could be removed. In addition, it was suggested that use of a better terminology, such as "treated with cold-pasteurization (irradiation) for improved safety" could enhance consumer confidence in the technology. Recent surveys conducted in the United States, India, and other countries have indicted willingness of consumers to buy

TABLE 10.9
Process Parameters for Irradiation of Fishery Products

Preirradiation control
  Species, size, grade, source
  Maximum initial microbial load
  Preirradiation treatment (evisceration, peeling, filleting, salting, cooking, etc.)
  Packaging requirements
  Preirradiation temperature
  Moisture content (dry fish, shellfish)
During irradiation
  Maximum and minimum dose, dose uniformity ratio, average dose
  Irradiation temperature (frozen/chilled products)
Postirradiation
  Storage temperature
  Insulated transport

irradiated foods even at an extra cost, in view of the improved hygienic quality of the products.[113]

10.13 INTERNATIONAL STATUS

The Food and Agriculture Organization, the World Health Organization, and a number of professional bodies in several countries have endorsed food irradiation. These include the American Society of Microbiology, Association of Food and Drug Officials, USA and the Center for Disease Control and Prevention, USA. The U.S. Food and Drug Administration has approved several food items for irradiation, as shown in Table 10.11. Table 10.12 shows clearances of irradiated foods by member states belonging to the European Council. About 40 countries have approved over 100 different food products including seafood for treatments at doses ranging from 1 to 10 kGy.[106] Over 30 countries are using irradiation of foods on a limited commercial scale including the United States, France, Netherlands, Belgium, Hungary, India, and South Africa. Irradiation of red meat to eliminate pathogenic bacteria has been initiated on a commercial scale in the United States, suggesting continued interest in food irradiation.[107]

The International Consultative Group on food Irradiation (ICGFI) established under the aegis of FAO, IAEA, and WHO in 1984 helped to evaluate the global developments in the field of food irradiation. Till its expiry on May 8, 2004, ICGFI provided advices on the application of the technology and furnished information to its members.[108] The recently created International Council on Food Irradiation (www.icfi.org) is intended to familiarize users with the benefits of irradiation. In 1993, the CAC, which is the Executive Organ of the FAO/WHO Joint Food Standards Program, adopted a General Standard for Irradiated Foods

TABLE 10.10
Areas of Future Research Needs in the Case of Food Irradiation

Pathogen reduction protocols	Currently there are no norms for reduction of pathogens in foods. These need to be established
Inactivation kinetics of food-borne viruses	Enteric viruses, responsible for several food-borne illnesses, are generally unaffected by radiation. Recent studies suggest that depending on the sample matrix, viruses can become sensitive to EB radiation at levels significantly lower than those produced with Cobalt-60 irradiation. Studies are needed to identify the conditions that can eliminate viral pathogens in ready-to-eat (RTE) foods
Radio-sensitization	A better understanding on sensitization of microbial pathogens can allow incorporation of specific "sensitizing" molecules directly to the food to attain a predetermined reduction of pathogens
Microbial stress conditions and radiation sensitivity	Recent studies have shown that the physiological state of the cells is critically important in its radiation sensitivity. The precise mechanism of protection or repair needs to be elucidated to adopt appropriate strategies when irradiating foods
Sensory attributes	Standardization of sensory changes with respect to irradiation is needed, since these changes are dependent on the radiation dose, irradiation conditions and product profiles
Multicomponent foods	RTE meals are becoming popular and therefore there will be significant opportunities for hygienization of these products by irradiation. The issues of dosimetry, pathogen reduction, and sensory changes would have to be optimized for the purpose
Combination of technologies	Optimal combinations of irradiation with MAP, antimicrobial coatings, antioxidant additions and others need to be designed
Product packaging	Research is needed on the next generation of packaging materials to retard negative sensory attributes or enhance desirable ones. The development of packaging materials that can visually denote an irradiated product or dose range or detect adverse changes in a product would also be beneficial

Source: Adapted from Smith, J.S. and Pillai, S., *Food Technol.*, 58, 48, 2004.

and the Recommended International Code of Practice for the Operation of Radiation Facilities Used for the Treatment of Foods.[10] The General Standard and the International Code give guidelines for designing irradiation facilities that meet the requirements of safety, efficacy, and good-hygienic practices. The application of irradiation to individual food products or groups of food products is covered in Special Codes for Good Irradiation Practice elaborated by the ICGFI. The quality control system of the plant has to be based essentially on the Hazard Analysis Critical Control Points (HACCP) concept (Chapter 2). According to Sanitary and Phytosanitary Measures (SPS), members of the World Trade Organization (WTO) have the right to take legitimate measures to protect the life and health of their population from food-borne hazards. Food irradiation can have a significant role

TABLE 10.11
Approval of Irradiation for Various Foods by the U.S. Food and Drug Administration

Product	Dose	Purpose	Date
Wheat, wheat flour	0.2–0.5	Insect disinfestation	1963
White potatoes	0.05–0.15	Sprout inhibition	1964
Pork	0.3–1.0	Control of *Trichinella* spp.	1985
Fruit	1.0	Disinfestation, delay in ripening	1986
Vegetable, fresh	1.0	Disinfestation	1986
Herbs	30.0	Microbial control	1986
Spicesa	30.0	Microbial control	1986
Poultry, fresh, or frozen	3.0	Microbial control	1990
Animal feed and pet food	2.0–25	*Salmonella* control	1997
Meat, uncooked, chilled	4.5	Microbial control	1997
Eggs	8.0	*Salmonella* control	2000
Seeds	3.0	Microbial control	2000
Raw and processed crustaceans	—	Microbial control	Under consideration
Ready-to-eat foods (Poultry, fruits, and vegetables)	—	Microbial control	Under consideration

Source: Adapted from Olson, D.G., *Food Technol.*, 52, 56, 1998.

to play in this respect. The irradiation process could be a critical control point in ensuring the microbiological safety of foods.[109,110] The know-how for installation of food irradiation plants has been gained by successful running of pilot plants in a number of countries including Belgium, China, India, Netherlands, and the United States. Clearances for irradiation of fishery products have been accorded and marketing trials have been reported in some countries. Unconditional clearance for irradiation of fish items for shelf life extension, disinfestation and frozen (hygienization) has been accorded by countries including Bangladesh, Brazil, Chile, Costa Rica, Croatia, Cuba, France, India, South Africa, Pakistan, Syria, Thailand, the United Kingdom, and Vietnam. Commercial scale irradiation of frozen shrimp for hygienization has been carried out in Belgium and Netherlands in the past several years. Successful inter-country transportation of packaged, sliced, dried Korean pollack irradiated at 5 kGy has been reported[111]. The current status of the radiation industry has been discussed in a recent international meeting[112]

In conclusion, the diverse nature of seafood production, processing and trade, and social practices in some parts of the world such as consumption of raw

TABLE 10.12

Authorization of Irradiation of Food and Food Ingredients by Member States of the European Council

Product	Authorized maximum overall average absorbed radiation dose (kGy)				
	Belgium	France	Italy	The Netherlands	United Kingdom
Deep frozen aromatic herbs		10			
Potatoes	0.15		0.15		0.2
Yams					0.2
Onions	0.15	0.075	0.15		0.2
Garlic	0.15	0.075	0.15		0.2
Shallot	0.15	0.075	0.15		0.2
Vegetable, pulses					1.0
Fruit (including fungi, tomato, rhubarb)					2.0
Dried vegetables, fruits		1.0		1.0	
Cereals					1.0
Cereal flakes				1.0	
Rice flour		4.0			
Gun arabic		3.0		3.0	
Chicken meat				7.0	
Deboned poultry meat		5.0			
Offal poultry		5.0			
Frozen frog legs	5.0	5.0		5.0	
Fish, shellfish, mollusks					3.0
Frozen peeled shrimp	5.0	5.0			
Shrimps				3.0	
Egg white		3.0		3.0	
Casein, caseinates		6.0			

Source: Article 4 (6) 1999/2/EC of the European Parliament and of the Council on the Approximation of the Laws of Member States concerning food and food ingredients treated with ionizing radiation. Reprinted from *MPEDA Newsletter*, Marine Products Export Development Authority (India), 7, 1, 2002. With permission.

fish, all of these warrant increasing caution with respect to the quality of fishery products. Conventional sanitation measures and good manufacturing practices cannot completely protect the consumers against seafood-borne health hazards. Radiation treatment can significantly enhance the hygienic quality of processed fishery products. Other major advantages are reduction of postharvest losses in fresh items and elimination of insects in dried items. The unique possibility of irradiation of prepackaged, ready-to-export foods provides added advantage of

prevention of postprocess contamination during handling. Integration of radiation treatment with conventional processing can enhance safety and shelf stability of fishery items to meet international quality standards. The irradiation process is cost-effective. At present intercountry trade in irradiated foods is not common due to lack of harmonized regulations. Harmonization of regulations can open international markets to irradiated seafood. These, in turn, can result in stabilization of quality, global availability of diverse seafood species, efficient use of marine and freshwater resources, consumer confidence, and food security.

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11 High Pressure Processing

11.1 Introduction

High hydrostatic pressure (HHP) treatment is one of the conventional techniques practiced in ceramics technology for development of electronic components having specific properties. Investigations on the applications of HHP for food processing started a century ago. Early investigations started with the work of Hite in 1899, followed by that of Cress in 1924 on high pressure processing (HPP) of fruits and vegetables. Later, its effectiveness on other foods was demonstrated. These included reduction of bacterial counts in milk, extension of keeping quality of meat up to 3 weeks, and coagulation of egg albumin. Serious attempts to apply HPP to food preservation were initiated in the 1980s by the Japanese Ministry of Agriculture, Forestry, and Fisheries in collaboration with several food industries. Close to this development, the European Union initiated a collaborative program for development of machinery for the processing. HPP treatment has been shown to produce shelf-stable foods with a pH of 4.2 or lower and extended shelf life of refrigerated foods. In the early 1990s high-pressure-processed fruit jams and grapefruit juice appeared in the Japanese market, representing commercial application of the process. High pressure has potential applications in other food commodities also, which include improvement of tenderness of prerigor beef muscle, removal of bitterness in orange juice, selective removal of β-lactoglobulin from whey concentrates, and acceleration of bovine milk curdling by rennet.

11.2 Basic Principles and Units of Pressure

Two basic principles determine the effect of high pressure on foods.[1,2] Pascal's isostatic principle indicates that pressure applied to a sample including biological products is transmitted in a uniform and quasi-instantaneous manner. In contrast to thermal processing, the pressurization process is independent of sample volume, product size, and geometry. The other principle is that of Le Chatelier which states that the phenomena of phase transition, chemical changes, etc., which are accompanied by decrease in volume are favored by pressure and vice versa. Reactions in which volume increase occurs will tend to be inhibited by pressure. Since most biochemical reactions including those that occur in foods often involve a change in volume, pressure has a profound influence on the rates of these reactions. Pressure may also inhibit the availability of energy by affecting energy-producing enzymatic reactions. The effect of hydrostatic pressure in a food product is dependent upon the amount of pressure applied, duration of compression, depressurization

rate, temperature of treatment, product pH, water activity, salt concentration, etc. As compared with thermal processing, HHP treatment consumes less energy. The energy required for pressurization at 400 MPa is comparable to that required for raising the temperature of the material from 0 to 30°C.

The unit of pressure usually designated in food processing is Pascal (Pa) (also referred as Newton/meter^2, N/m^2), which is equivalent to 10 dynes/cm^2. One dyne is equivalent to 9.869×10^{-7} atmospheres. One MPa (Mega Pascal) is equivalent to 145.038 psi or 9.869 atmospheres. Generally, pressures applied in food processing operations are in the range of 300 to 600 MPa. Definitions and terminology for high pressure-low temperature processing employed for food has been provided recently.[3]

11.3   Effect of HHP on Water

Since water is the main component of fresh foods, the influence of pressure on it has been examined in detail. Unlike gases, HHP causes minimum decrease in the volume of water. For example, a maximum decrease of 15% in volume of water is observed at a pressure of 600 MPa. Foods that contain much water and little gas have a compressibility similar to that of water. Pressure treatment can also cause a decrease in pH of the foods containing significant amount of water; a treatment at 400 MPa causing a reduction of pH up to 0.7. The effect of high pressure on the solid–liquid phase diagram of water was elucidated in the classic work of Bridgman, as illustrated in Figure 11.1.[3] As shown therein, freezing point of water decreases with increasing pressure; the freezing point is −5°C at 70 MPa, −10°C at 125 MPa, −20°C at 200 MPa, and −22°C at 207 MPa. Release of pressure enables rapid and uniform nucleation and growth of ice crystals causing minimum damage (path "abcd" in Figure 11.1). To enable complete freezing of water, additional cooling is needed because of the large heat of fusion of ice formation. This also helps in increased freezing rate as compared to a conventional freezing process. Table 11.1 summarizes the advantages of HHP processing in terms of behavior of water.

11.4   Significance of High Pressure in Food Processing

The freezing rates of food products or living cells are highly system-dependent. Conventional freezing techniques are known to have detrimental effects on the food depending upon the methods. Slow freezing of food results in larger ice-crystal formation, which may cause extensive mechanical damage, accelerated enzyme and microbiological activities, as well as potentially increased oxidation rates, resulting from the increasing substrate concentration and the insolubility of oxygen in ice (Chapter 4). The solid–liquid phase transition of water depending upon the pressure (Figure 11.1) has significance with respect to food processing. The major advantages are pressure-assisted freezing (pressure-shift freezing), pressure-assisted thawing (pressure-shift thawing), and possibility for storage of food under
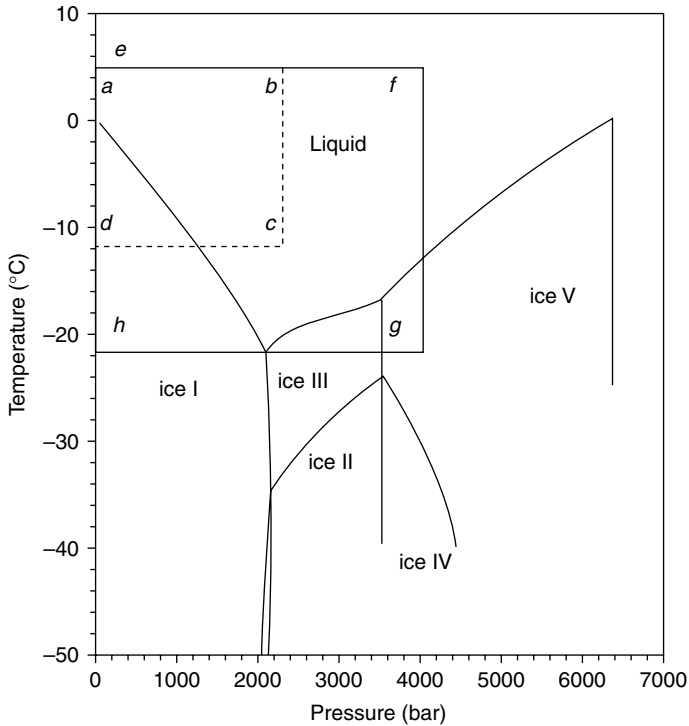
FIGURE 11.1   Effect of high pressure on liquid–solid phase transition of water (From Indrawati, A. et al., *Food Biotechnol.*, 12, 263, 1998. With permission from Marcel Dekker)

TABLE 11.1
Advantages of HHP Processing Acquired through Water in the Foods

1. Superior frozen quality due to formation of different polymorphs of ice
2. Rapid freezing by enhancement of ice nucleation by fast pressure release
3. Facility to maintain food products at subzero temperature without freezing
4. Glassy state of water because of fast cooling under pressure
5. Provision for rapid thawing of frozen foods below 0°C

nonfrozen conditions at subzero temperature under pressure.[4] Pressure-assisted freezing occurs due to the pressure-induced melting point depression, which enables water to remain in liquid phase at high pressures. As shown in the phase diagram (Figure 11.1), the melting temperature of ice I is lowered to −22°C when pressure increases to 207.5 MPa (2075 bar). Release of pressure enables rapid

and uniform nucleation leading to freezing of the sample. The pressure-assisted freezing produces smaller ice crystals rather than stress-inducing ice front moving through the sample (path "abcd" in Figure 11.1). The pressure-assisted thawing involves pressurizing a frozen sample up to the liquid area of the phase diagram, thus increasing the thawing rate if the appropriate heat of fusion is provided (path "dcba," in Figure 11.1). The use of high pressure on water offers the possibility to obtain other ice polymorphs instead of normal ice (ice I) (e.g., ice II, III, IV, and V) all of which have a smaller volume as compared to the liquid state. Other ice polymorphs can be obtained by direct pressurization at subzero temperatures (e.g., path "hg," Figure 11.1) or alternatively by pressurizing a liquid sample above 200 MPa and subsequently cooling the sample (e.g., path "efg," Figure 11.1). Depressurization of the sample will result in the formation of ice I. The influence of pressure on water can be beneficially applied for foods without causing significant damage.[5] The phase transitions occurring during the pressure treatment under different conditions may also beneficially influence the functional properties of the food constituents. The use of moderate hydrostatic pressure in combination with subzero temperatures has been proposed as a means of storing food products without the formation of ice, avoiding damage due to freezing and microbial spoilage. Further, significant energy could be saved using pressure-assisted storage rather than freezing. Product deterioration due to freezing and thawing effects is also avoided.[6]

11.5   INFLUENCE OF HIGH PRESSURE ON FOOD

11.5.1   Food Components

In order to evaluate the potentials of HPP in processing of food, it is important to understand its effect on food components. The effect of HPP on food components depends upon the nature of the constituents, pH, and treatment conditions such as temperature and presence of oxygen. Proteins are generally stable at high pressures. The treatment does not lead to covalent bond breakage and therefore, formation of newer compounds is insignificant, unlike in the case of thermal treatment. However, changes in the functional properties of proteins are possible at pressures in the range of 400–600 MPa, due to changes in noncovalent interactions such as hydrogen bonds and electrostatic interactions as a result of the treatment. These reactions eventually influence denaturation, gel formation, precipitation, association, aggregation, polymerization, and coagulation of proteins. A recent study showed that while pressure treatment at 50–300 MPa for 10 min at 20°C did not modify myofibrillar proteins, at above 300 MPa aggregation of proteins occurred with increase in hydrophobic bonding.[7] Purified myosin denatures at 100–200 MPa and actin at 300 MPa, with only a few soluble proteins surviving 800 MPa.[8,9] Modification of the tertiary and quaternary structures of proteins may induce a molten globular state and changes in their susceptibility to digestion by proteolytic enzymes.[10] Enzymes are generally stable to pressure treatment.[3,8,10,11] While activities of methyl esterase, peroxidase, and polyphenol oxidase are negligibly

affected, enzymes such as fish myosin ATPase and lipoxygenase are activated.[11] Cathepsin C, collagenase, chymotrypsin, and trypsin-like enzymes in fish are more susceptible to high pressure at 100–300 MPa.[9] Decrease in autolytic activity has been reported at pressures over 200 MPa in octopus.[12] Nevertheless, residual enzyme activities may affect the quality of some food and therefore they may require an initial blanching to inactivate the enzymes before pressure treatments.

Pressure treatment can affect meat color depending on the pressure level. When beef samples were treated to 130 MPa and stored at 4°C, there was an increase in redness, which was maintained up to 3 days of storage. However, the color of samples treated at 520 MPa decreased gradually. Cooking for 1 h at 65°C after pressurization nullified the color differences observed between the nontreated and pressurized samples.[13] The influence of pressure could be attributed to dimerization of metmyoglobin as a result of the treatment.[14] Subjecting fillets of fish such as those from cod, mackerel, etc. to high hydrostatic pressure can result in changes in their color and visual appearance. This is due to destruction of the myofibrils and release of the bound pigments, although no degradation through destruction of covalent bonds in the myofibrillar proteins occurs.[9] Lysosomes are cellular organelles containing digestive enzymes such as proteases. High-pressure treatment can disrupt the lysosomes and release the bound digestive enzymes.[15] In the case of carp, a freshwater fish, a decrease in the content of inosine monophosphate in the fish muscle treated at 350 and 500 MPa has been noted. Exposure to 50 MPa resulted in a decrease in total sulfhydryl contents and exposure of hydrophobic residues. At 100 and 150 MPa there was unfolding of myosins associated with increase in surface hydrophobicity. Myosins formed intermolecular disulfide bonds at 100 to 200 MPa. Increasing pressures decreases Ca–ATPase activity of myosin.[16] Carbohydrates are sensitive to changes in conformation leading modification in gelation characteristics. Starch components undergo gelatinization and become susceptible to enzymatic hydrolysis, while unsaturated lipids undergo oxidation. Polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid and docosahexaenoic acid of marine lipids are sensitive to oxidation. The effects of HHP on major food components are summarized in Table 11.2.

11.5.2 Microorganisms

Since microbial inactivation is a major effect of high pressure processing, this aspect has been studied in detail.[17–20] The sensitivity of microorganisms to pressure is extremely variable. The extent of inactivation depends on the type of microorganisms, the pressure level, the process temperature, exposure time, and the composition of the dispensing medium as indicated in Table 11.3. Gram-positive bacteria are more resistant than gram-negative bacteria. Vegetative cells in the early growth phase are more sensitive than cells in the stationary phase. Vegetative microorganisms have, in general, a decimal reduction time of 2–11 min at 300 MPa and 20°C. Vegetative cells and also spores of yeast and mold are inactivated at 200–300 MPa. Bacterial spores can survive pressures as high as 1000 MPa. Viruses are very resistant to pressure.[20–21]

TABLE 11.2
Some Effects of HHP on Major Food Components

Component	Pressure range (MPa)	Effect
Proteins	100–300	Unfolding of molecules, changes in hydrophobic and electrostatic interactions, changes in ionic bonds, denaturation, dissolution or precipitation, modified susceptibility to enzymes
Enzymes	400–500	Comparatively less sensitive
Starch	400–500	Gelatinization, modification in susceptibility to α-amylase
Lipids	300–500	Lipids in food system are generally stable. Isolated lipids are more prone to oxidation, which are further sensitised by metal ions

Source: Venugopal, V., Kamat, A.S., and Bongirwar, D.R., *Indian Food Ind.*, 20, 65, 2001. With permission from Association of Food Scientists and Technologists (India), Mysore.

TABLE 11.3
Factors Contributing to Microbial Inactivation by HHP

1. Pressure applied
2. Nature of pressure treatment (cyclic or continuous)
3. Temperature
4. Time of exposure
5. Microbial species and strain
6. Growth stage (vegetative cells or spores) and age of culture
7. Properties of suspending medium (ionic strength, pH, composition, etc.)

Optimization of pressure processing requires detailed studies on resistance of microorganisms present in the food to the treatment. Guidelines for laboratory practices for conducting experiments on inactivation of microorganisms at high pressure have been provided recently.[22] Pressure-induced inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens*, and *Listeria inocua* in beef muscle by HHP exhibited first-order kinetics. The inactivation kinetics of *C. freundii* was given by log n = 7.017 − 0.068 t, where n is the number of survivors after pressurization for 7 min at 230 atmospheres. The time required to reduce the

survivor population by 1 log cycle at 150 atmosphere and 20°C ($D_{150\ atm,\ 20°C}$) was 14.7 min. Similarly, the value for *P. fluorescens* under the same conditions was 23.8 min. For *L. inocua*, the inactivation time was 6.5 min at 330 atmosphere and 20°C.[23] However, a recent study suggested that HPP at 250 MPa did not inactivate *L. monocytogenes*, but only extended the lag phase of the organism up to 17 days at 5°C.[24]

High hydrostatic pressure inactivation of *Vibrios* spp. has received good attention essentially because of the interest in hygienization of oysters, which harbor these organisms in significant levels. *Vibrio vulnificus*, *V. parahaemolyticus*, and *V. cholerae* varied in their sensitivities to high pressure processing. *V. vulnificus* exhibited a decimal reduction time of 26 sec at 200 MPa, while *V. cholerae* was more sensitive requiring 14.9 sec for decimal reduction. A pressure treatment of 250 MPa for 120 sec achieved a 5-log reduction of *V. parahaemolyticus* serotype 03:K6 suspended in buffers. However, inactivation of the bacterium at the same level in oysters required treatment for 180 sec at 300 MPa.[25]

Pressure treatment reduced spoilage causing and pathogenic bacterial species present in shellfish, which included mussels, prawns, scallops, and oysters. The shellfish were pressure treated at 300–600 MPa for 2 min at 20°C and stored up to 28 days at 2°C. Pressure treatment inactivated psychrotrophic bacteria, coliforms, and pseudomonads. There was predominant inactivation of gram-negative bacteria leading to an increase in the proportion of gram-positive species. The main types of bacteria isolated from pressure treated shellfish were *Bacillus*, *Acinetobacter/Moraxella*, and lactic acid bacteria. Together these made up 96% of the bacteria isolated from the pressure-treated samples.[17] Pressure processing is beneficial for inactivation of several pathogenic microorganisms. Treatment of microorganisms in meat homogenate at neutral pH and 400 MPa caused a 6 log cycle inactivation of *Escherichia coli*, *Campylobacter jejuni*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *Sacharomyces cerevisae*. *S. typhimurium* was more sensitive, which was inactivated to the extent of 7 log cycles by exposure to 200 MPa for 10 min. While bacterial spores are highly resistant to pressure inactivation, a combination of gamma irradiation (2–4 kGy) and 400 MPa could reduce bacterial spore load more effectively.[21] The potential for HHP for sterilization of food has been examined, using spores of *Bacillus sterothermophilus* as the test organism.[26] The treatment achieved complete inactivation of 10^6 spores per g suspended in an enriched medium when repeatedly (five times) exposed to 600 MPa, each treatment involving an exposure at a temperature of 70°C.[27] Table 11.4 shows the pressure required for inactivation of microorganisms.

The mechanism of inactivation of microorganisms by HHP has been studied in detail with particular reference to their nucleic acid and membrane components. Nucleic acids are more resistant to pressure than proteins. However, DNA transcription and replication are disrupted by high pressure due to involvement of enzymes. The disruption is reversible at low pressure and irreversible at high pressures. Another site of inactivation is the cell membrane. The most important functions of cell membranes are providing barriers to diffusion, supporting

TABLE 11.4
Pressure Ranges for Inactivation of Microorganisms

Type	Species	Pressure (MPa)
Gram-negative bacteria	*Campylobacter*	300
	Pseudomonas	
	Salmonella	
	Yersinia	
Gram-positive bacteria	*Micrococcus*	600
	Staphylococcus	
	Streptococcus	
Bacterial spores	*Bacillus* spp.	600 with heating at 60°C for 40 min
Bacterial toxins	*C. botulinum*	Partial inactivation at 600 MPa

Note: Treatment conditions, 25°C, 10 min. Suspending medium, pork meat.
Source: Adapted from Ronner, U., in *Food Preservation by Combined Processes*, Leistner, L., and Gorris, G.M., Eds. Final Report, FLAIR Concerted Action No. 7 B, EUR 15776, EN, p. 31. With permission.

receptors, enzymes, and ion channels (the ion channels are involved in selectively transporting amino acids and other solutes across membrane), supply of lipid precursors for the cell's internal signal system, and maintenance of the shape of the cell. Treatment at high pressure adversely affects one or more functions of the cell membrane. Other reasons for microbial inactivation include denaturation and displacement of ATPase, and lowering of pH in the microbial cells. If the applied pressure is low, the cell regains the original permeability. The destruction of the cell wall is irreversible when the applied pressure is relatively high.[28]

Recent studies have shown that adding air or CO_2 during HPP can sensitize *E. coli* and *L. innocua* to high-pressure inactivation.[29] This has significance in the use of HPP in combination with modified-atmosphere packaging (MAP) for food preservation. This is also illustrated by the inactivation of *L. monocytogenes* in vacuum packed and modified-atmosphere packed, ready-to-eat poultry products.[30] Instead of modified atmosphere, HPP could be used along with bacteriocin to destroy *E. coli* O157:H7 as shown in vacuum packed frankfurters.[31] Apart from many microorganisms, HHP is also capable of killing *Anisakis simplex* nematodes in fishery products.[32] The requirement for pressure inactivation of some bacterial species is shown in Table 11.5.

11.6 Merits of Food Processing Employing HPP

The salient effects of high pressure on foods include modification of biopolymers such as proteins leading to changes in enzyme activities, gel formation, etc., changes in product functionality such as density, freezing/melting temperature,

TABLE 11.5
Pressure–Temperature Conditions Required for Inactivation of Common Bacterial Contaminants of Fish

Bacteria	Pressure (MPa)	Temperature (°C)	Treatment time (min)	Cell viability
B. subtilis	585–690	—	5	Vegetative cells killed
E. coli	290	25–30	10	Most cells killed
	100	30	0	9×10^8 cells/ml
	100	30	1440	10^2 cells/ml
	100	30	1800	4 cells/ml
	100	40	720	Sterilization
L. monocytogens	240	—	20	<10 cells from 10^6 cells/ml
P. aeruginosa	195	—	720	Sterilization
S. typhimurium	410–550	—	5	Sterilization
V. parahaemolyticus	170	—	10–30	<10 cells from 10^6 cells/ml
V. cholerae	195	—	720	10^6 cells/ml sterilization

Source: Adapted from Barbosa-Canovas, G.V., Pothakamury, U.R., and Palou, in *Non-Thermal Preservation of Foods*, 1997, p. 9. With permission from Marcel Dekker.

and textural attributes, inactivation of microorganisms, and retention of initial quality.[28] The microbial inactivation can enable hygienization and shelf life extension of foods.[21,33,34] In addition, the phase behavior of water under high pressure offers distinct advantages in application of HPP to food products. Possibilities for development of unique textured products and preservation of natural flavor are favored by pressure treatment.[21,35] The process can be combined with other food processing operations and can eliminate the requirements of chemical additives.[35]

The advantages of HPP are applicable to a variety of food items. The low pH fruit juices have been made shelf stable by inactivation of vegetative bacteria, with the pressure-resistant bacterial spores being suppressed by the pH. Pressure treatment of nonpasteurized citrus juice resulted in a flavor close to fresh juice with no loss of vitamin C and a shelf life up to 17 months. Strawberries and tomatoes retained fresh taste and color for several days under 50 to 200 MPa at −5 to −20°C, without significant loss in vitamin C. Egg yolk formed a soft gel when subjected to a pressure of 400 MPa for 30 min at 25°C, while maintaining its original color. Harder gels were obtained at 500 MPa. Pressure-treated rabbit meat kept its native color and flavor. Cured and noncured pork increased in hardness when subjected to 400 MPa for 30 min at 25°C. The digestibility by trypsin was increased in pressure-treated raw pork. Chicken could be preserved at −8 or −15°C under 170 MPa for 50 days without significant change in texture[18–20]. Table 11.6 summarizes the merits of high-pressure treatment of food.

TABLE 11.6
Advantages and Disadvantages of High Pressure Treatment of Food

Advantages	Disadvantages
Treatment at low or ambient temperature results in minimum change in food components. Unlike heating, no covalent bond breakage and formation of newer compounds occur. Minimum changes in vitamins and other nutrients. Microbial inactivation helps shelf life extension of fresh muscle foods	Food enzymes generally require very high pressure for inactivation. At usual range of pressures used, an increase or decrease in enzyme activity may result, affecting the food quality. A blanching process needs to be included to inactivate the enzymes.
Opportunity to reduce the use of food additives	Dissolved oxygen results in oxidative degradation of food components
Less energy requirement	Most pressure-processed foods need low temperature storage and distribution to retain their sensory properties
The treatment is isostatic (uniform throughout the food). The effect on the food is instantaneous	Bacterial spores are resistant
Minimum damage to food packaging due to isostatic effect	
Environmentally friendly process	
Possibility to produce foods with novel texture (e.g., meat, fish, dairy products)	

Source: From Venugopal, V., Kamat, A.S., and Bongirwar, D.R., *Indian Food Ind.*, 20, 65, 2001. With permission from Association of Food Scientists and Technologists (India), Mysore.

11.7 HPP of Fishery Products

The perishability and safety concerns with respect to fishery products have attracted detailed investigations on their amenability to HPP.[1,20,28,34,36–41] Most studies have shown significant beneficial effects of HHP treatment in seafood processing. The behavior of water under pressure offers scope for application of HHP in seafood processing, employing pressure-freezing, pressure-thawing, and preservation at subzero temperatures under non-frozen condition. The sensitivity of spoilage causing and pathogenic microorganisms can be exploited for shelf life extension of fresh fish and also their hygienization. Furthermore, high pressure processing offers novel texturized products making use of its effect on fish myofibrillar proteins. These aspects are discussed below.

11.7.1 Pressure-Assisted Freezing and Thawing

Pressure-assisted freezing and thawing are benefits that can be accrued based on the phase diagram shown in Figure 11.1. These have significant application in

processing of fishery products to obtain superior quality products.[42,43] Pressure-assisted freezing helps rapid freezing of fishery products without formation of large ice crystals that can have adverse effects on the product. The pressure-assisted thawing being faster is associated with less amount of drip formation, as compared with conventional thawing. From a microbiological point, thawing at a lower temperature and under pressure is safer, because low temperature and high pressure can have a synergetic effect on the lethality of microorganisms.[33] Pressure-assisted thawing at 50 to 210 MPa and at initial temperatures ranging from −5 to −20°C was examined in the case of whiting. A pressure of 210 MPa was found to be optimum for thawing based on the heat transfer criterion.[44] Higher temperature and higher pressure resulted in partial melting during pressure loading causing the sample temperature to decrease.[42,43] The latent heat of water decreases with increase in pressure (from 333 kJ/kg at atmospheric pressure to 240 kJ/kg at 200 MPa). Therefore, there is a need to raise the temperature of the thawed sample by a few degrees above its freezing point at atmospheric pressure to avoid partial freezing of the sample. From a thermal point of view, high pressure thawing of fish is faster because the larger difference between the heat source and the phase transition temperature. Higher pressure could alter the structure of the Ice I crystals, which is not desirable because of the possibility of textural damage of the food. The different enthalpy changes associated with crystallization of water to ice III and ice V would affect the overall duration of the process.[45]

Quality of fish subjected to pressure-assisted thawing at 200 MPa has been compared with that of sample conventionally thawed in water at 15°C at ambient pressure. Visual differences in the pressure-assisted and water-immersion thawed fillets have been observed. Organoleptic characteristics of the high-pressure treated fillets were better compared to the conventional water thawed samples. Differences were also observed in microbial counts, pH value, water binding ability, instrumentally evaluated texture parameters, and in thermo-analytical behaviour of the two differently thawed samples. The variations in these parameters were dependent on the fish species. It was concluded that the specific properties of samples have to be taken into consideration when designing pressure supported processes.[46] In another study, the effect of high pressure processing on color, drip loss, and texture of Atlantic salmon frozen by different methods was compared with thawing. The freezing methods included conventional air freezing, plate freezing, and liquid nitrogen (LN) freezing. The frozen samples were subjected to different thawing treatments. These included water immersion thawing (WIT) at 4 and 20°C and high-pressure thawing (HPT) at 100, 150, and 200 MPa employing water (containing 2 g oil/100 g) as pressure medium at 20°C. Temperature and phase-change behavior of fish samples were monitored during freezing and thawing. The phase-change temperatures of frozen salmon were lowered to −14, −19, and −25°C for the HPT processes at 100, 150, and 200 MPa, respectively. These phase-change temperatures were lower than those for pure ice at the same pressures, possibly due to the presence of solutes in the fish. The time required for HPT were 22.6 ± 1.4, 18.1 ± 1.4, and 17.0 ± 1.3 min at 100, 150, and 200 MPa, respectively, whereas the WIT process required 26.6 ± 2.1 and 94.3 ± 3.4 min

at 20 and 4°C, respectively. Employing pressures above 150 MPa caused notice-
able color changes in salmon during the HPT process and the product texture was
significantly modified during HPT at 200 MPa. Different freezing rates prior to
thawing resulted in differences in drip loss in salmon samples, but these did not
induce specific color and texture changes. A significant reduction of drip loss by
the HPT process was observed only for the LN frozen samples in which mechanical
cracking occurred and much of the drip appeared after WIT process.[47]

11.7.2 Storage Under Subzero Temperature Under Nonfrozen State

The use of moderate hydrostatic pressure in combination with subzero temperat-
ures has been proposed as means of storing high-value seafood items without the
formation of ice, which can avoid damage due to freezing and microbial spoil-
age. Further, significant energy could be saved using pressure-assisted storage.
Product deterioration due to thawing is also eliminated. However, progress in this
area is hampered essentially by lack of cost-effective equipment for storage of the
treated products.

Commercial applications of high pressure for freezing, thawing, and holding
of fishery products under nonfrozen conditions at subzero temperatures demand
sufficient knowledge on the thermo-physical properties of the processed mater-
ials under pressure. Modeling heat transfer in high-pressure food processes can
be a useful technological tool for process development. Advances made in this area
have been reviewed recently.[43,48] When modeling high-pressure processes at sub-
zero temperatures, pressure/temperature phase transition data and latent heat are
also needed. While those for water are well studied, the data are insufficient for
food components. Moreover, the precise mechanisms that rule high-pressure shift
freezing and induced thawing are not yet clear.[4,42]

11.7.3 Extension of Shelf Life

High pressure treatment significantly inactivates several spoilage causing micro-
organisms, enabling scope for enhanced shelf life of fishery products. However,
it may be noted that at the pressures usually employed for food processing, ster-
ilization cannot be achieved, because of extreme resistance of microbial spores
to the treatment.[17] Therefore, such pressure-treated products should be stored
below 3.3°C to control the growth of surviving microorganisms and also to ensure
safety from spores of *Clostridium botulinum* type E that might have contamin-
ated the samples. A number of fish and shellfish items have been examined for
shelf life extension under chilled conditions using HHP treatment. In the case of
pressure-treated tilapia fillets, the *K*-value increased less rapidly during storage,
as compared with nonpressurized fillets, suggesting lower spoilage rate as a result
of the treatment.[16] Subjecting squid mantle flesh and tuna meat to 450 MPa at
25°C for 15 min lowered the initial bacterial counts from 5.2×10^3 and 1.9×10^3
to about 300 organisms per g, respectively favoring enhanced chilled shelf life.

Salmon has been studied in detail for high pressure treatment.[49,50] A pressure level of 150 MPa was found ideal for shelf life extension and also for retaining the color of modified atmosphere stored Atlantic salmon.[49] Pressure treatment has been found useful for preservation of cold-smoked salmon fillets.[50] Recent studies have shown that pressurization is more effective when applied in consecutive pulses than when applied continuously, essentially because of higher sensitivity of microorganisms to pulsed pressure treatment than a continuous exposure.[12]

Apart from pressure-induced microbial inactivation, suppression of activities of a number of muscle enzymes by high pressure may also contribute toward the quality of chilled stored fishery products.[3,51,52] Darkening of shellfish due to melanosis is a problem during their prolonged chilled storage (Chapter 2). Oyster meat may turn dark during chilled storage, the darkening being caused by rupture of roe or kidney or by melanosis. Effect of HHP on the problem has been examined. Oysters treated with and without 4-hexyl resorcinol were pressurized at 400 MPa at 7°C for 10 min, subjected to either vacuum or aerobic packaging and then stored for 21 days under chilled conditions. It was observed that activities of polyphenol oxidase (PPO) and autolytic enzymes increased by pressurization. PPO activity was inhibited by 4-hexyl resorcinol chiefly in the vacuum packed samples.[53] However, pressurization reduced autolytic activity in oysters.[12,52] Enzymatic degradation of ATP occurred at appreciable levels during pressure-chill storage.[52]

Carp could be preserved at −8 or −15°C under 170 MPa for 50 days, without significant changes in texture.[51] A pressure of 100 MPa was optimum for preservation of turbot fillet without any changes in physicochemical properties. Both high pressure and holding time were important parameters to achieve a desirable effect.[54] High pressure at a level of 150 to 200 MPa for 5 min resulted in a cooked appearance in trout, cod, carp, plaice, and pollack, whereas octopus retained its natural appearance even at 800 MPa.[55] Subjecting squid mantle and tuna meat to 450 MPa at 25°C for 15 min caused reduction of initial microbial counts from 5200 to 300 per g. Combination of processing techniques such as modified-atmosphere storage salting, heat pasteurization, and gamma irradiation along with HHP has potential to enhance chilled storage life of several fishery products, because of the synergistic effect of the microbial hurdles on viability of the surviving microorganisms (Chapter 6).

Cephalopods are one of the major fishery products consumed in the Mediterranean countries followed by those in Asia. Spoilage of cephalopods during chilled storage is a serious problem because of the high level of proteolytic activity, which also favors microbial growth in the muscle. Continuous or pulsed HPP treatment at 7 and 40°C resulted in a reduction in autolytic activity and reduction of microbial flora in octopus (*Octopus vilgaris*). The reduction was more significant in the step-pulse pressurized at 400 MPa. The shear strength values of the meat remained unchanged during storage at 2.5°C. Pressurization also reduced drip formation and extended shelf life of octopus at 7°C, 43 days longer than the nonpressurized counterpart.[12] Pressures as high as 800 MPa affected the physicochemical characteristics of oyster muscle.[53,60]

11.7.4 Hygienization

The sensitivity of microorganisms to HHP can be advantageously used for hygien-ization of perishable foods including fishery products.[35,56–59] The operational goal of HPP pasteurization is to achieve a reduction of 5 to 6 log cycles of vegetative bacterial pathogens. *L. monocytogenes* is a major problem in the salmon industry, particularly in cold smoked salmon. High pressure treatment could be applied to inactivate this bacterium in the product.[50] Several epidemics of severe illness have been associated with the consumption of raw oysters in many countries. Major-ity of the incidences is attributed to *Vibrio* spp. (Chapter 2). Recent studies have shown that HHP processing can significantly reduce total plate count of aerobic and anaerobic bacteria including pathogens in oysters while maintaining good sens-ory quality.[60] Optimum reduction of *V. parahaemolyticu*s in oysters was achieved when exposed to 345 MPa for 90 sec.[58] Nevertheless, variations in resistance to hydrostatic pressure among foodborne pathogens needs to be considered in using the technology.[59]

11.7.5 Shucking of Oysters

Shucking oysters, clams, mussels, and scallops under natural conditions has been in use on a commercial scale, particularly in the case of oysters. Traditionally, the adductor muscle in oyster is cut by hand with a sharp knife, which is a time consuming job, although machines are now available for the purpose. Microwave heating may be used to open oyster shells, but it gives a heat treatment that changes the sensory characteristics. HPP can facilitate shucking, since being a cold process it does not cause normal heat-associated changes in the muscle. In the process, oysters are placed into holding vats where hydrostatic pressure is increased to 200 to 300 MPa. As a result, the adductor muscle detaches and the shellfish releases its muscle from its shell, essentially shucking itself. The process also significantly reduces bacteria, including pathogens, and extends the shelf life while preserving the taste and texture. At the commercial level shucking oyster using high hydrostatic pressure is being practised in the United States.[61] Some of the HPP treated oyster products include whole oyster, half shell, and shucked oyster meat. HPP treatment of oysters involves optimization of pressure conditions to prevent physicochemical changes such as denaturation and increase in moisture content of adductor muscle.[60]

11.7.6 Pressure-Induced Texturization of Fish Proteins

The possibilities for pressure-induced gelation of proteins through changes in noncovalent interactions offer potentials for development of fish products having novel textural attributes.[7,9,38,50,62,63] Pressure-assisted gelation depends on the pro-tein characteristics, its concentration, environment such as pH and ionic strength, as well as pressurization conditions namely pressure level, continuous or pulse mode, temperature, and exposure time.[38,62,63] The changes result in modific-ation of functional properties of the proteins. Pulsed pressure has been found

more effective than continuous pressure treatment. While pressure is transmitted in uniform (isostatic) and quasi-instantaneous manner throughout a biological material, heat transmission by conduction is a rather slow process, so that a temperature gradient develops within the food.[64] The heating rate depends on the initial and final temperature of the sample, temperature of the processing medium, and sample size. In some fish myofibrillar proteins, the sol–gel transition can take place even at 4°C. Particular attention must therefore be paid to sample preparation and pretreatment conditions.

Responses of fish myofibrillar proteins to HHP can have significant applications in the *surimi* industry for development of novel products. *Surimi* forms a gel with excellent texture when exposed to mild heat in the presence of 2.0 to 2.5% salt (Chapter 8). Instead of heat, pressure can be used to induce gelation of *surimi*. The pressure-initiated gelation may also be completed by mild heating of the proteins. Alternatively, gelation using HHP could also be augmented by the enzyme transglutaminase that enhances cross-linking among the proteins.[65] Pressure induced *surimi* gels from marine species are smoother and more elastic than those produced by heat. Excellent gels could be produced from pollock, sardine, skipjack, and tuna meat at 400 MPa and from squid at 600 MPa. Pressure treatment is also effective in producing highly appealing kamaboko from *surimi*. Low-fat fish is ideal for HHP treatment, since the treatment may induce oxidation of lipids in fatty fish.

Milkfish was employed as a model system to obtain fundamental data on high pressure induced gelation and also inactivation of actomyosin Ca–ATPase. It was observed that the gel characteristics differed according to the treatment conditions and also presence of food additives.[66] The gelling properties of blue whiting mince in presence of several hydrocolloids including locust bean gum, guar gum, xanthan gum, carboxymethylcellulose, iota-carrageenan, Kappa-carrageenan, or alginate has been examined under different pressure–time-temperature conditions employing multivariate statistical methods. The main differences among different gel characteristics were attributed to the process.[67] Reddish flesh of mackerel meat could be changed to opaque, as if it had been grilled or boiled. Similarly, the transparent cod meat was converted into opaque when subjected to 600 MPa for 15 min.[56] The effect of high pressure (100–400 MPa for 30 min) followed by storage at 5°C caused whitening and changes in jelly strength of the minced sardine meat. Denaturation of proteins during pressure treatment, which adversely influences gel strength, can be controlled by the use of cryoprotectants. Muscle tissues from four species of fish from different habitats, namely, orange roughy, tilapia, pollock, and hoki, were exposed to 50–200 MPa. At 200 MPa, there was denaturation of the proteins and a cooked appearance to the flesh. Stability of the proteins from the different species seemed to vary primarily according to body and habitat temperatures. Sugars and polyols increased the muscle protein stability during the treatment, sorbitol being the most effective agent. The degree of protection was however less during pressure-assisted freezing of the fish species.[68]

The effects of HHP on gel strength of Pacific whiting and Alaska pollock *surimi* were determined by torsion. Pacific whiting gels were made with and without

1% beef plasma protein (BPP) as protease inhibitor. HHP treated whiting and pollock gels in presence of BPP showed higher strain values at all pressure and temperature combinations compared with heat-set counterparts. Stress values for the same samples were variable depending on treatment and species. A threefold increase in strain and stress was found for HHP treated whiting gels made without inhibitor.[69] There were no significant changes in breaking strength, when the pressure-induced walleye pollock *surimi* gel was subjected to heat treatment at 90°C for 30 min after storage at 5°C up to 120 days. The subunit compositions of myofibrillar proteins were also practically unchanged.[70] The superior quality of HHP treated *surimi* gel was demonstrated in another study. Frozen *surimi* from Alaska pollack was ground with 2.5% NaCl and exposed to HHP. Strong gel was formed from the salted paste by pressure treatment at 200–400 MPa and 0°C, for 10 min. The gel obtained by treatment at 300 MPa had the highest gel strength. In addition, the pressure-induced gel was also characterized by its transparent nature in comparison to the heat-induced gel.[71]

11.8   Sterilization of Foods

Sterilization of food employing pressure has been examined only on a limited scale.[26] This was essentially due to the requirement of very high pressures for inactivation of bacterial spores, as mentioned earlier. Nevertheless, HHP-assisted sterilization of a number of foods including fish has been investigated using *B. stearothermophilus* as a model bacterium. It was observed that sterility could be achieved using pulsed high pressure in conjunction with heat. The critical factors for achieving sterility were two or more cycles of pressurization, a minimum temperature of 105°C, and the duration of treatment, the levels of the variables being determined by the initial level of bacterial load. For high-pressure sterilization of low-acid foods including seafood, a pressure of 1172 MPa and a temperature of 60°C have been suggested.[26] However, the amenability of the seafood to such high pressure needs to be ascertained in addition to equipment that can withstand such stringent treatment conditions.

11.9   Equipment

For HHP treatment, the food packaged in containers such as conventional plastic materials is charged into a pressure vessel filled with normal tap water. The vessel is closed and the pressure therein is increased to the required value and then decompressed to ambient pressure again. The treated package is dried, which is ready for further processing or serving. The holding time in the pressure chamber depends on the type of food and process temperature. In development of equipment for HHP processing, it is important to note that a 50 l equipment is comparable to 200 l heating vessel and a time of 15 min for the treatment is equivalent to 1 h in heating and cooling operations.[72] The early equipment used for high pressure processing were
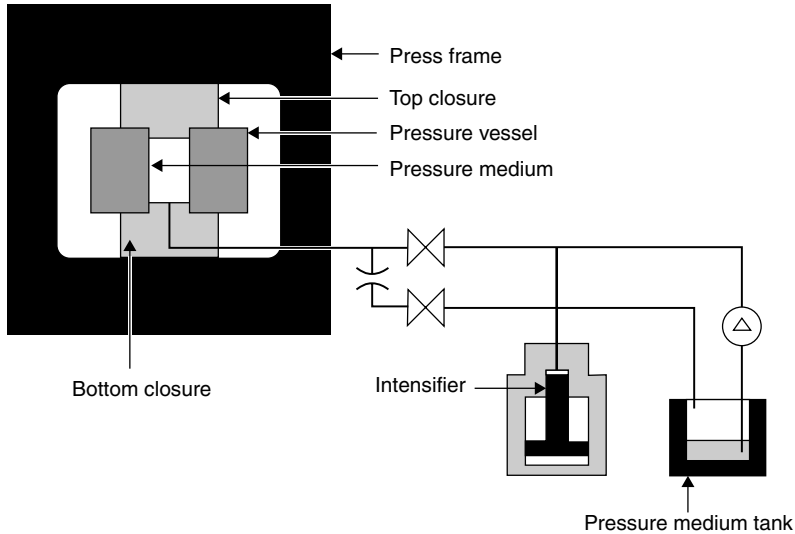
FIGURE 11.2 Generation of high pressure by indirect compression of the pressure-transmitting medium (From Barbosa-Canovas, G.V., Pothakamury, U.R., and Palou, in *Non-Thermal Preservation of Foods*. New York, Marcel Dekker, 1997, p. 9. With permission)

those used in ceramic processing, which were modified with additional sensors. Later, batch type machines having limited volumes were developed.

High pressure can be generated by: (i) direct compression by pressurizing a medium with the small-diameter end of a piston, (ii) indirect compression using a high-pressure intensifier to pump a pressure medium from a reservoir into a closed high-pressure vessel, or (iii) heating of the pressure medium, utilizing its expansion with increasing temperature to generate high pressure. Most industrial isostatic pressing systems employ the indirect compression method, as shown in Figure 11.2.[19] Advances in equipment design and construction have brought down the processing costs to be competitive with retorting and freezing.[26] The important engineering challenge of the application of high pressure in the food industry is the fabrication of pressure vessels to handle required volumes of food. The pressure vessel should have a short cycle time, be easy to clean, and be safe to operate, with accurate process control.[19] A pressure indicator for hydrostatic processing of food has been developed recently.[73] Some of the companies manufacturing high-pressure process equipment have been indicated in Annexure. The Quintus high pressure research food system developed by ABB Autoclave Systems, Sweden, consists of prestressed, wire-bound isostatic pressure containment system, intensifier-based electrohydraulic pumping system, water circulation system, and control module. The equipment can withstand up to 900 MPa and a temperature of 80°C. The commercial opportunities for high pressure processing of foods has been discussed recently.[74]

11.10 Packaging

Plastic films are generally amenable for HHP, which may not be generally suitable for high-temperature processing. However, metal cans and glass containers are not suitable for the process. Packaging materials for HHP treatment should preferably be oxygen impermeable to avoid the adverse interactions between food components, such as oxidation of unsaturated lipids, under the treatment conditions. There is a need for development of improved packaging materials that prevent oxygen permeability and light exposure for keeping fresh color and flavor of HHP processed foods including fishery products.[75] Specific packaging and equipment requirements for pressure-assisted freezing and thawing have been discussed.[42]

11.11 Commercial Potential and Consumer Attitudes

The first generation commercial high-pressure processed foods were shelf stable, low-acid foods. High-pressure processing is being commercially used, although on a limited scale, for pasteurization of seafood, meat, and other food.[26,60] The HHP-pasteurized low-acid foods including fishery products have potential for distribution under refrigeration as Extended Shelf Life (ESL) products. The technology enables processors to make novel, minimally processed ESL, convenient food items with fresh-like attributes and natural-looking colors.[40] A consortium of professional bodies and manufactures of high-pressure equipment was formed to pursue regulatory acceptance, market development and equipment to demonstrate the commercial potential of high-pressure processing of foods including low-acid foods.[40]

Consumer attitudes to HHP processed products are important in determining the success of the technology. In a survey, a consumer population of 3000 adults aged 14 years and above, from France, Germany, and the United Kingdom was interviewed in order to compare their response to high-pressure processing with conventional techniques. The survey revealed that HPP was acceptable to majority of consumers in France and Germany. The overall average acceptability value of 67% suggested consumers willingness purchase HPP food products. The main concerns expressed by the consumers were cost and health benefits. Young educated people were more in favor of the technology.[76] These studies demonstrated commercial potential and research challenges in high pressure processing.

In conclusion, HHP processing has potential for value addition of seafood. The main benefits of HHP include inactivation of contaminant microorganisms, texturization of proteins, shucking of oysters, and improved freezing and thawing operations. Pressure-assisted hygienization has found commercial application for products such as oysters. Development of novel textured products such as *surimi*-based seafood analogs is another use. High pressure can help extension of shelf life of fresh seafood in nonfrozen conditions under subzero temperatures. Combination of other processes such as low-dose radiation, heating, MAP, etc. together with

high pressure could have multiple benefits in seafood processing. Improvement in equipment for continuous treatment of large volumes of fishery products would be beneficial for commercialization of this technology.

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12 Value Addition of Freshwater and Aquacultured Fishery Products

12.1 INTRODUCTION

Freshwater fish and shellfish constitute an important component of global fish production. This has been attributed to rising aquaculture operations, which has been growing at about 11% per annum since the 1980s. More than 80% of the world's total aquaculture output is from Asia. The prominent farmed shellfish are black tiger prawn (*Penaeus monodon*), white leg shrimp (*Penaeus vannamei*), and scallop (*Pecten yesoensis*). Of the total finfish production of 16.6 mt in 2000, freshwater fish contributed as much as 86%, the rest being marine and diadromous fishes.[1] The major cultured finfish species include grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), big head (*Aristichthys nobilis*), crucian carp (*Carassius carassius*), mud carp (*Cirrhinus molitorella*), black carp (*Mylopharyngodon piceus*), Nile tilapia (*Orechromis niloticus*), rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhinus mrigala*), Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), milk fish (*Chanos chanos*), Channel catfish (*Ictalurus punctatus*), Japanese eel (*Anguilla japonica*), and yellow tail (*Seriola quinquer adiata*).[1] However, in general, unlike their marine counterparts, freshwater products attract consumers from only limited market segments, perhaps because of their peculiar flavor and other characteristic features. Nevertheless, in view of the stagnant marine stocks, there is a need for value addition to freshwater fish, both wild and cultured, to meet the increasing demand for fishery products.[2–4]

12.2 ADVANTAGES OF RAISING FISH THROUGH AQUACULTURE

The rapid growth in aquaculture owes to the advantages associated with farming operations. Farming enables the producer to maintain better control of the product from farm to market. Apart from scope for domestication and improvement of the cultured species, the process parameters could be carefully controlled to deliver products having the required quality. Such controls could be operated at the production environment, stocking density, feed quality and feeding time, harvesting conditions and practices, and during postharvest processing.[5] These controls help

to ensure a higher quality product with a longer shelf life compared with wild-caught species. While controls at the farming level are not within the purview of this chapter, the discussions will focus on techniques for value addition, marketing, and quality aspects of processed freshwater and aquacultured fishery products.

12.2.1 Influence of Environment on Quality

Similar to wild fish, freshness of fish is the single most important attribute in assessing the quality of cultured fish. Sensory methods offer the most satisfactory means of assessing the freshness quality of fish. The taste of freshwater fish depends mainly on the quality of the water habitat and on food. For example, carp living in dirty and muddy ponds, have an unpleasant flavor. The flavor of wild trout from streams is better than that of the aquacultured fish. Aquacultured eel has a more tender tissue and thinner skin. In some cases, wild and cultured fish may have a comparable sensory quality, as observed in the case of sea bass (*Dicentrachus labrax*), the fish from both the habitats giving a chilled shelf life up to 18 days.[6] The important factors affecting the quality of cultured shrimp are the environmental conditions of the pond (such as salinity, temperature, microbial population, as well as organic and inorganic matter), handling, and processing methods.[7] Farming operations, if not carefully executed, may give rise to fishery products that can be carriers of health hazards. For a product free from any health hazard, careful management and monitoring of the growth environment is essential. The importance of selecting farm sites with a reliable water supply free of chemical hazards and microbial pathogens is of foremost importance.

Harvesting of fish is subject to management decisions and can be timed to get fish of required quality, processing yield, and demand. However, since these decisions are often aimed at preserving the stock, the processing plant receives fish with wide variations in quality.[8] Influence of season on physical (length–weight relationship), biological parameters (males, females, and combined sexes) and gonadosomatic index (GSI) with respect to processing yield of wild carp (*C. carpio*) has been studied recently.[9] A resting period and period of rapid growth (April–September) were observed in gonads of female carp, which influenced the processing yields. Spawning takes place in August–September. Physical and biological parameters obtained for carp were comparable to those of hake (*Merluccius hubbsi*).[3] More care is needed when fish are marketed live or eaten raw. Gentle harvesting slows the onset of postmortem changes, and thus, effectively prolongs the shelf life of the product.[6] It is possible to quickly move the fish to the factory before the fish entering rigor mortis. Handling of fish prerigor has the advantage of delaying the onset of deteriorative processes in the flesh. However, for product development, fish are best processed once rigor has resolved, otherwise the flesh will shrink or come apart during freezing and thawing (Chapter 2). Immediately after harvest and slaughter, the flesh characteristics begin to deteriorate through biochemical and microbiological processes (see Chapter 2). Many of these strategies that are used in aquaculture to arrest these processes are the same as those for the handling of seafood in general.

12.2.2 Color and Flavor of Aquacultured Fish

The color of flesh is an important marketing attribute for many fishery products including aquacultured items.[10] Dietary sources of xanthophylls, commonly found in yellow corn and corn gluten meal, can lead to objectionable concentrations of yellow color in the muscle of some fish such as catfish. Atlantic salmon requires a deep pink color for optimal acceptability. Smoked salmon is sold in markets that have different preferences for flesh pigmentation, but usually a deep red/orange color is preferred. Feeding pigments, flavor volatiles, and natural antioxidants have been resorted to impart "fresh sea" flavor and color to enhance the marketability of salmonid fishes.[10–13] However, dietary supplementation of carotenoids is expensive and less than 20% of the supplemented compounds contribute to flesh pigmentation. Addition of carotenoids at small concentrations (about 35 mg/kg meat) during processing of rainbow trout has been suggested as an alternative.[10]

In contrast to saltwater fish, many freshwater fish and shellfish from ponds, rivers, and lakes, such as carp, catfish, and trout, have an earthy odor. The off-flavor is mostly associated with absorption of certain compounds usually found in freshwater or water of low salinity (<3/1000 parts). The muddy off-flavor is usually caused by geosmin (*trans*-1,10-dimethyl-*trans*-(9)-decanol) or MIB (2-methylisoborneol).[14] These compounds are synthesized by blue green algae growing in the seawaters. Fish absorb these compounds directly from the water through the gills and the skin. Eventhough, the muddy flavor usually disappears within several weeks, it is a serious inconvenience to the industry. Sensory thresholds of geosmin and MIB in fish flesh are 8.4 and 0.8 ppb, respectively.[15] The earthy odors of fresh carp and salmon are derived also from pyrrolidine and piperidine and their complexes with ethanal, while their combinations with TMA are responsible for the faint odor of saltwater fish[14]. High piperidine contents (up to 13 mg per 100 g muscle) have been reported in salmon and carp just after spawning.[14] There is no satisfactory control for production of the odorous compounds in the culture system. However, off-flavors can be purged from the fish by holding it in clean water for 3 to 7 days.[15]

12.3 HANDLING OF LIVE AQUACULTURED FISH AND SHELLFISH

The live fish market has become an important niche market, not just in Southeast Asia and the Far East but also in other parts of the world such as the United States, Australia, and Europe. Many restaurants in these countries keep fish in aquarium and prepare them for the table as per the consumer choices. A well-managed approach in harvesting gives aquaculture a greater scope for "stress-free" harvesting, unlike in the case of wild fish. The technology for harvesting and transportation of live fish has been sophisticated in the recent years.[16,17]

12.3.1 Conditioning

For live transportation, the fish are "conditioned" by starving them up to 24 h by placing them in clean running water in separate tanks. There are several advantages of

conditioning the fish. The process removes food remains from the alimentary ducts and lowers the oxygen demand. It reduces stress, inhibits metabolism, and removes off-flavor from fish. Conditioning may also improve texture of the meat and limit the excretion of ammonia and carbon dioxide from the fish. Besides, the treatment reduces mortality and aids in increasing packing density for transportation.

During conditioning, oxygen content of the water and its temperature should be properly maintained to supply the required oxygen for the starving fish. For 1 kg of fish at a temperature of 10°C, the oxygen demands for eel, carp, and pike are 25, 45, and 50 mg, respectively. Young fish need more oxygen than older fish. The water temperature needs to be adjusted depending upon the season. For carp, the temperature should be not less than 10–12°C in summer and 5–6°C in spring and autumn. For trout, the optimal temperatures for conditioning and transportation are 5–6°C and 3–5°C in summer and spring, respectively. During winter, fish can tolerate temperatures as low as 1–2°C. In the short conditioning process 1 m^3 of water is sufficient for 50–60 kg of carp, 30–40 kg of pike, and 20–25 kg of trout or pike-perch.[18]

12.3.2 Sedation

The conditioned animals are anesthetized by bubbling carbon dioxide into the water, maintaining low water temperature. Fish may be treated with 500 ppm of carbonic acid or 150–650 ppm of sodium bicarbonate for up to 5 min for the purpose. Tricaine methane sulphonate (MS 222) and carbon dioxide are two anesthetics cleared by the U.S. Food and Drug Administration (FDA) for use in food fish. MS 222, however, needs a withdrawal period of at least 21 days before the fish can be released for human consumption. Sodium chloride or calcium chloride at 0.2–0.3% concentrations is often used to reduce handling stress and delay mortality of fish.[16,17]

12.3.3 Transportation

Special tanks with aeration system often alongwith cooling (using ice) and filtering systems (activated coal, biological filters) are used for transportation of live fish on trucks. Blowers fitted to the transport trucks are commonly used for aeration during transport of tilapia. Lowering the temperatures reduces the metabolic rate of the fish and thus prevents stress during transportation. For tropical fish, a temperature between 15 and 18°C has been recommended. Cost-effective life-support systems for display and holding fish have been designed. Care should be taken in handling live fish to avoid exposure to high temperature and direct sunlight.

The traditional polystyrene box used for live fish transport is gradually being replaced by more functional aerated boxes made of fiberglass or high-density polyethylene (HDPE). The double wall rotary molded containers manufactured from low- and medium-density polyethylene with a polyurethane core, approved by the U.S. FDA, provide additional structural strength and insulation. These containers can hold more fish per unit volume of water, although usually fish and

water are maintained in equal quantities. Such containers are often equipped with an oxygen cylinder for aeration. Even if the temperature and oxygenation levels are properly maintained, at 10°C or lower, some weight loss occurs, which varies from 1 to 6%. About 10% of carp and 20% of trout die during transportation in winter extending for 6 days.[16,17] Once the fish arrives, it is acclimatized in holding tanks containing aerated water having comparable temperature of the transfer pouches. The fish are then carefully transferred to the tanks, which should be kept dark and covered to avoid stress of the fish. They should be fed after 1 day of their arrival and transfer to the tank. The main purpose of the holding tanks is to keep the fish in good live condition and to display them to maximum advantage. The holding tank can be of either fixed or mobile types, closed, semi-closed, or open systems with provision to maintain water quality through filtration.[3,16,17]

Air transportation of live fish is becoming increasingly popular. For air transport of large high-value species, plastic bags may be used. Generally, the gross weight of the pack should not exceed 20 kg as per airline requirements. Double-layered plastic (polyethylene) bag around 100 μm thick is suitable for packing the fish. About 3 kg of conditioned fish can be packed in a bag containing 10–12 l of chilled water with ideally an equal volume of head space filled with oxygen. The bag is then placed in a polystyrene box of approximately $50 \times 50 \times 50$ cm^3 in size and thickness less than 3 cm. Ice may be added to the box to keep the temperature low. Commercial gel ice products can be ideally used in live air freighting of fish. The gel ice comes in roll form. It is prepared for use by placing it in water for 12–15 min. The product soaks up water like a sponge. Once hydrated, the product is drained of excess water and then placed in a freezer. The product is then ready for use in live fish packaging in place of ice to keep the transport water cool. A recent Japanese technology for live transportation involves virtually freezing the fish such as sole prior to air transport. The fish held in seawater tanks is cooled to freezing point. At this stage, water is drained. After air lifting, the fish is revived at destinations by immersing in water at room temperature. A method employing partial paralysis of the fish has also been suggested for live transportation.[3,16]

A few species of such as kuruma prawns, black tiger shrimp, and giant freshwater prawns are suitable for marketing alive, since they can survive up to 10 h out of water. These are packed in closed, oxygenated plastic bags or in open tanks with aerators. In many Southeast Asian countries, live black tiger and other species of shrimp are fairly densely packed in aerated tanks of 900–1600 l capacities, and are usually delivered to buyers within 6–8 h of harvest. Ideally prawns should be harvested at night to reduce temperature stress and transferred into holding tank with adequate aeration and water temperatures at 8–12°C or lower. Cooling at this temperature for 20–30 min induce hibernation. Shrimp in sawdust are packed in Styrofoam boxes, which are placed in cartons for air shipment. Mollusks (oysters, mussels, clams, and cockles) and crustacea (crabs and lobsters) are mostly marketed in the live form. Being filter feeders, they need to be depurated to make them safe for human consumption. Crabs and lobsters could be held in moist bags or wooden boxes for several days in cold circulating water. Lobsters and soft-shelled

TABLE 12.1
Some Techniques for Live Marketing of Aquacultured Fishery Products

Item	Methodology	Remark
Finfish	Condition the fish by starving for several hours. Hibernate by bubbling CO_2 in the water, and immobilized by lowering water temperature to 10°C, packed in polystyrene boxes in minimum cold water and transported	Helps dense packaging and lower transport costs. No need of feeding. Better product quality
	Electric shock followed by severing the spinal cod from the brain	As above
Crustacea	Crabs and lobsters, mostly marketed alive, by holding in presence of moist straw, seaweed, or inert wooden shavings in precooled insulators. Hibernation can be applied	As above
	Some species of shrimp such as *Penaeus japonicus*, *P. monodon*, and *Macrobrachium rosenbergii* are hibernated and packed in moist, chilled sawdust	As above
Mollusks	Mostly marketed in live form after depuration in clean water. Oysters with shell are packed, sitting flat and cup down. Shucked oysters and oyster meat are vacuum packed and chilled in ice	As above

Source: Adapted from Subasinghe, S., *Infofish Int.*, 3, 45, 1996. With permission from *Infofish*.

crabs are packed in moist straw, seaweed, or wooden shavings. The steps involved in live marketing of aquacultured fish are summarized in Table 12.1.

12.4 Preliminary Processing of Freshwater Shrimp

Further processing encompasses transformation of a raw aquatic commodity into an item that is designed to meet consumer need. Both intrinsic and extrinsic parameters determine the quality of finished products (Chapter 2). In addition, the time of processing after harvest is also important. Shrimp need to be iced well immediately after harvest. Non-iced or insufficiently iced shrimp lose their prime quality due to black-spot formation, development of strong ammoniacal odor, and soft texture. Viable bacterial counts of cultured shrimp are generally higher than in their wild counterparts. This is a serious cause of concern for processors and exporters. Loss of quality can be retarded, if shrimp are handled under sanitary conditions and iced well promptly. Washing and disinfecting in 100 ppm chlorine reduces the total plate counts and total fecal coliform counts in cultured *Penaeus indicus*. During ice storage, there could be some loss of soluble nitrogen compounds such as amino acids. Washing delays the onset of black discoloration at least by a day. The shelf lives of unwashed, washed, and chlorine-treated shrimp in ice were 12, 16, and 18 days, respectively. For every hour delay in icing, the loss

TABLE 12.2
Freshness Score Sheet for Farmed Shrimp (*P. indicus*)

General appearance	Odor	Texture	Score
Body transparent, head completely attached to body	Seaweedy, characteristic of species	Firm, elastic, hard shell, body	10
Body less transparent, head still attached to body, tail and elastic pleopods with light red stripes	Slightly seaweedy, fresh cut-glass	Fairly firm, slightly elastic	9
Body slightly translucent, slight blackening in head, and tail with light red stripes	Moderately characteristic odor of the species	Slightly soft body/shell	8
Body moderately translucent, slight blackening in head, and tail with light red stripes	Slightly characteristic odor of the species	Moderately soft body/shell	7
Body translucent, head with blackening near eye region, slightly loose carapace, tail reddish	Slightly ammoniacal	Slightly soft body/shell	6
Body translucent, head almost completely black and slightly loose, slight occurrence of black spot on body, tail brownish	Urea smell	Soft body and shell	5
Body translucent head almost completely black and loose, moderate occurrence of black spots on body, tail, brownish to brownish-black	Offensive, sulfide	Soft body and shell	4
Head completely black and slightly detached from body, heavy occurrence of black spots on body, tail black	Ammoniacal, sulfide	Body sponge-like	2
Head completely black and detached from body, heavy occurrence of black spots on body, tail black	Strong sulfide	Body sponge-like, mushy	1
Black spots all over the body, head completely black, tail black	Fecal, strong ammoniacal	Very soft, papery shell, mushy	0

Source: Adapted from Karthikeyan, M. et al., *J. Food Sci. Technol. (Mysore)*, 36, 173, 1999. With permission from Association of Food Scientists and Technologists (India), Mysore.

of shelf lives for washed, chlorine-treated, and metabisulfite treated shrimp, were approximately 0.9, 1.2, and 1.8 days, respectively.[19] Table 12.2 presents freshness score sheet for quality evaluation of farmed shrimp (*P. indicus*).

12.5 PRELIMINARY PROCESSING OF FRESHWATER FINFISH

Finfish species are subjected to preliminary processing immediately after harvest. The duration of rigor in freshly harvested fish depends on the fish species,

harvesting technique, and temperature. During rigor mortis, fish temperature should be as low as possible. In carp held at 0°C, rigor starts after 48 h. In the case of roach and perch held at 0°C, it starts after 24 h and lasts for 72–80 h, while at 35°C, rigor starts 20–30 min after death and completes after about 3 h. Rigor starts faster in swimmers such as trout, but lasts for a shorter duration than in slow swimmers like carp.[18] The Quality Index Method (QIM) employed for marine fish (Chapter 2) can also be used for freshwater fish species with suitable modifications. Table 12.3 indicates the QIM for farmed salmon.

Broadly, the technological value generally depends on two parameters, the yield on preliminary processing and the quality features of fish meat and by-products. The yield of edible parts of the fish depends on the species and constitution, and also on age and consequently, size and maturity. Yield is affected by the ratio between edible and inedible parts of the fish, which is a decisive factor with regard to the technological value of the fish. This ratio depends on the species. For most fish species, this parameter ranges from 50 to 60%, and can be as high as 75% in species belonging to the Salmonidae family. On the contrary, in the case of perch and most of the Cyprinidae family the yield is less than 50%.[18]

12.5.1 Sorting and Grading

Freshwater fish are graded according to size, larger individuals usually being preferred to small ones. Popularity is also dependent on the bone content. For example, trouts weighing about 300 g are very popular as single portions, and prices increase with popularity. The most expensive are fish weighing over 500 g, which are also destined for smoking. The best market values are for carp weighing 1–2 kg, but those exceeding 3 kg have less customer appeal. The processing sequence starts from sorting and grading the fish by species and size. Sorting the catches on the basis of freshness and physical damage are still manual processes. Mechanical grading yields better sorting precision and efficiency, perhaps 6–10 times more than manual grading. Size grading is very important for processing such as smoking, freezing, heat treatment, salting, etc., as well as for marketing.

12.5.2 Washing

Washing with potable water is intended primarily to clean the fish and to remove slime and bacteria. Slime accumulating on the surface of the skin of dying fish is a protection mechanism against harmful conditions. In some freshwater species, slime constitutes 2 to 3% of body weight. Slime excretion, which stops before rigor mortis, creates a perfect environment for microbial growth.[18] Eel, trout, and carp require special care with regard to slime removal. Even small amounts of slime, which frequently remain after manual cleaning, result in visible yellowish-brown spots, particularly in smoked eel. Fish is generally washed in an equal amount of potable water. However, in practice the water used can be twice the quantity of fish. Mechanized washers can be used to wash whole, headed, or gutted fish, and also boneless fillets because the washing action generates no physical damage to

TABLE 12.3
Quality Index Method Scheme for Farmed Salmon

Body part	Quality parameter	Description	Score
Skin	Color/appearance	Pearl-shiny all over the skin	0
		The skin is less pearl-shiny	1
		The fish is yellowish, mainly near the abdomen	2
	Mucus	Clear, not clotted	0
		Milky, clotted	1
		Yellow, clotted	2
	Odor	Fresh seaweedy, neutral	0
		Cucumber, metal, hey	1
		Sour, dish cloth	2
		Rotten	3
	Texture	In rigor	0
		Finger mark disappears rapidly	1
		Finger leaves mark over 3 sec	2
Eyes	Pupils	Clear and black, metal shiny	0
		Dark grey	1
		Mat, grey	2
	Form	Convex	0
		Flat	1
		Sunken	2
Gills	Color	Red/dark brown	0
		Pale red, pink/light brown	1
		Grey-brown, brown, grey, green	2
	Mucus	Transparent	0
		Milky, clotted	1
		Brown, clotted	2
	Odor	Fresh, seaweed	0
		Metal, cucumber	1
		Sour, moldy	2
		Rotten	3
Abdomen	Blood in abdomen	Blood red/not present	0
		Blood more brown, yellowish	1
	Odor	Neutral	0
		Cucumber, melon	1
		Sour	2
		Rotten/rotten cabbage	3

Source: MPEDA Newsletter, 9, 17, 2004. With permission from Marine Products Export Development Authority, Cochin, India.

the product. Horizontal-axis drum washers are particularly suitable for production lines requiring constant product flow due to their continuous operating cycle. However, this type of washing is ineffective for fish such as eel.[18]

Further processing of freshwater fish usually consists of the unit operations, which include scaling, evisceration, heading, removal of fins and belly flaps, slicing of whole fish into steaks, filleting, skinning, or mincing. Currently, these steps are done manually in small plants, for supply of fish to the local markets. In future, with increase in freshwater fish products, the level of mechanization of fresh fish processing is likely to increase with associated reduction in production costs and improved economic performance. The different aspects of preliminary processing of freshwater and the equipment that are available for the purpose have been discussed recently by the FAO.[18,20,21]

12.5.3 Scaling

Manual scaling of the washed fish is an extremely labor-intensive process. Fish such as perch, bream, pike-perch, and carp are particularly difficult to scale manually. The process can be simplified by blanching the fish in boiling water for 3–6 sec before scaling by hand. Electrical hand-held scaling equipment is available for fast scaling.[18] For this, the fish (usually 30–40 kg) is loaded from the top of the scaling machine and unloaded through the door in the side. Scales catch on small contoured slits cut in the bottom and side of the device, and are pulled out of the skin. Fish that are for skinning and filleting or to be smoked or minced is not scaled.

12.5.4 Heading

Head constitutes about 10–20% of the weight of the fish, which is cut off as an inedible part. While mechanized heading machines are available for processing marine fish, a common machine that would cover a broad spectrum of freshwater fish sizes, ranging from 20 to 110 cm is not available at present. For larger fish, a heading machine with a guillotine cutter is available for heading. The speed of the heading device depends on the size of fish processed and is usually 20–40 fish per min. Economical cuts such as contoured cut or cut around operculum can be performed by changing the cutters. A cut around the operculum, a so-called round cut, results in lowest meat loss. This technique is 4–5% more efficient than the straight cut commonly used in mechanized systems. The contoured cut, which runs perpendicular to the fish's backbone and then at an angle of 45° is also advantageous. This heading technique is used when fillet, mainly boneless and skinned, is the final product. Along with the head, pectoral bones and fins are also removed. The amount of meat lost during the heading procedure depends not only on the type of head cut but also on the experience and skill of the operator.[18] Recently, machine vision has been employed to develop a prototype machine that removes head from salmon and other fish with minimum wastage.

12.5.5 Gutting

Gutting consists of cutting down the belly portion, removal of internal organs, and cleaning the body cavity of the peritoneum, kidney tissue, swim bladder, and blood.

Fish is cut longitudinally up to the anal opening taking special care to avoid cutting the gall bladder. Evisceration of freshwater fish is labor-intensive and is usually performed by hand, keeping the fish on a table made of hard material. Simple mechanical devices consisting of rotating brushes and water sprays are common. Cutting off the belly tissues decreases the muddy odor. To improve the effectiveness of the cleaning procedure, various mechanized scrubbing devices are utilized that can remove up to 90% of the initial bacterial contamination.

12.5.6 Removal of Fins

Fins are cut away after gutting during the production of headed whole fish and fish steaks. Manual removal of fins is labor-intensive and very strenuous. An automated device consists of rotating disc knives with a slit-cutting edge, powered by electric motor. The knife slot has a horizontal opening through which the dorsal and ventral fins are passed manually and cut out.

12.5.7 Steaks

The gutted, headed, or whole fish is sliced into steaks of 2.5 to 4.0 thickness with cuts perpendicular to the animal's backbone. A knife or a band saw is used to slice the fish. Smaller and medium-size fish are cut manually in concave basins, which have evenly spaced slots to facilitate slicing into steaks of equal thickness. Sometimes a band saw is used to remove the head and cut the body into two parts, one of them retaining the backbone. The present level of mechanization is low because of the overall limited production, seasonal availability of the raw product, and lack of inexpensive, efficient mechanical equipment adaptable for processing of various fish species.[18]

12.5.8 Fillets

A fillet is a piece of meat consisting of the dorsal and abdominal muscles and is the most sought-after fish product in the retail market. Filleting efficiency depends upon species, sex, size, and nutritional condition of the fish. Manual filleting is labor-intensive and largely depends on the skill of the workers. Filleting machines for marine fish are expensive and therefore, filleting of freshwater fish is not as widely applied as for marine fish. The simplest filleting machine for gutted and headed fish has two disc knives set from each other at a distance equal to the thickness of the fish's backbone. Filleting speed of these devices is 30–40 fish per min giving fillets a size range of 20–45 cm. Expensive multifunction devices are available for some important fish such as trout.[18] Different species (trout, perch, pike-perch, pike, cyprinids, etc.) having the same size can be processed using a common filleting device. As the demand for freshwater fish fillets increases, simple and inexpensive single-purpose machines for filleting of headed and gutted fish will have to be made.[18] Ribs and pin bones are manually removed from the fillets, and sometimes as in the case of cyprinids, perch, and roach, the bones are

cut by machine. Generally, the yield of the fish fillet depends upon the structural anatomy of the fish. The fillets are skinned for which machines are available. The muddy flavor in some freshwater fish can be significantly removed by skinning their fillet. The yield of fillet from fish with large heads is lower compared with fish having smaller heads and frames.[20]

Among the commercially cultured species, tilapia is the most difficult to process and has the lowest fillet yield (33%) compared to salmon (>50%), catfish (>38%), and striped bass (>40%). Fish such as sea bream and sea bass also give higher fillet yields than tilapia. Freshwater eel gives maximum fillet yield of 60%. For development of a good quality product, the fish should be properly purged and bled, headed, and gutted. Fillets should also not have excess fat as well as pin bones. Skinning and filleting can improve the marketability of finfish such as tilapia and catfish. Both subjective and objective sensorial traits are used to evaluate the quality of fillets from aquacultured fish.[22] Fillets of carp, perch, pike-perch and smoked, single or block fillet of salmon, and trout are some of the popular products. Besides fillets, other forms are processed, such as block fillets retaining some bones. The simplest type of processed carp is the headed whole fish cut into two halves, one retaining the backbone. Table 12.4 gives preprocessing yields of some freshwater fish species.

TABLE 12.4
Preprocessing Yield of Some Freshwater Fish Species

Species	Minimum weight of fish (kg)	Preprocessed product form	Yield (%)
Trout	0.35	Gutted only	74–82
Trout	0.35	Headed and gutted	62–74
Trout	0.35	Fillet with skin	50–55
Carp	3.0	Gutted only	76–82
Carp	1–3.0	Gutted only	73–79
Carp	3.0	Heading and gutting	55–61
Carp	3.0	Fillet with skin	41–49
Pike-perch	1.0	Gutted only	79–89
Pike-perch	1.0	Headed and gutted	66–74
Pike-perch	1.0	Fillet with skin	52–64
Pike	1–3.0	Gutted only	76–84
Bream	1.0	Gutted only	68–76
Bream	0.5–1.0	Headed and gutted	56–64
Tilapia	0.2–0.4	Headed and gutted	60–65
Tilapia	0.2–0.4	Fillet	33–35
Tilapia	0.2–0.4	Steak	40–45
Freshwater eel	0.2–0.4	Fillet	60

Source: Adapted from Freshwater fish processing, Fisheries Global Information System, Food and Agriculture Organization of the United Nations, Rome, Italy and Badonia, R. and Quereshi, T.A, *Appl. Fish. Aquacult* 2, 21, 2002. With permission.

12.5.9 Preparation of Mince

Freshwater fish and frames after filleting are a good source of minced meat.[18] The production of mince from freshwater fish is similar as in the case of marine fish, by squeezing the gutted and split fish through orifices of a cylinder under pressure, exerted by a conveyor belt (Chapter 8). Eventhough, the orifice in the cylinder has usually a diameter of 3 to 7 mm, the ideal dimension for processing of freshwater fish is 5 mm. The smaller the orifice, the stronger the grinding action. The ground meat collected can be stored at −25 to −28°C for up to 6 months. Mince separation by mechanical deboning of freshwater species such as perch and bream offers a new perspective on production of novel products such as fish burgers and *surimi*-based seafood analogs. The technological efficiency attained during the production of ground meat from bream not larger than 1 kg is 40% of total body weight. Up to 50% meat left on the fish's backbone after filleting can be recovered to a high degree using a meat-bone separator.[23]

12.6 TECHNOLOGY FOR VALUE ADDITION

The technology available for value addition of marine fish and shellfish could also be applied to freshwater fishery products with suitable modifications. Individual quick freezing (IQF) of fillets, modified-atmosphere packaging (MAP), breading and battering, retort pouch packaging, and others, are techniques that have potential for value addition of freshwater fish and shellfish. Nevertheless, while developing products, amenability to individual species to the processes needs to be assessed. The various technologies for value addition of freshwater and aquacultured fish and shellfish are indicated in Table 12.5.

TABLE 12.5
Some Technologies for Value Addition of Freshwater and Aquacultured Fishery Products

- Breading and battering
- Composite fillets
- Canning
- Cook-chill processing
- Extrusion cooking
- Fermentation
- High-pressure treatment
- Individual quick freezing
- Marinades
- Modified-atmosphere packaging
- Smoking
- *Sous-vide* processing
- Retortable pouch packaging

12.6.1 Chilling

Chilling is very important in handling of freshwater fish and shellfish as in the case of marine fishery products. The raw material, intermediate products, and in many cases, the final products are all almost exclusively chilled in ice. The chilled storage lives of a number of freshwater fish species have been examined using chemical, microbiological, and sensory methods of quality evaluation. The parameters used for quality evaluation included measurements of the nucleotide degradation products, K-value and texture, using the Intelectron fish tester (VI) in addition to microbial counts, volatile bases, and sensory analysis.[24] The K-value increased linearly with storage time in ice. Catla and rohu maintain 20% K-value (a reference level for very fresh fish) up to 6 and 8 days, respectively, and exceeded 50% after 12 and 15 days, respectively. The concentration of inosine monophosphate (IMP) increased steadily with a content of 1 mole of IMP per g of meat at the terminal storage lives of the fish samples. At this stage, hypoxanthine concentration was quite low in both cases. The Intelectron fish tester readings decreased at a constant rate to less than 20 on the 20th day. Tenderization of the muscle was observed at 8 to 10 days, but the flavor loss was negligible. The total viable counts reached 10^5 per g only after 20 days. Total volatile nitrogen content was less and did not indicate the quality of the carps. Based on the adenonine triphosphate (ATP) degradation products, catla and rohu could be classified as inosine-forming species. The objective indices, IMP level, K-value, and fish tester readings, correlated significantly with the storage time and sensory score in the case of the two fish species examined.[24] The amounts of volatile basic nitrogen compounds are much less during terminal spoilage of fresh water fishes as compared with marine species. The shelf lives of several species of carps were found within 16–21 days in ice[37]. A shelf life of 14 days for ice-stored rohu (*L. rohita*) was observed in another study, whereas steaks of the fish had a longer chilled shelf life of 20 days. Exposure of the fish to gamma radiation at a dose of 1 kGy enhanced the shelf life to 28 and 30 days in the case of whole rohu and the fish steaks, respectively. The shelf life of whole rohu was further extended up to 35 days by a dose of 2 kGy.[25] Coating of the rohu steaks with dispersion prepared from the same fish meat followed by irradiation at a dose of 1 kGy, could further enhance the shelf life up to 40 days.[26]

Providing a gelatin-based antimicrobial edible coating has been reported to enhance the shelf life of tilapia fillets. In the process, skinless tilapia fillets are covered with a gelatin coating containing benzoic acid as an antimicrobial agent. The content of gelatin is in the range of 6.3 to 17.3 mg/g fish fillets. After 7 days of refrigerated storage, coated fillets have an acceptable quality and lower microbial counts than uncoated fillets.[27] The quality changes during ice storage of northern squawfish (*Ptychocheilus oregonensis*) were studied in terms of the Torrymeter readings, K-values, and descriptive sensory evaluation. The data suggested a shelf life of 15 days for the fish. During ice storage, the fish retained good gel-forming ability, which decreased by only 10% after 24 days.[28] The maximum shelf life for iced whole salmon is about 20 days as per evaluation by QIM method.[29]

TABLE 12.6
Chilled Shelf Life of Some Freshwater Fish Species

	Shelf life (days)
Temperate freshwater	
Channel catfish (*Ictalurus* spp.)	12
Whiting (*Gadus merlangus*)	9–12
Yellow walleye (*Stizostedion* spp.)	20
Trout (*Salmo* spp.)	10
Perch (*Perca* spp.)	13–17
Tropical freshwater	
Tilapia (*Oreochromis* spp.)	14–28
Major carps	35
Catfish (*Bagrus* spp.)	20
Lung fish (*Protopterus* spp.)	25

Source: Adapted from Ninan, G., in *Product Development and Seafood Safety*. Central Institute of Fisheries Technology, Cochin, India, 2003, p. 44. With permission.

Turbot species (*Psetta maxima*, also known as *Scophthalmus maximus*) are highly valued marine flat fish found in northern waters. In recent years, farming of these species has been practiced to make them available to consumers from European countries. The farmed turbot has a shelf life of 19 days as revealed by sensory and biochemical analyses. When the fish is stored under ice for 40 days, formation of total volatile basic nitrogen compounds in the fish reach levels as low as 40 mg/100 g muscle only. This is due to low microbial growth in the fish, the total aerobic counts reaching levels below 7-log colony forming units per g during this period. The slow bacterial growth together with relative predominance of lactic acid bacteria over gram-negative microorganisms has been suggested to be responsible for the extended shelf life of the farmed fish.[30] Table 12.6 gives chilled shelf life of some freshwater fish species.

Chilling may be combined with vacuum, MAP or irradiation to enhance shelf life of freshwater fish such as carp, tilapia, trout, salmon, as well as shellfish.[31,32,98] The products may be skinless and boneless fillets as well as fresh or cooked shellfish. Products that are particularly sensitive to oxygen are vacuum-packed. A laminate of nylon/polythene is commonly used as the packaging material. This type of packaging is used for smoked trout. Several machines are available for vacuum packing having single, double, or continuous chambers. Vacuum-sealing machines can additionally be equipped with MAP system. Usually the mixture consists of 30% nitrogen, 40% carbon dioxide, and 30% oxygen.[18] In the case of fatty fish, oxygen is replaced by nitrogen. The MAP products have to be stored at temperatures lower than 3°C to control *C. botulinum* hazard. Cultured salmon has

been found to remain for several days when packaged in MAP having a CO_2 to N_2 ratio of 75:25.

Chilled freshwater fishery products such as seafood, should be transported maintaining a temperature close to 0°C, using both the most simple isothermal vehicles and mechanically cooled containers. Fish and fish products should reach the buyer without delay. Products need to be delivered direct to shops where they should be placed in cold stores and if necessary ice should be added. Good trade practice indicates that retailers should only keep a one-day stock of chilled fish or fish products such as fillets or headed and gutted fish (see Chapter 3 also).

Indian major carps offer distinct advantages such as white color of meat, good taste and low fat content offering good scope for value addition[37]. Improved red-colored hybrids of tilapia are sold whole or filleted as "freshwater snappers" or "freshwater perch." Channel catfish are processed in plants near the farm, primarily into whole dressed (less head, skin, viscera) or fillets.[15] The fillets or steaks may be individually wrapped in polyethylene films and held in polystyrene trays. They may also be packed under vacuum or modified atmosphere. Most of the products are transported by road in refrigerated containers.[3] Air transportation is also becoming popular. For the latter, cooling pads are used instead of conventional ice. The pads could also be used in the container to absorb drip or fluid oozing out from the fish. In the case of large fish, the pads could be stuffed into the belly cavity of the fish to give additional protection from heat. Temperature monitors including time-temperature tags are available to record the temperature profile of the products.

Another technology for retailing is based on "cook-chill" process, which offers convenience, freshness, and quality to the product. The fish items are subjected to minimal processing that include salting, mild heating, rapid cooling, packaging, and chilling. In *sous vide* processing, the food is prepared, seasoned, vacuum sealed, and subsequently cooked at controlled temperatures followed by immediate chilling to 4°C. The treatment gives the products a shelf life of about 3 weeks. However, the products are retained in supermarket shelves for a much shorter duration from a microbiological safety point of view (see Chapter 5). The technology can as well be used for preparation of fish-based ready-meals. The packaging conditions need to be standardized as per requirement.

12.6.2 Freezing

Freshwater fishery products, whole, steaks, or fillets can be candidates for freezing, their amenability depending upon the species. Similar to marine products, lower the freezing temperature, the higher the quality of the products. Low temperatures inhibit microbiological decomposition but do not protect against fat oxidation and loss of water. The stability of frozen fish depends on the initial quality of the raw material, the fat content, the freezing process, and the storage temperature. The stability of different species of carp after frozen storage was examined at -5, -12, and -18°C. After 2 months of storage at the different temperatures, quality of the fish species varied as flower carp > straw carp > carp.[2] Sensory analysis correlated with texture measurements using Steven's LFRA Texture Analyzer. The fish was acceptable after 7 months' storage at -20°C.[2] Glazing effectively

prevents loss of water from fish tissue and prevents rancidity (see Chapter 4). This method is used especially for freezing whole fish or fish/fillet blocks. The quantity of glaze on frozen fillets should not be excessive.[33] Individual portions of fish or individual fillets are packed in plastic material having low permeability to water vapor and oxygen, to prevent rancidity and dehydration. Generally, glazed fatty fish have a storage life of 9 months at $-24°C$, while lean fish can remain up to 12 months at this temperature.[18]

Individual quick freezing employs cryogenic freezing or by the more common mechanical refrigeration (Chapter 4). Fluidized-bed freezers are normally used for small-size shrimp, with handling capacities in the range of 250 to 1000 kg/h. Spiral-belt freezers are also used. Freshwater fish fillets and prawns including black tiger are potential candidates for IQF processing of larger products. Freezing time for whole 125-mm thick salmon in a blast freezer at $-40°C$ with an air velocity of 5 m/sec is 5 h. For salmon fillets, the time required is less than 90 min in the same type of freezer at $-35°C$ with an air velocity of 4 m/sec. Oyster meat is frozen at $-160°C$ for 45 sec using liquid nitrogen. Oysters are marketed half-shell, six or more on a tray or in a skin pack. Frozen mussel in shell is also marketed vacuum-packed in clear laminated pouches. Frozen soft-shell crabs are also retail-packed in plastic polyvinyl chloride (PVC) trays with a secondary packaging of cardboard cartons. Lobsters are skin packed in special plastics giving a very attractive pack, quite different to conventional "Popsicle" pack. Carp fillets are popular frozen products.[34] Black tiger (*P. monodon*) is the major shrimp variety raised in Asia including India. IQF black tiger can have great market potential.

12.6.3 Canning

Thermal sterilization of fish in sealed cans eliminates bacterial as well as autolytic spoilage. The products have a shelf life of 1 to 2 years at ambient temperature. The dressed fish after the necessary preprocess operations is packed in metal cans in a fluid packing medium followed by exhausting, sealing, and heat processing in steam at $121°C$ for the required duration to attain commercial sterility and immediate cooling. Prior to filling in the cans, the dressed fish is blanched for a few minutes in brine to enhance the flavor of the final product. The filling medium accelerates heat transfer to the fish and avoids overcooking at points closest to the can walls. The cans normally will have a 5 mm vacuum after processing. There are three essential rules for the safety of canned products, namely, container-seal integrity, adequate thermal processes to eliminate the most dangerous and heat resistant microorganisms including *Clostridium botulinum* spores, and postprocess hygiene.

The duration of heat treatment depends on the size and dimensions of the can. Optimization of thermal processes of canning include determinations of (i) thermal death curves (TDC) and (ii) F_0 value. (See chapter 7). F_0 values commonly used for medium and low-acid products range from 6 to 14 in order to ensure safety of the products. The details of the canning process have been discussed.[35,36]

Freshwater ide (*Leuciscus idus*) and chub (*Leuciscus cephalus*) have been examined in detail for canning. Heat processing equivalent to F_0 value of 7.5 with a sterilization time of 57 min at $115°C$ was required for these fish. The time was

reduced to 35 and 25 min at 120 and 125°C, respectively. Increase in precooking time in brine containing tartaric acid or polyphosphate significantly increased the shear-force value of the canned products as measured by Kramer shear cell. The texture measured by instrumental methods correlated with sensory panel scores.[38] In the case of freshwater fish rohu, a treatment of the fillet in 15% brine containing 0.25% of $CaCl_2$ imparted a firmer texture to the product. The sterilization time at 121°C was 25 min.[39]

Tuna is one of the most sought-after raw material for canning.[30] The U.S. canned tuna industry processed more than 220,000 t of fish in 1997 to produce 37 million cases of light-meat tuna, the majority from skipjack tuna (*Katsuwanas pelamis*). During the last few years, a variety of canned tuna products, other than the traditional tuna in oil/brine/vegetable broth, have found their way into the market. These products include tuna salad with garden vegetables, tuna salad in Italian sauce, tuna in sweet and sour sauce, and tuna spread.[40] However, the increase in variety and availability of other pre-prepared foods has led to a decline in consumption of canned tuna, forcing the tuna industry to seek improvement in product quality and yield.[6] Carp (*C. carpio linnaeus*) is an important aquacultured fish for canning.[34] Canned big-head carp loins that had been cooked either in steam or convection oven has been accepted equal to canned tuna by a consumer panel.[41] Hybrid *Clarias* catfish is used for canning, in addition to processing as smoked and sausage-type products. Canned crisp catfish in chilli sauce and green curry are popular in Thailand. Other freshwater fish suitable for canning are roach, perch, vendace, and whitefish. These canned products are acceptable up to 18 months storage when stored at room temperature.

Apart from freshwater fish, several small marine species that are good candidates for canning include anchovy, herring, mackerel, sardine, scad, sprat, pilchard, fish belonging to Elasmobranch, and a variety of shellfish and mollusks.[35,36,42] Canning has been employed to preserve fish balls, pastes, and spreads (see Chapter 9). Freshwater bream (*Abramis brama*) has been found suitable for processing as canned fish ball. The product had firm texture and sensory textural scores correlated well with instrumental readings. Tenderness and juiciness scores could be predicted from the instrumental data. The heat treatments in the blanching and sterilization processes associated with canning did not significantly affect the amino-acid composition, particularly, lysine, leucine, and threonine in carp. The fish components need to be thoroughly cooked before chopping and mixing with other ingredients to achieve spreadable consistency. Incorporation of gelling agent such as gelatin may be required to get a pate that is sliceable and spreadable.[2]

12.6.4 Products from Freshwater Fish Mince

Mince technology offers scope for development of a number of conventional products including pastes, sausages, patties, balls, etc. as discussed in Chapter 8. In India, carp (*C. carpio*) contributes more than 90% to total freshwater fish production, however, their market value as a food and consumer acceptability is low mainly due to bone content in the flesh. In order to enhance consumer acceptability, value-added convenience foods, namely, *surimi*, fish patties, fish fingers,

and fish salad were prepared from mince of freshly caught two-year-old carp. The products were compared with a reference product ("fish pakora") prepared from the fish chunks. The ingredients in the fish patties and fish fingers contained corn flour, spices, salt, and monosodium glutamate and were covered with breadcrumbs. Fish salad contained macaroni, mayonnaise, vegetables, canned pineapple, honey, limejuice, and American spices, while "fish pakora" contained Bengal gram flour, salt, thyme seeds, and garlic. The products were prepared using conventional formula. Acceptability ratings of the products in terms of mean sensory scores were fish patties > fish fingers > fish salad > "fish pakora." Fish patties were the most preferred products in terms of appearance and color.[43] The study showed potential for using carp meat as a raw material for value-added products. Fish noodles were made from Indian carp (*L. rohita*) by mechanical mincing of the fish meat with ingredients followed by passing the fish and a cutter. The fish noodles obtained were then steamed, dried under the sun, and packed in polyethylene bags. Organoleptic tests showed that fish noodles made from fillets with the dark meat removed were the most acceptable.[44]

Silver carp (*Hypophthalmichthys molitrix*), which is widely grown in the composite cultures due to its quick growth and resistance to stress, disease, and rough handling, are seldom processed or preserved. As a result, postharvest losses are very high, in spite of the fact that the fish contains more than 16% high-quality nutritious proteins.[2] *Surimi* production from silver carp has been investigated in detail with a view of value addition of the fish.[45,46] The fish, having an average total length of 40.8 cm and weight of 875 g yielded a mince with a proximate composition of 81% moisture, 16.7% protein, and 1.4% lipid. Frozen storage of mechanically deboned silver carp mince for 180 days resulted in about 20% decrease in soluble myofibrillar proteins, while peroxide value, free fatty acids, and total volatile base nitrogen increased significantly throughout the storage period. Repeated washing of the mince resulted in a loss of about a half of the total extractive nitrogen. The main nitrogenous compound lost was creatine followed by histidine, glycine, taurine, alanine, and proline. Jelly strength of the *surimi*-based products increased by washing and mild heating. Washing reduced the odor, lightened the color, and increased the firmness of *surimi*-based products.[45] The gel strength of kamaboko prepared from silver carp decreased significantly during the storage period. Texture of the mince was correlated with the decrease in the myofibrillar proteins and jelly strength. Based on the texture of the mince, acceptability for 180 days at $-18°C$ was suggested. However, for production of sausages, the mince should not be stored for more than 90 days at $-18°C$. Figure 12.1 shows changes in kamaboko-forming ability (jelly strength, expressible water, and folding test grades) of silver carp mince stored at $-18°C$. The study indicated that production and freezing of silver carp for limited period could offer a potential method for utilization of the fish for sausage and *surimi* production.[46]

In recent years, tilapia has become a predominant product for the food service sector, especially casual and family-style restaurants and institutional catering. Therefore, amenability of the fish for *surimi* making was examined. Tilapia (*Orechromis mossambicus*) gave a *surimi*, which had higher gel strength and compressibility. Fresh or one-day chill-stored tilapia could be used for production of
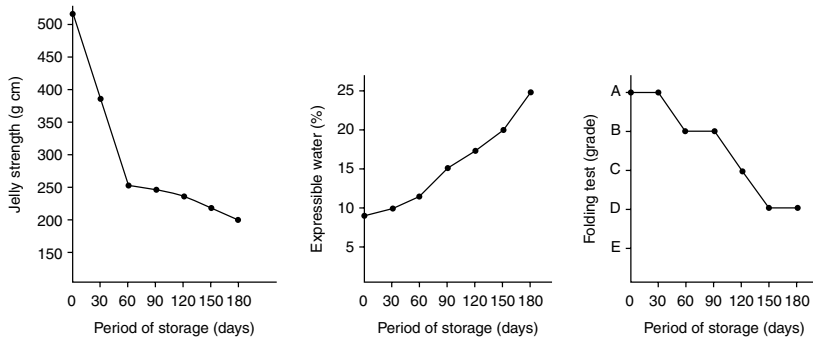
FIGURE 12.1 Changes in kamaboko forming ability (jelly strength, expressible water and folding test grades) of silver carp mince stored at −18°C (Reprinted from Siddaiah, D. et al., *Food Res. Int.*, 34, 47–53, 2001. With permission from Elsevier)

surimi of acceptable quality.[47] The quality of *surimi* prepared from tilapia has also been compared with those prepared from Alaska pollock and Pacific whiting.[48] Other examples of value-added tilapia products are sausages, patties, loins, smoked items, various breaded, battered, and marinated products. It is possible to make formed products from the tilapia meat separated form the discarded rib-bones.[33] *Surimi* has been made from freshwater Northern squawfish (*Ptychocheilus oregonensis*). The yield after two washings averaged 17.9%, with moisture and protein content of 72.10 and 17.54%, respectively. Measurement of stress and strain indicated that it was feasible to produce *surimi* from squawfish stored in ice up to 9 days. Freshness of the fish affected initial *surimi* gel strength and color and had variable effects on loss of gel strength during frozen storage.[49] Acceptable fish cakes from *A. nobilis* and *Nemipterus* spp. have been developed.[50] Burgers from freshwater fish having less than 3% fat and rich in proteins have been developed[2].

Process for sausages from freshwater fish has been developed. The variables examined in the process included pH (2.0–9.2), salt concentration (0.8–6.0%), storage time (0–12 months), starch type (corn, sweet potato, potato), concentration of starch (5–15%), and processing methods. Gel strength of ground fish flesh was highest (158.3–163.1 g/cm^3) at pH 7.2–6.8. Optimum salt concentration for maximum gel strength was 2–3%. With respect to starch, the best quality sausage was obtained with potato starch. For 100 g mince, the optimal proportions of ingredients were: water, 50; starch, 15; salt, 4; sugar, 3.5; and sodium glutamate, 0.85 g. For processing procedure, the key points were grinding at 0–10°C for 10–15 min and the sequence for adding supplements is NaCl, starch, water, and others.[51] A new casing-tube having antibacterial properties for fish sausage or salmon pate has been developed. The tube is coated inside with some spice or spice-mixture. During cooking of the sausage, the spice is transferred to the emulsion and is incorporated in the protein rind of the emulsion. After cooking and cooling, the casing can be peeled off very easily, but the spice rind remains in place providing antibacterial protection. The products are then vacuum packaged to provide protection against oxygen and water-vapor transmission. Alternately,

the casings could be laminated with polyamine or polyethylene, to ensure shelf life up to 4 months for the sausage.[52]

12.6.5 Smoked Products

Smoking is a part of curing that encompasses a combination of processes such as salting, drying, pickling, and marinading. It is a popular method for value addition of fish species, including underutilized fish. The basic principle involved in curing is rendering the medium unsuitable for the growth of microorganisms by the control of water activity (a_w) of the product.[53,54] Reduction of a_w by combination of salting, dehydration, and the addition of chemical preservatives helps retention of food stability and safety even without refrigeration, based on the "hurdle concept" (see Chapter 6). Generally at 25% moisture level, bacterial activity of muscle foods is inhibited and at 15% moisture mold growth is also arrested.[54]

In wet salting, fish are immersed in strong brine, usually 36% concentration of salt for 15 to 20 min. Depending upon the fish type, it may be salted whole (e.g., anchovies and small herring), eviscerated and split open (e.g., mackerel) or in small pieces, or as fish mince. For dry salting, fish are split open, gills and intestines removed, washed, and salt is scrubbed into it, at a fish to salt ratios ranging from 1:3 to 1:10 depending upon the size of fish. Injection salting is a rapid curing method compared with brine and dry salting, and it gives substantially higher processing yields. The salted fish are given a light rinse in fresh potable water to remove the adhering salt crystals. Fish can be dried by air (sun, solar) or by contact (where heat is transferred to the fish from heated air or a heated surface), vacuum (by enhancement of evaporation rate by reduced pressure), or freeze-drying (employing very low pressures to sublime ice from frozen fish). The disadvantage of sun drying is that the fish may get contaminated with sand, microorganisms, and insects. Significant differences do not exist between sun and solar-dried products.[55] Theoretical aspects of salting, brining, and sorption isotherms for predicting the course of drying and selection of optimum storage conditions for dried fish have been discussed.[56–58]

The common defect of cured fish products is "dun," "rust," and formation of pink or red patches on the product. "Dun" is the formation of brown or yellowish brown spots on the flesh and is caused by the growth of pigmented, salt-tolerant microorganisms at ambient temperatures. "Rust" is characterized by the color of rusted iron and is formed through the reaction of oxidized lipids with fish proteins. The high fat content in some fish may result in development of rancid flavors, which is also enhanced by salt.[59,60] For good quality product, raw materials should be as fresh as possible, wholesome, and if necessary, gutted, headed, cleaned, and washed prior to curing. If artificial dryers are employed, drying should be carried out as swiftly as possible to reduce the risk of spoilage. The humidity in the air needs to be controlled to avoid case hardening. The storage life of the product is also influenced by the storage temperature.[61]

Smoking is defined as the process of the penetration of volatile compounds resulting from incomplete burning of wood into food products. Freshly salted fish

are hung on racks or in a kiln and exposed to smoke under careful controlled temperature and air velocity. Fresh fish are ideal for smoking for good quality products, since prolonged frozen storage prior to the treatment can affect the final quality. The wood used for smoke is usually sawdust or chips of wood such as maple, oak, etc. Hardwood is preferred because it imparts a milder flavor, whereas softwood such as fir and pine imparts a more resinous flavor. Before smoke treatment, the fish is salted through wet, dry, or injection salting. The former two methods have been most commonly used by the industry.[62–64] The rate of uptake of salt in the fillet depends on the method used, species, fat content, fillet size and thickness, brine concentration, brining time, and the ratio between fish and salt.[65] Storage stability of smoked fish is due to combination of four factors: (i) salting, which reduces water activity and inhibits the growth of many spoilage-causing organisms including pathogens; (ii) drying, which provides a physical barrier to the passage of microorganisms; (iii) creation of a hostile environment for microbial proliferation by deposition of antimicrobial substances such as phenol; and (iv) deposition of phenolic antioxidant substances from smoke, which delays auto-oxidation and rancidity development. The vegetative forms of microorganisms are the most sensitive to smoke but spores of molds are relatively resistant.

The process of smoking may be "cold" or "hot" depending upon the temperature of the treatment. For cold smoking, the temperature is usually below 30°C; sometimes the temperature is raised to 40°C toward the end. A relative humidity of 60–70% is most satisfactory for cold smoking to control dehydration. Cold-smoked fish, containing about 5% salt and smoked for 7 h can be kept for about 2 months at low temperature. Such products are not necessarily cooked before consumption. Unlike cold smoking, hot smoking is carried at higher temperatures in the range of 50–90°C for 4–12 h. The treatment is done in three stages: (i) a tempering stage where the fish is smoked at 30°C for about 1 h (which allows toughening of the skin), (ii) a heating step where the temperature is raised to 50°C for 1 h, and (iii) finally a cooking stage at a temperature of 70–90°C for 1–2 h. During hot-smoking thermal treatment should be continued until the temperature inside the thickest part of the fish reaches about 70°C. The preferred relative humidity during the smoking process is about 70%. In some situations, the fish may be contaminated with *C. botulinum*. To control possible contamination of this pathogen, the fish is treated to absorb a minimum of 3.5% salt content and heated at 82.2°C for about 30 min. The hot-smoked products are rapidly cooled and stored at a temperature below 4°C or preferably frozen.[18,66,67] Usually 25–30% weight loss takes place during hot smoking and 40–45% during cold smoking. Vacuum packaging, modified or controlled atmosphere packaging can increase the shelf life of the smoked products.

Smoked seafood may contain up to 0.5 g of smoke constituents per 100 g tissue, which include formic, acetic and butyric acids, ethanol and methanol, formaldehyde, octyl aldehyde, acrolein, methyl ethyl ketone and other carbonyls, hydrocarbons such as naphthalene, stilbene and phenanthrene, ammonia, carbon dioxide, carbon monoxide, esters, furans, nitrogen oxides, phenols, and sulfur compounds, among others.[68] Some of the volatiles may be carcinogenic,

the prominent one is benzopyrene, which, nevertheless, may decrease during storage.[69] Significant improvements have been made in fish smoking relating to temperature control, electrostatic filtration, and development of liquid smoke to control the formation of carcinogens. Mechanical and automated processing equipment such as automatic briners and controlled mechanical kilns is also available. Many types of dried and smoked fish with traditional seasonings are used in Japanese foods.

The characteristic golden color of the smoked products is due to the interaction of carbonyls with amino components on the flesh surface. Flavor is the most typical feature of smoked products. It is generally considered that phenol compounds and other components soluble in water are the most important factors imparting acceptable flavor in smoked products. The influence of smoking on the proximate and fatty-acid composition of commercially important freshwater fish has been studied. Palmitic acid ($C_{16:0}$) accounts for 68 to 90% of the total saturated fatty acid. Linoleic acid, linolenic acid, eicosapentaenoic acid ($C_{20:5}$), and docosahexaenoic acid ($C_{22:6}$) were the predominant polyunsaturated fatty acids in lake-superior fish. Brining, smoking, and refrigerated storage caused a reduction in total lipids and only a small loss in n-3 fatty acids.[70] Influence of dry salt curing followed by sun drying or wet curing/salting and subsequently by smoking on nutritive value of two African freshwater fish species, has been reported. Sun drying was carried out for 9 days and smoking was done continuously for 3 days over charcoal. Control samples were oven-dried at 60°C for 48 h. Nutritive value of the product, as measured by protein-efficiency ratio, protein-retention efficiency, biological value, and net protein utilization was not significantly different for the fish samples processed by the two methods. The study concluded that traditional processing methods did not adversely affect the protein quality of the fish.[55]

A number of freshwater fish including salmon, catfish, and tilapia have been examined for smoking. Cold smoking has been employed for salmon, trout, and eel apart from marine fish, including herring, haddock, and cod. Cold-smoked Atlantic salmon is a lightly preserved product and a treasured food item in many countries. It is estimated that almost half of the farm-reared Atlantic salmon from Norway reaches the final consumer as a cold-smoked product. The smoked product has a salt content of 2 to 3.9%. Some of the problems in cold-smoked salmon are variations in appearance due to biological differences and aquaculture practices, gaping of the fillets, and variations in textural characteristics.[62,71] The quality of smoked salmon may be improved by freezing the fish before smoking, which results in an increase in product yield and water content, but gives a softer texture.[72] In cold-smoked salmon, some degradation of texture has been reported as a result of favorable temperature that enhances muscle proteases. Nevertheless, smoking process and storage time did not affect lipid hydrolysis and formation of free fatty acids.[70] Recently, application of high-pressure treatment has been suggested to improve the quality of cold-smoked salmon[62] (see Chapter 11). A popular salmon product is the nugget, which is naturally smoked, flash frozen, and vacuum packaged on a Styrofoam tray package for retail or food service. It can be baked, grilled, or served in a preparation dish. Figure 12.2 presents a scheme for cold smoking of salmon.

Raw material
Fresh fish
(If frozen, thaw, wash in potable water)

↓

Fillets

↓

Brining
(Liquid brine in bath, injection brining, or dry salt)

↓

Drain and rinse with freshwater
Place fish on hooks or racks

↓

Cold smoking
Arrange the fillets to allow uniform smoke absorption,
heat exposure, and dehydration

↓

Cooling
(Cool to 10°C within 3 h and to 3.0–3.3°C within 12 h)

↓

Packaging
(Air, MAP, or vacuum)

↓

Storage and distribution

FIGURE 12.2   Flow diagram for preparation of cold-smoked fish. *Note:* Temperature during smoking, not to exceed 49°C for 6 h, 32°C for 20 h or 10°C for 24 h. Final salt content required in the product: above 2.5% in air packaged product; above 3.5% or a combination of 3.0% salt and 100–200 ppm sodium nitrite in MAP or vacuum-packaged product. Storage conditions: product must be maintained at 3.0–3.3°C at all times. If the species has been identified as representing a parasite hazard and the incoming raw material was not previously frozen, then product should be subjected to freezing (Adapted from Lakshmanan, R., Piggott, J.R., and Paterson, A., *Trends Food Sci. Technol.*, 14, 354, 2003. With permission from Elsevier)

Trout, eel, and to some extent, carp are also smoked on a commercial scale.[36] Smoked catfish, semidried under modified atmosphere or vacuum can remain in acceptable shelf life up to 28 days at 4–6°C.[73] A smoking process has been developed for Mediterranean mussel (*Mytilus galloprovincialis*). Fresh mussel are washed in running water and steamed at 80°C for 10 min. The shells are then removed and the meat was dipped in 4% brine for 15 min, drained, and smoked at 60°C for 13 min, 65–80°C for 17 min, vacuum packed in pouches and cold stored at 2°C. The product has a shelf life of 70 days. The season of harvest h ad no effect on the physiochemical and microbiological parameters of the smoked product.[74]

12.6.6 Fermented Products

Fermentation of fish has been employed in Southeast Asian countries for development of popular flavored products[75,76] (see Chapter 9). The process can be incorporated to develop fermented fish sausages and pastes, which are widely

TABLE 12.7
Some Fermented Fish Products

Product and origin	Raw material	Process in brief
Momone (Africa)	Catfish, seabream, threadfin bream, croaker, mackerel, herring	Dressed fish is dry salted, fermented for 1–6 days and then sun dried for 1–3 days
Kako (Africa)	Sharks, skates, and rays	Dry salting and fermentation
Tambadiang (Africa)	Bonito	Dressed fish is kept in concrete vats with alternate layers of salt and fish, fermented for 103 days, scaled, washed, and dried
Sauces and pastes (Southeast Asia)	Anchovies, mackerel, sardines	Dressed fish is mixed with coarse salt grains in salt to fish ratio of 1:3, allowed to mature in vats. The fish is beheaded that removes entrails at the same time
Mediterranean style fermented anchovies	Anchovies	Fish is dry-salted and fermented at 15–20°C. After a few days, further salt is added. After 6 months of storage, the fish is chilled, washed in brine, filleted by hand, and the fillets are blotted dry. The product is canned in oil

Source: Adapted from Gall, K., Reddy, K.P., and Regenstein, J.M., in *Marine and Freshwater Products Handbook*. Martin, R.E. et al., Eds. Technomic, Lancaster, PA, 2000, p. 819 and Saisithi, P., Fisheries Processing: Biotechnological Applications, Martin, A.M. (Ed) Chapman & Hall, Newyork, 1994, p.111. With permission.

produced and consumed in many countries. *Pediococcus* spp. and other lactic acid bacteria are involved in acid production during fermentation. Traditionally, pastes are fermented for a shorter period than sauces. Fish pastes are widely produced and consumed in Southeast Asian countries, perhaps to a greater extent than fish sauces, as condiments for rice dishes. Mackerel mince was homogenized and was subjected to fermentation by lactic-acid bacteria for 2 days at 37°C. The fermentation was indicated by decline in pH and suppression of native microorganisms.[77] The process has potential for extension to freshwater fish species. Table 12.7 shows some fermented fish products.

Fish protein hydrolyzates are prepared by digesting fish using proteolytic enzymes such as papain, ficin, trypsin, pancreatin, pronase, or enzymes isolated from proteolytic microorganisms at the optimal temperature and pH required by the enzymes. The hydrolyzate is decanted and centrifuged to remove scales and bones and subjected to spray drying to prepare the protein powders.[78,79] Peptides in fish hydrolyzates have functional roles. The activity of these biopeptides depends on the raw material and hydrolysis conditions. Small peptides from cod stomach hydrolyzates have been shown to enhance disease resistance of aquacultured salmon.

12.6.7 Marinading

Marinades are usually made from fatty fish having a lipid content of 5–15%. In marinading, the fish is immersed for at least a week in 5–10% acetic acid solution containing 10–15% salt. In the process, the bacterial action is controlled and the protein of the flesh is coagulated. Maintaining a palatable level of preservative for a reasonable period of time is also essential. A final product in 1–2% acetic acid and 2–4% salt will keep the fish in a good condition for at least 3 months at chilled temperatures.[80] Marinades are highly popular in Northern Europe. Marinading has been successfully employed to preserve farmed trout.[81] Marinated trout fillets are prepared by pickling in common salt and acetic acid for one week followed by transferring the fillets into a second pickle of the salt, malt vinegar and seasoning, and sugar for 2 weeks.

12.6.8 Novel Combination Processes for Value Addition

Potentials for some novel combination processes for value addition of fishery product including those from freshwater sources have been examined. A cook-freeze technology involves freezing raw fillets after adding a cooled sauce, and is cooked prior to consumption. Trout salads are prepared by grilling or hot smoking skinned and deboned trout and then putting into salads consisting of potatoes, tomatoes, apples, onion, and seasoning. Both the products can have high acceptability. Similar products can also be prepared from other species such as carp. Dried products from some freshwater fish have an extremely hard consistency. Recently, a process has been developed for soft, dehydrated steaks from rohu (*L. rohita*), which can give crispy products upon frying in oil.[82] The process consists of tenderization of fresh rohu steaks with 0.01 to 0.03% papain at ambient temperature for 3 h, salting the treated steaks in equal amount of 10% brine for 1 h and drying in a tunnel or solar dryer at 60°C. Dehydration in solar dryer took 16 h as compared to 11 h in tunnel dryer to give a product having 15% moisture content. The tenderization process also enhanced rehydration capacity of the dried steaks. Sophisticated machinery is available for unit operations in value-addition processes. Forming machines are available for production of fish balls and burgers. Automatic frying systems and cookers allow food products to be fully fried or cooked under controlled conditions. Table 12.8 presents some value-added products from farmed salmon.

12.6.9 Packaging

The developments in packaging technology are of immense help in value addition of freshwater fishery products. For retail marketing, the processed products are packaged in consumer friendly packages. Expanded polystyrene is frequently used but it is partly oxygen-permeable and so those products, which are sensitive to rancidity, have to be additionally overwrapped or skin-packed with suitable film. Trays used for packing are generally overwrapped with a protective film, which shrinks. The film shrinking is achieved by use of hot air or hot water.[18] Polyester

TABLE 12.8
Some Value-Added Products from Farmed Salmon

Whole, gutted fish in MAP for the chilled retail market
Boneless fillets, with or without skin, packaged in bulk
Fillet portions and steaks, mainly in MAP lidded-tray formats
Portions combined with marinades, in MAP lidded-tray formats
Fish in sauce for microwave or oven cooking
Low fat burgers
Salmon, flavored, roasted
Smoked salmon chowder and spread
Salmon pate
Lightly smoked salmon
As component of ready meal

Source: Adapted from Moller, A.B., FAO/GLOBEFISH, Special market study, Food and Agriculture Organization of the United Nations, Rome, p. 93. With permission

can be used as a packing material for heating of the product in the traditional and microwave ovens, but this material cannot be used for microwave cooking. Stretch wrapping is often used for products that are heat-sensitive. The film is stretched over the product manually or by machine. Foils used as wrapping or bags for packing of trays with product must be puncture-proof, extensible, and impervious to gases like oxygen. Plastics such as polyethylene film or copolymer of ethylene and vinyl acetate are very often used for packing of frozen products. An impulse or bar sealer is used to seal the bags, which are hand-filled. In order to improve the barrier properties of packages laminates are used, for example polyester/polythene. "Shrink packaging" is now popular in most developed countries. The film material used for shrink packaging is made of special plastics, 100 to 150 μm thick and puncture resistant. IQF shrimp is usually packed in such bags in quantities ranging from 1 to 30 lbs. Vacuum-shrink packaging has been successfully employed in the United States to market. Vacuum-skin packaging is becoming more common for packaging smoked fish. In this process the wrapper is heated and wrapped over the product, the film mold completely to the product shape and seal the product completely, forming an extra skin. Fresh or frozen boneless, deep-skinned fillets of freshwater fish such as tilapia can be packed in styrofoam boxes to ensure maximum protection.[83] It is important to provide necessary information on the pack for the purpose of traceability of the product (see also Chapter 5). The labels are required by law to carry basic information such as name of the product, net weight/volume, name and address of the producer. The regulations also insist on information to be supplied on ingredients and additives, date of manufacture or expiry, and health claims.

12.7 MARKETS FOR AQUACULTURED FISH AND PROSPECTS FOR EXPANSION

International trade statistics often do not distinguish between fishery products from wild and farmed origins and hence actual volume in trade of farmed species is difficult to determine. However, the situation is likely to change gradually as producers' associations in producing centers will begin to keep records and also in view of environmental labeling requirements that distinguish between farmed and wild products. In the recent past, only a few species of freshwater and aquacultured fish were popular, that too, in certain limited market segments of countries engaged in fish farming. In such places where freshwater fish are popular, marine fish are less favored. At present there is good demand for freshwater fish in many Asian countries. In India, rohu and catla are the common preferred species, particularly in the east cost of the country. Major carps such as rohu, catla, and mrigal from India are also exported to Bangladesh. Grass carp, silver carp, crucian carp, and common carp are the favored species in China. In Philippines, tilapia is preferred, while in Thailand, market exists for tilapia, silver barb, snakehead, and catfish. Recently, the demand for aquacultured fishery products is increasing throughout the world. However, aquaculture in the western countries is not considered viable because of variable weather and high feed and labor costs. Therefore, these countries have to depend on Asian countries, where fish farming is flourishing because of yearlong favorable growing season, abundance of natural resources, low labor costs and government support. Some freshwater fish species such as tilapia are increasingly favored as substitute for ground fish, the supply of which is often dwindling. The main traded products from aquaculture are shrimp and prawns, catfish, salmon, tilapia, seabass and seabream, and mollusks. Demand for salmon is especially high during festive seasons such as the Chinese New Year, when a combination of raw fish such as salmon and carp together with vegetables and ground peanuts are popular.[84,85]

The extensive farming operations in China have made the country a major exporter of the cultured products to countries including Japan, United States, and Europe. Eel, shrimp, seaweed, carp, shellfish, clam, scallop, mussel, oyster, yellow croaker, ornamental fish, pearl, and crab are the major aquacultured products exported by China.[85] Japan imports aquacultured items from Australia which include frozen southern blue fin tuna (*Thunnus maccoyii*) for sashimi and live kuruma prawns (*Penaeus japonicus*). Tuna is the most valuable aquacultured item for export. In Australia, tuna are fattened over a four-month period in large sea pens. Clarias catfish, hybrid of Thailand and African catfish species, contributes 15% of total freshwater fish production in Thailand. Recently, cultured abalone and rock lobster are also traded, the main product forms being live and canned.

Asia is the major producer of tilapia. Until the early nineties tilapia was regarded as a low value fish, but this concept has changed in recent years. The fish flesh has a firm texture with a mild sweet flavor, and adapts easily to value addition. In recent times, tilapia is marketed in a great variety of product forms, including live and also as frozen fillets together with a wide range of value-added

products.[86] Nile tilapia (*O. niloticus*) is increasingly being farmed due to its high demand, with a total production of about 1.5 mt of the fish in 2001, China contributing more than one third of the production. The major emerging markets for tilapia are the United States, Canada, and Europe. In the United States, the boneless, odorless, mild-flavored species is regarded as a white fish substitute. Red tilapia is grown in ponds under controlled conditions for special fillets. One of the popular tilapia products is the frozen and fully trimmed 12 oz fillets that are individually wrapped for convenience. The product has been well received by the food service sector in Europe. Japan imports fresh or frozen tilapia fillets from China and other countries. In Japan, vacuum-packaged fresh tilapia is sold for *sashimi*.[86] The acceptance of aquacultured fish such as tilapia and carp in many parts of the world needs to be exploited for enhancing marketability through value addition.[49,83]

Salmon, perhaps, is one of the most popular fish species. Trade in salmon, consisting mainly of Atlantic salmon, has grown rapidly during the past few years, and has reached an amount of one million tons. The leading exporters of salmon are Norway, followed by Chile, United States, and Canada.[87] Presently, Norwegian salmon is finding good markets in some Asian countries. Some of the commercial products from farmed salmon popular in Europe are indicated in Table 12.8. International trade in trout is smaller than salmon, with exports in 2,000 reaching 140,000 t of total farmed trout production of 511,000 t. Commercial value-added catfish products include IQF fillets, marinated, and coated fillets in retail tray packs.[87] Catfish is the 5th most consumed fish in the United States. Milkfish are harvested and marketed mostly fresh or chilled, whole or deboned, but some are canned or smoked. Milkfish is also traded in different product forms, such as dried, canned, smoked, and marinated. There is a growing market for quick-frozen deboned milkfish fillets. However, over the past 20 years the relative importance of milkfish has declined with the expansion of tilapia, tiger shrimp, and seaweed farming.[88]

Shrimp is already the most-traded seafood product internationally. Since the late 1980s, farmed shrimp has tended to act as a stabilizing factor for the global shrimp trade. In 2000, about 1.1 mt or 26% of total available shrimp came from aquaculture.[89] Farm-raised shrimp are marketed in similar forms as ocean-caught shrimp. Black tiger shrimp (*P. monodon*) is the major product of export from aquaculture. It is usually transported from the production site, as headless and frozen, to a further processing plant, where they are prepared into a variety of marketable products. The most common marketed-form shrimp is raw, headless, with shell on. Raw peeled shrimp is sold in a variety of ways — peeled and undeveined (PUD), peeled and deveined (PD), tail-on, butterfly cut (cut along the vein), cooked, etc. Guidelines for shrimp farming, information on further processed shrimp, value addition, packaging, market demands and trends in marketing, and regulatory measures in shrimp trade are available.[89] With increased interest in more value-added products, novel processing lines have been developed to process shellfish more efficiently and economically. One single machine could be used to process more than one species ensuring a high standard of quality[90] (see Chapter 4).
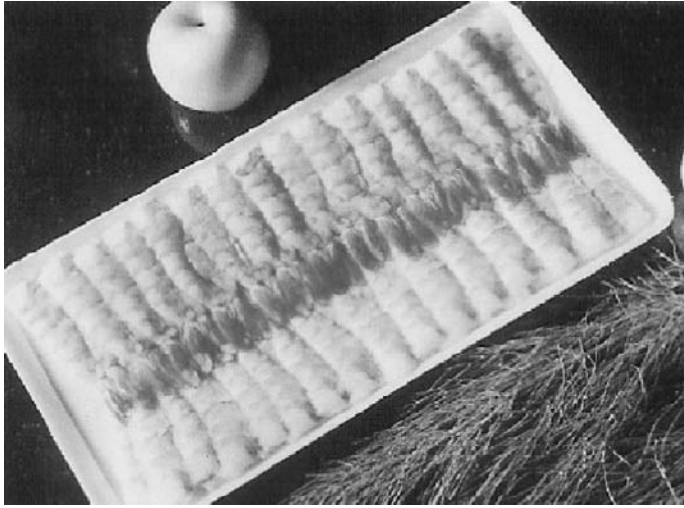
FIGURE 12.3   Stretched shrimp

12.8   QUALITY ISSUES IN MARKETING OF AQUACULTURED FISHERY PRODUCTS

Freshwater fish and shellfish, whether wild or farmed, are caught from waters close to human activities, which may expose them to significant biological, physical, and chemical hazards.[91–93] The biohazards include presence of parasites such as trematodes and nematodes, Enterobacteria including *Salmonella* spp., *Escherichia* spp., *Vibrio* spp., and viruses in the harvested items (see also Chapter 3). Brackish water-raised fishery products are generally contaminated with *Salmonella* spp. and *Vibrio* spp. Chemical hazards include veterinary residues, hormones, growth regulators, and environmental pollutants, while physical hazards may be glass, wood, metal pieces, etc. Food-borne diseases associated with fishery products can be largely prevented and controlled through appropriate food-safety measures, including implementation of Hazard Analysis Critical Control Points (HACCP) in farming operations.[94] The Critical Control Points with respect to fish farming are site of the pond, water supply, feed supply, and the rearing of the shellfish or fish.[94] The U.S. FDA recommends visual examination by a process called candling and physical removal of the parasites, nematodes, and cestodes. Recently, application of low-dose irradiation has been suggested to reduce bacterial and nematode parasite contaminations of farm-raised fishery products[98] (Chapter 10).

It is a common farming practice to use antibiotics such as chloramphenicol to control diseases in aquacultured fish. Using drugs in aquatic animals for both curative and preventive purposes may not only lead to environmental pollution, but also affect human health due to drug residues in the cultured fish. A withdrawal period is needed for complete removal of drug residues from the animal.[16]

Nevertheless, occasional presence of drug residues in imported fish has become a recent concern. The maximum residue limits for antibiotics cannot be established because these compounds even in traces constitute a hazard to the health of the consumer. The development of antibiotic resistance by microorganisms in aquacultured products is another problem in farming operations.[95] Presence of antibiotic residues, particularly chloramphenicol, has resulted in rejection of farmed products exported to the European Union. The European Union recently introduced legislation pertaining to chloramphenicol contamination in shrimp.[96] Maximum permissible level of chloramphenicol in shrimp, prawn, and fish, according to the European Union is 0.3 ppb. The United States, Australia, and New Zealand also stipulate the same limit. A maximum level of 1 ppb is allowed for nitrofuran compounds in the countries.[94] Most of the hazards can be controlled by the choice of culture site and proper pond management.

12.9 ORGANIC FARMING

One of the recent approaches to culture safe fishery products is organic farming. In the last decade, the organic food sector has made significant advances and is entering international food markets. Organic aquaculture is relatively new compared to land-based organic farming of agricultural and animal products and includes various elements that ensure the farming activity is in harmony with nature, and keeping in mind the good health and welfare of the cultured organisms. The elements are the following: intensive environment monitoring, natural breeding without the use of chemicals, integration of natural plants in the farm, use of feed and fertilizer from certified organic agriculture, application of only natural medicines and nonuse of synthetic drugs, and adoption of other environment friendly measures. Some species, which are currently the subject of organic farming are trout, carp, and prawn from freshwater and salmon, seabass, seabream, turbot, mussel, and shrimp from salt water.[97]

In summary, with global rise in interests of freshwater fishery products and aquaculture operations, there is a need for parallel progress in technology for their processing and value addition in order to enhance their marketability. Processing of these fishery products, like in the case of other food raw materials, should assure best possible market quality, provide proper forms of semiprocessed and value-added products, assure health safety of products, apply the most rational processing method, and reduce waste to the extent possible.

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13 Gel Formation of Fish Structural Proteins by pH Changes and Its Applications

13.1 INTRODUCTION

Ability to form gel is an important functional property of macromolecules including proteins. A protein gel is an intermediate form between solid and liquid phase in which strands of protein chains are cross-linked to form a continuous three-dimensional network. Gelation of fish myofibrillar proteins essentially involves three steps namely, dissociation of myofibril structure, partial unfolding of native myosin structure caused by mild heat, and irreversible aggregation of unfolded myosin to form a three-dimensional structure. During aggregation head portions of myosin molecules interact through disulphide bonds when significant helix-coil transitions take place in the tail parts of the molecules. The bonds involved in stabilization of the gel structure include both covalent (disulphide) and non-covalent (hydrophobic, hydrogen bonding, and electrostatic) linkages. During the aggregation process water, oil, and flavoring compounds may be entrapped in the gel matrix. Rigidity of the gel is dependent upon the presence in the matrix of ingredients such as salt, starch, polyphosphate, or proteins from other sources.[1–6]

Fish muscle structural proteins including myosin and actomyosin are highly unstable, sparingly soluble in water, and require extractants having high ionic strength for their dissolution. In addition, the proteins undergo rapid denaturation even on mild heating.[1–3] These properties limit the use of fish proteins in product development. The *surimi* process enables use of washed fish meat for product developments, through the gel-forming ability of the proteins in the meat (Chapter 8). During gelation partial denaturation of the *surimi* proteins is achieved by mild heating in the presence of a small amount of salt which helps dissolution of myosin. Gelation is observed when *surimi* is incubated overnight at 0–4°C or when subjected to mild heat for several minutes. The gel set at low temperature is weak and possesses high elasticity and transparency. Raising the temperature of the mild heat-set gel to nearly 90°C makes the gel opaque and rigid due to further cross-linking of the proteins. *Surimi* initially set at 40–50°C gives a stronger gel if subsequently heated to 80–90°C.[3,4] The washed fish meat has neutral or slightly alkaline pH and therefore conventional *surimi* gelation takes place under nonacidic conditions.

13.2 Gelation of Fish-Muscle Proteins under Mild Acidic Conditions

Unfolding of native structure of major myofibrillar proteins, myosin and actomyosin, essential for gelation in *surimi* is brought about by mild heating. Logically, it should also be possible to make the protein unfold under controlled, nonthermal conditions. Mild acidic conditions created by the presence of weak organic acids are also capable of causing unfolding of the protein molecules.[7] Gelation of fish-muscle structural proteins under acidic pH has not been studied in detail. The advantages of this type of gelation are mainly two. First, there is no need to add small amounts of salt to dissolve the myosin as in the conventional gelation process, which offers salt-free (low sodium) products. Second, because of the acidic pH, the product has comparatively higher microbial stability. Organic acids, such as acetic and lactic acid, are the common acidulants that can be used for the purpose. Acetic and lactic acids are generally recognized as safe (GRAS) and have been approved for use as food additives by the U.S. Food and Drug Administration. These are weak acids having ionization potential ideal to slow lowering of the pH required for unfolding of the myofibrillar proteins. Inorganic acids cannot be used since they bring about drastic fall of pH causing precipitation of the proteins. The proteins that unfold as a result of mild acidification can aggregate among themselves to form the gel. The aggregation can be accelerated when the acidified proteins are subjected to mild heat treatment.[8] Similar to conventional *surimi*, in mild acid-induced gelation too the fish mince needs to be thoroughly washed to remove low molecular weight components to eliminate their interference with the gelation process. Before the acidification and heating, suitable additives could be incorporated to modify the texture of the gelled proteins. However, since ionic compounds including common salt interfere with the gelation process at acidic pH, these compounds should be avoided. Nevertheless, salt to taste can be added after the gelation process is complete.

13.2.1 Process of Making Acid-Induced Fish Protein Gel

Mechanically deboned fish mince is used in the process. Alternatively, headed, eviscerated boneless fish meat cut into pieces of 4–5 g each could be used. The fish mince or meat pieces are repeatedly washed in cold water as in conventional preparation of *surimi*.[8–10] Usually three-step washing is recommended for meat pieces. In the first step, the pieces are held overnight in a cold room in excess (usually three times the weight of meat) of cold (<10°C) water. If mince is used, it has to be soaked in cold water for 1–2 h. In the case of Indian dog shark (*Scoliodon laticaudus*) the skinned meat cut into pieces (4–5 g, average size) are subjected to repeated washing in cold water. For low fat fish such as threadfin bream or Bombay duck, washing is repeated twice with fresh cold water keeping a holding time of 1 h for each wash.[11,12] While washing fatty fish, such as mackerel and herring, it is advisable to use 0.5% aqueous solution of sodium bicarbonate in the second washing to remove lipids adhering to the meat. The alkaline pH of the bicarbonate solution significantly aids removal of the lipids. Fish mince that

Fresh fish

↓

Evisceration, heading

↓

Mechanical/manual deboning

↓

Washing in excess of cold water, decanting

↓

Washing in 0.5% sodium bicarbonate solution
(for fatty fish), decanting

↓

Washing in excess of cold water, decanting

↓

Lowering of pH to 3.5 by drop-wise addition of acetic acid

↓

Gel

↓

Homogenization of gel in water

↓

Heating 45 to 50°C

↓
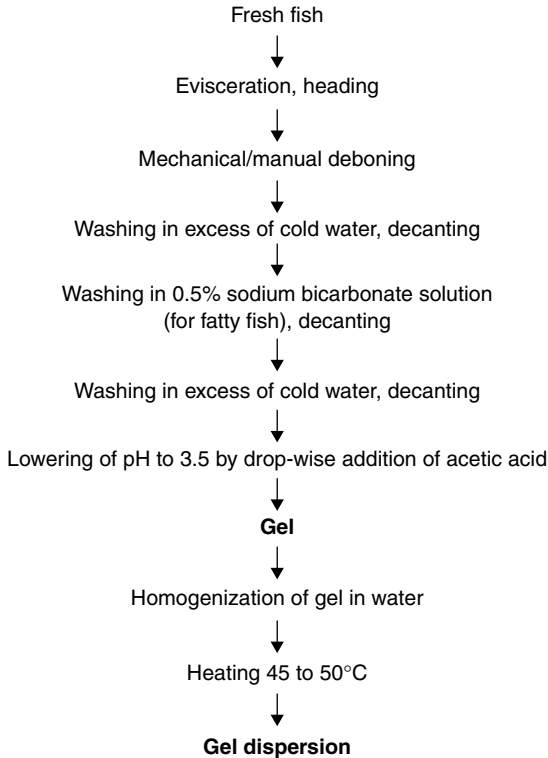
Gel dispersion

FIGURE 13.1 General process for preparation of acetic acid-induced gel and thermostable water dispersion of fish meat (Reprinted from Venugopal, V., *Trends Food Sci. Technol.*, 14, 39, 1997. With permission from Elsevier)

has an unappealing dark color such as that of capelin may be decolorized by an initial washing with 0.5% aqueous solution of sodium chloride.[13] The proteins in the washed fish mince have significant solubility in water.[8,14]

The washed meat is homogenized in fresh cold water, usually at a ratio of 1:1. To the aqueous slurry a few drops of glacial acetic acid is added with gentle stirring to bring down its pH to about 3.5 in order to induce gelation of the myofibrillar proteins. Usually an amount of acid equivalent to 0.5% of the slurry is sufficient for the purpose. Proteins in the fish Bombay duck are highly labile. Homogenization of the washed meat followed by acidification may result in precipitation of the proteins. Therefore, in this case, the washed meat in equal amount of cold water is held overnight at 0 to 2°C in the presence of 0.5% acetic acid to induce gelation.[11] Figure 13.1 shows a general process for preparation of acetic acid-induced gel and its dispersion.

Acid-induced gelaton of shark myofibrillar proteins is indicated by increase in viscosity of the slurry, as shown in Figure 13.2. If the acidification is carried out at ambient temperature, the gelation as indicated by thickening is completed within
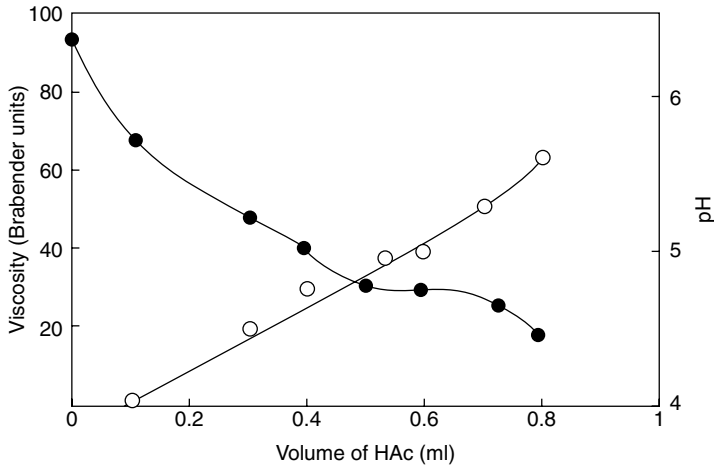
FIGURE 13.2   Effect of acetic acid addition on pH and viscosity of washed shark meat slurry in water (Reprinted from Venugopal, V., Doke, S.N., and Nair, P.M., *Food Chem.*, 50, 185, 1994. With permission from Elsevier)

a period of 3 h. The gelation of the proteins in terms of increase in viscosity could be measured if the acidification is carried out in a Brabender viscoamylograph, as depicted in Figure 13.3.[8] The increase in viscosity will be slow if the slurry is cold (<10°C), but can be enhanced if it is warmed to 50°C in the equipment during the acidification process.[8] The moisture content of the acid-induced shark gel varied between 88 and 93% when washed shark meat was homogenized with water using meat to water ratios of 1:1, 2:3, or 1:2, with respective gel strengths of 134, 147, and 158.[14] However, compared with conventional *surimi*, the strength of acid-induced gel is lower suggesting its weaker nature.[15] The expressible water, a property of gel, is as high as 30% in the preparation.[15] This is in contrast with conventional *surimi*, where the expressible water content may not exceed 10%.[1] Instead of acetic acid, lactic acid could be used whereas citric, tartaric, or hydrochloric acids were ineffective.[8] The presence of sodium chloride was, however, detrimental to mild acid-induced gelation of shark meat proteins.[8]

As compared with gelation of shark meat, mild acid-induced gelation of fish species such as threadfin bream, Bombay duck, Indian mackerel, Atlantic mackerel, and freshwater fish rohu was not characterized by thickening of their meat slurries.[11,12,16,17] Capelin is unique in this respect, since the slurry of washed capelin mince in water forms a gel when subjected to mild heating. Acetic acid was not needed for gelation in this case.[13,17] The differences in behavior could be due to variations in structural characteristics of the fish myosins. Research in this area with a view to modify the gelation characteristics of the fish proteins could be useful for development of desired products.

The acid-treated washed meat could be suitably diluted with water to get dispersions of the meat (Figure 13.1). While acidification of water slurry of washed fish mince results in a fall in viscosity, mild heating of the acidified slurry results
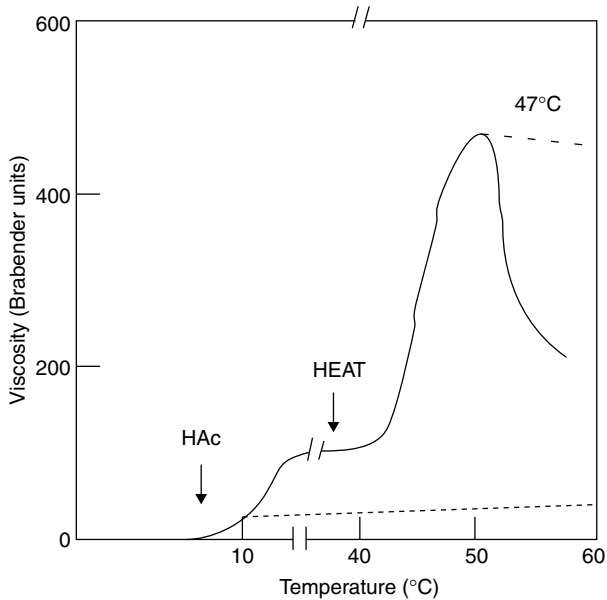
FIGURE 13.3 Viscosity changes of washed shark myofibrillar protein (protein 16 mg/ml) during acetic acid-induced gelation followed by heating. Viscosity was measured using Brabender viscoamylograph. The broken line at the top represents viscosity of the gel when heating was stopped. The broken line at the base shows viscosity of heated sample in the absence of acetic acid (Reprinted from Venugopal, V., Doke, S.N., and Nair, P.M., *Food Chem.*, 50, 185, 1994. With permission from Elsevier)

in drastic fall in its viscosity to give a dispersion of proteins in water. Further, the meat proteins are highly soluble in the acidified and heated dispersion, as shown in Table 13.1 (see also Section 13.2.5). Therefore this phenomenon offers a method to prepare thermostable dispersion of fish meat in water.[17]

13.2.2 Entrapment of Water in the Gel Matrix

The washing of fish meat is associated with an increase in its wet weight, despite the removal of the sarcoplasmic fraction of the muscle. This could be attributed to enhanced affinity of the washed proteins to water. This is true even in the case of fish such as Bombay duck, the native muscle of which has moisture content as high as 90%.[11] In the case of Bombay duck, the washing process enhanced the weight of the meat by more than 30% suggesting significant hydration of the fish proteins. On a dry weight basis, in the acid-induced gel, up to 11 g of water per g protein were imbibed by the meat.[11] Hydration of muscle proteins has also been found during mild acid-induced gelation of proteins from other fish species including freshwater fish rohu.[16] In all the cases water was firmly entrapped in the gel matrix.[17] The bound water could not be separated from the gel or gel dispersion by heat treatment and centrifugation. No water could be separated from

TABLE 13.1
Soluble Proteins in Thermostable Dispersions of Mince from Different Fish Species

Fish species	Protein content of unacidified fish mince homogenate in water (%)	Protein content of gel dispersion (%)	Solubility of proteins in dispersions (%)
Atlantic mackerel	2.40	2.35	98.0
Atlantic herring	1.30	1.28	99.0
Indian mackerel	1.50	1.30	86.0
Capelin	2.50	2.10	84.0
Shark	2.94	2.16	73.0

Source: Reprinted from Venugopal, V., *Trends Food Sci. Technol.*, 14, 39, 1997. With permission from Elsevier.

the shark gel even when centrifuged at $12,100\times g$ for 30 min; in contrast as much as 50% of water could be separated by centrifugation of unacidified shark slurry. Furthermore, there was no separation of water when the gels were subjected to heat treatment at temperatures as high as 100°C. The stability of the proteins in the dispersion is, however, influenced by the presence of ionic compounds. Addition of traces of common salt to the washed meat prior to acidification and heating prevented gelation or collapse of the gel network resulting in precipitation of the proteins and separation of water (see Section 13.2.4.3).

13.2.3 Chemical Changes During Gelation

Mild acid-induced gelation is associated with decrease in sulfhydryl contents and formation of disulfide bonds. This has been verified with respect to gels from threadfin bream, shark, and Atlantic herring.[12,15,17,18] This is also comparable with that in conventional *surimi*.[3] Another structural change is the disappearance of myosin heavy chain (MHC), as shown by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) data.[12,15,18] The degradation of MHC in threadfin bream was also indicated by concomitant appearance of a protein band of about 186 kDa. Solubility of the gel in various solvents containing sodium dodecyl sulfate, urea, and β-mercaptoethanol also suggested structural changes in myosin during gelation.[12]

13.2.4 Rheology of Mild Acid-Induced Gel and Gel Dispersion

13.2.4.1 Viscoelasticity of gel

Rheology is the study of deformation and flow of matter. In foods, deformation is a measure of mouth feel while flow is associated with viscosity.[19] Rheological

TABLE 13.2
Some Basic Rheological Expressions

Expression	Description
Viscosity	The flow behavior of a substance under the influence of stress to become irreversibly deformed
Apparent viscosity	Viscosity of non-Newtonian fluids
Elasticity	Property of a material to recover its original size and shape immediately after removal of force causing deformation
Viscoelasticity	Attributes of substances that are both elastic and viscous. Viscoelastic substances save a part of the deformation energy
Newtonian flow	Viscosity at a constant temperature and pressure is constant regardless of the applied shear rate and time. The shear rate is directly proportional to the shear stress. Therefore a single viscosity measurement will give its true value
Pseudoplastic behavior	The shear rate is not directly proportional to the shear stress. Therefore, several viscosity measurements against different shear rates are necessary to establish a rheological profile
Yield value	Minimum shear rate needed to induce flow. Expressed as $N\,m^{-2}$ or Pa. Above this value, materials exhibit plastic flow behavior
Bingham system	A type of plastic flow behavior, where there is no movement below a characteristic yield value. Above this yield value, the system acts like a Newtonian liquid
Casson relation	Another type of plastic flow involving a special form of relationship between shear stress, shear rate, and yield value
Thixotropy	Viscosity decreases under constant shear stress. If the stress is removed, the viscosity increases again
Dilatancy	Viscosity increases with rising shear rate. Dilatant behavior (shear thickening) is opposite of pseudoplasticity

Source: Adapted from Holcomb, D.N., in *Encyclopedia of Food Science and Technology*, Vol. 4. John Wiley & Sons, New York, 1991 and Bieleman, J., in *Additives for Coating*. Wiley VCH, New York, 2001, p. 24, 25.

properties are important in determining the functional properties of food. Some basic rheological expressions are given in Table 13.2. One of the important properties of fish protein gels including the conventional *surimi* is their viscoelastic character, which make the gels behave as elastic solids and exhibit viscous flow. These properties have direct relations with the texture of the products developed from the gels. Measurement of viscoelasticity involves identification of responses of the gels to both large and small stresses. The response to large stress is usually determined by "texture profile analysis" using texturometers such as the Instron

Universal testing machine.[20] Whereas behavior of gels toward large stress at failure is comparatively easy to study, the data may be subject to variations depending upon the conditions. A major advance in this field is dynamic measurements of small deformations in the gel under either constant or sinusoidal oscillating stress.[19] The controlled stress approach, where the measurement is based on displacement (rotational speed) in response to an applied torque (stress), provides subtle changes in the gel indicative of its viscoelasticity. To measure the controlled stress, the sample is subjected to rotational strain between parallel plates or between a cone and a plate at controlled temperatures. The moving cone or plate has an air bearing with very low friction and is rotated by a computer-controlled induction motor and the movement is measured optically. The controlled stress rheometers can measure viscosity versus rate of shear, creep, stress relaxation, the bulk modulus, and storage modulus, etc. The validity of these measurements is suggested by the fact that real time processes such as gravity flow are mostly stress driven and the consumer perception of a food is strongly related to its yield point and initial deformation during consumption. Dynamic rheological tests based on controlled stress are widely used to study heat-induced gelation of *surimi*.[21-23] However, it has also been cautioned that the fundamental rheological properties measured at low strain may bear no relation to the behavior and texture at high strains.[21]

The viscoelastic properties of acid-induced shark gel have been studied by dynamic rheological analyses.[15] Shark gel showed higher storage modulus G' (a measure of elasticity or energy stored), than loss modulus, G'' (a measure of viscosity, or energy lost). The G' increased with decrease in moisture contents suggesting higher rigidity of the gels at lower moisture contents. The G' and G'' profiles indicated structural changes in the gels at temperatures above 50°C. Gelation process was monitored by measuring changes in the stress–strain phase angle during oscillatory testing where the loss tangent (referred as $\tan \delta = G''/G'$) gave information on sol–gel transition. Relatively low values of $\tan \delta$ indicated elastic nature of the gel.[15]

13.2.4.2 Flow behavior of gel dispersions

Viscosity, the important rheological property of protein gel dispersions, is governed by the composition of proteins, their molecular shape, size, and charge as well as by factors such as temperature, concentration, pH, and ionic strength.[24,25] These properties are important in process optimization such as heating, pumping, mixing, and concentration. Rheological properties of gel dispersions have been studied in the case of Bombay duck, capelin, Atlantic mackerel, Atlantic herring, Indian mackerel, and threadfin bream using LVT Brookfield Synchro-lectric viscometer.[11-15,17,18] The apparent viscosity of washed capelin mince homogenate in water decreased with increasing temperature and also with increasing shear rate. Analysis of the data suggested that the protein dispersions exhibited non-Newtonian flow behavior. The influence of shear rate on the apparent viscosity also indicated pseudoplastic nature of the dispersion.[13] The apparent viscosity

TABLE 13.3

Influence of Acetic Acid on the Apparent Viscosities of Washed Fish Homogenates in Water

Fish species	Protein content of water homogenate (%)	Viscosity without acetic acid	Viscosity after addition of acetic acid (pH 3.5)	Viscosity after addition of acetic acid followed by heating (50°C)
Atlantic herring	1.6	3.4	1.4	0.07
Atlantic mackerel	2.4	5.9	0.3	0.06
Capelin	1.9	3.0	A	0.04 (no acetic acid required)
Indian mackerel	1.5	4.0	0.5	0.05
Shark	2.3	12.0	600	>1200
Threadfin bream	2.3	6.2	1.2	0.04

The viscosity values are denoted as apparent viscosity values, and expressed as Pa.s. Viscosity of shark was measured using Brabender viscoamylograph and expressed as Brabender units. "A" indicates samples precipitated in presence of acetic acid.

Source: Reprinted from Venugopal, V., *Trends Food Sci. Technol.*, 14, 39, 1997. With permission from Elsevier.

decreased significantly on heating to 35 to 40°C. Apparent viscosity of homogenate of washed Bombay duck muscle in water showed initial increase between 20 and 35°C followed by rapid fall at 40°C and above.[11] Table 13.3 shows the influence of acetic acid (0.5%, v/v of the slurry) and heating (50°C) on apparent viscosity of washed mince homogenates from some fish species.

13.2.5 Stability of Proteins in the Dispersions

Interest in stable proteins is increasing not only to understand their structure–function relationships, but also to explore their potential areas of applications.[26,27,29] Weak acid-induced fish meat gel dispersions are low viscosity fluids in which the proteins are highly thermostable. Whereas mild acidification and heating decreased the viscosity of gel dispersions, the proteins were highly soluble in the heated dispersions. Most of the proteins in the washed meat were retained in the dispersions.[11,15,17,28,30] Furthermore, the proteins in the dispersions were not precipitated by a combination of heating (100°C, 15 min) and centrifugation (up to $135,000 \times g$). Table 13.1 shows the solubility of proteins in thermostable dispersions prepared from different fish species. Nevertheless, almost all proteins in the dispersions were precipitated when they were heated after increasing their pH from 3.5 to 7.0. In addition, presence of salts such as NaCl and $CaCl_2$ adversely affected the stability of proteins in the dispersions.[15,17,30]

13.2.6 Mechanistic Aspects of Protein Stability of Proteins in the Dispersion

Before discussing the mechanistic aspects of protein stability in the fish meat gel dispersion, it is pertinent to briefly discuss the nature of myosin, the major structural protein of vertebrate muscle including fish muscle. The protein has a molecular mass of about 500 kDa and plays a major role in the functionality of the muscle.[10,31–33] Myosin is composed of two heavy (∼200 kDa each) and four light (∼20 kDa each) chains. The *N*-terminal halves of each heavy chain fold into two globular heads, and the *C*-terminal ends fold into distinct α-helices that wrap around one another to form a long (160 nm), fibrous, coiled-coil tail. Each globular head is composed of ∼850 amino acid residues contributed by one of the heavy chains and two of the light chains. The globular heads contain the nucleotide-binding sites (for adenosine-5′-triphosphate hydrolysis) and regions required for binding actin to form the actomyosin complex (present in postmortem muscle), whereas the long rod-like portions of myosin form the backbone of the thick filament of the muscle structure. More than half of the amino acids that constitute myosin are hydrophilic. Most of these residues are exposed to the molecular surface, which may be in contact with water. The myosin head is highly asymmetric, the secondary structure being dominated by many long α-helices.[10,32,33] The high degree of flexibility of the myosin molecule arises through the rotation of the amino acid side chains and the torsional motions of the polypeptide backbone, which give rise to numerous conformations both at the head–tail junction and at locations within the tail end.[32,33] The internal flexibility provides the protein with a dynamic foundation for biological functions in foods, ranging from enzyme catalysis to gelation.[34]

Fish muscle myosin and actomyosin are soluble in aqueous salt solutions of high ionic strength, and the extractability in these solutions is considered as an index of the fish quality.[9] These proteins are also soluble in water at very low ionic strength.[35,36] In the native fish muscle, several low molecular weight compounds and enzymes are adhered to myosin and actomyosin, which hinder their interactions with water, suggesting the poor solubility of these structural proteins under physiological conditions. Washing removes the soluble components such as pigments, enzymes, and lipids adhering to the proteins liberating polar sites for interactions.[9] The removal of soluble components also alters the electrostatic balance. This results in unfolding of the myosin molecules bringing the buried nonpolar side chains into contact with water. The "cages" of water molecules that surround the nonpolar side chains are stabilized by hydrogen bonding.[37] The surface hydration shells provide the flexible matrix that enables the polypeptide chain to respond efficiently to environmental changes (such as alterations in pH and temperature), and also helps to stabilize the hydrated proteins. Such stabilization involves a multitude of noncovalent interactions including hydrophobic interactions, van der Waals' forces, hydrogen bonds, ionic interactions, and other weakly polar bonds as well as the covalent disulfide bonds. Available data are consistent with the conclusion that the structural organization of water

molecules that are adjacent to nonpolar side chains protects the protein.[38] It has been verified by electrostatic measurements in *surimi* that the total amount of bound water increases as the polar amino acids are dispersed more homogeneously in the gel.[39]

The above features throw some light on the mechanistic aspects of stability of myosin and actomyosin in the dispersions. The relative sensitivity of the myofibrillar proteins to variations in pH could be related to their content of ionizable amino acids. Release of carboxylic groups due to acidification induces a net positive charge, which enhances electrostatic repulsions in the protein and leads to changes such as loss of viscosity of the dispersion. Hydration water also protects the proteins against denaturation under the mild acidic conditions. During the gelation process, water or solvent molecules also become entrapped within the matrix enhancing hydration of the protein molecules. In addition, hydrophobic interactions of head and tail portions of the molecules as well as formation of disulfide linkages in the gel could also favor stability of the proteins.[40] A shift in the pH or presence of salts affects the balance of the electrostatic forces causing aggregation and precipitation of the proteins.[40,41] Modulations of these conditions favor formation of different gels, as illustrated in Figure 13.4. Figure 13.5 shows a model of the behavior of myofibrillar proteins in fish mince during their transformation into thermostable dispersions and of the destabilization of the proteins under adverse pH and temperature conditions. The microstructure of intact muscle (Figure 13.5[a]) is disrupted during mincing and washing. Following the removal of soluble components (depicted as dots in the figure) during washing, the structural proteins undergo some conformational changes imbibing water molecules (depicted as circles) into the intermolecular spaces (Figure 13.5[b]). The resultant water homogenate of washed muscle is highly viscous. Heating of the sample to 80°C or above causes random aggregation and precipitation of the proteins (Figure 13.5[c]). Mild acidification causes structural changes that are associated with stronger interactions with water and results in the formation of a less-viscous dispersion (Figure 13.5[d]). However, more studies are needed to explain the properties of fish muscle proteins in acidified water dispersions.

13.2.7 Functional Properties

Most functional properties of proteins in foods are related to their interaction with water.[41,42] Water, that is bound to the proteins, can be in one or more of six basic forms: structural water (which is unavailable for chemical reactions), hydrophobic hydration water, monolayer water, nonfreezable water, capillary water, and hydrodynamic water.[41] Solubilization of proteins, as a result of hydration, helps them to maintain their structural integrity so as to render them functionally active.[41] The salient features of acid-induced fish meat gel dispersions are their high protein solubility, significant thermal stability, and low viscosity. Solubilization of the proteins was not due to hydrolysis since trichloroacetic acid, salts, or adverse pH conditions precipitated the proteins. Nevertheless, the proteins are susceptible to proteolytic enzymes such as pepsin and trypsin.[13,15] Table 13.4 shows comparison
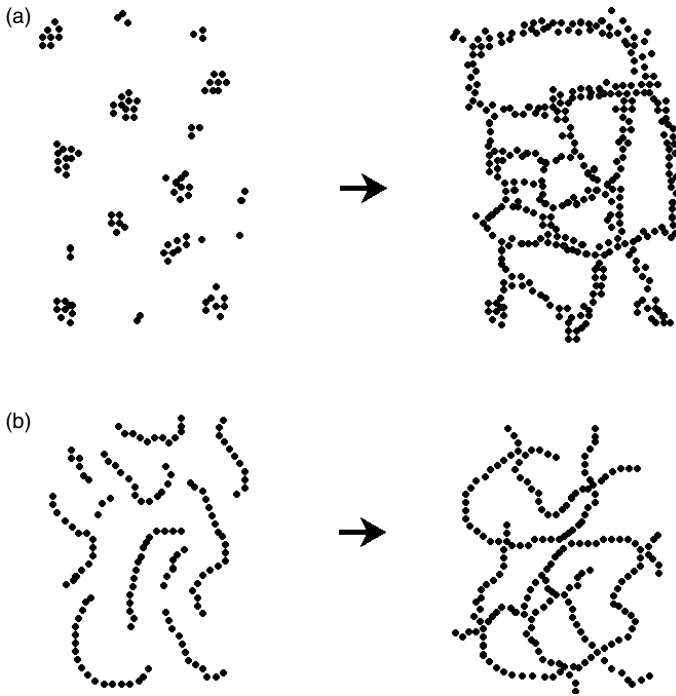
FIGURE 13.4   Schematic representation of gels formed under different conditions. Two types of gel network formed by aggregation of globular proteins. (a) Random aggregation of molecules and (b) aggregation of "string-of-beads" polymers (Reprinted from Oakenfull, D., Pearce, J., and Burley, W., in *Food Proteins and Their Applications*, Damodaran, S. and Paraf, A., Eds. Marcel Dekker, New York, 1997, p. 111. With permission from Marcel Dekker)

of conventional *surimi* gelation with weak acid-induced gelation of fish structural proteins.

13.3   APPLICATIONS OF WEAK ACID-INDUCED GELS

13.3.1   *Surimi*-Type Restructured Product from Shark meat

Recent studies have indicated that the low mild pH-induced gelation of fish proteins could be used in product development. An important possibility is development of restructured shark meat products from the acetic acid induced shark gel.[14] The gel could be suitably molded into desired products. The hardness of the product is dependent upon the moisture content of the gel, which can be varied by changing the proportions of washed shark meat and water in the slurry. Figure 13.6 shows influence of moisture content on hardness of the gel. Generally, an equal proportion of washed shark meat and water gives an acceptable hardness. The process of
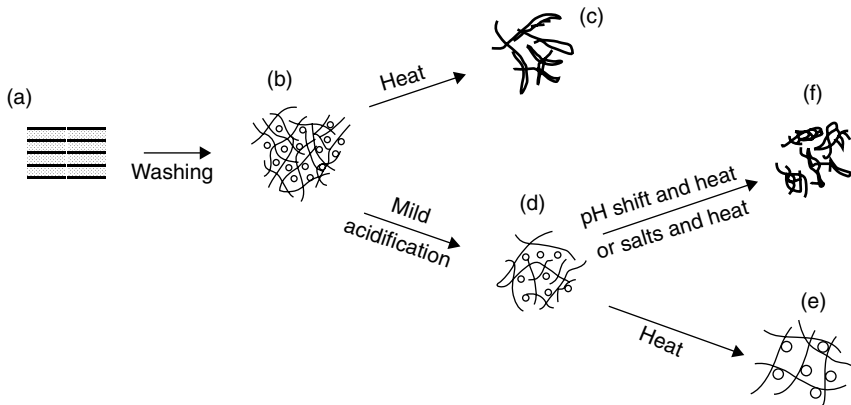
FIGURE 13.5 Tentative model for protein network formation in fish muscle dispersion. (a) Fish mince (insoluble), (b) washed fish mince (soluble and viscous), (c) cooked gel (aggregation and precipitation), (d) pH reduction by acetic acid (soluble, less viscous), (e) pH reduction followed by heating (soluble, least viscous), (f) heating after a pH shift or in the presence of salts (aggregation and precipitation). (. . .), soluble components and (○), water molecules (Reprinted from Venugopal, V., *Trends Food Sci. Technol.*, 14, 39, 1997. With permission from Elsevier)

preparation of restructured shark steaks is depicted in Figure 13.7. The gel is filled in appropriate molds and steamed for 15 min, cooled, aerobically packaged in polyethylene pouches, and stored at refrigerated temperature. The product is stable against microbial growth when stored at 10°C even up to 2 months due to the presence of small amounts of acetic acid. Prior to consumption the product is deacidified and salted to taste by dipping for 20 min in an equal volume of aqueous solution of sodium bicarbonate and common salt at concentrations of 5% each. The salted product can be battered, breaded, shallow fried, and kept frozen. The product can be consumed after heating as any coated seafood product (see Chapter 9). Elasmobranchs contain significant amount of urea. Since the washing process removes urea in addition to other soluble components, the washed meat is free of characteristic odor of shark meat. Consumer studies indicated good acceptability of the product. Alternately, the shark gel cut into desired shapes could be used as paneer (coagulated milk proteins).[14] The process can be extended to other species belonging to elasmobranchs.

13.3.2 Low Sodium *Surimi* from Alaska Pollock

Normal *surimi* processing involves heat-induced gelation of myosin in the presence of salt. The acid-induced gelation technique, which does not require salt, has been recently applied for development of low sodium *surimi* from Alaska pollock.[43] The salt-free *surimi* may have acceptance among consumers who prefer lower salt contents in foods because of health reasons. The acetic acid-induced Alaska pollock *surimi* can have physical characteristics dependent on the concentration

TABLE 13.4

Comparison of Conventional *Surimi* Gelation with Weak Acid-Induced Gelation of Fish Muscle Structural Proteins

Characteristics	Conventional *surimi* gelation	Weak acid-induced gelation
Gelation pH	Neutral or slightly alkaline	Acidic pH in the range of 3.5 to 4.0
Agents for gelation	Mild heat in presence of NaCl	Weak organic acids such as acetic or lactic acid
Chemical changes	Formation of covalent (disulfide) and noncovalent linkages, degradation of myosin heavy chain, decrease in α-helix content, and increase in hydrophobicity of myosin	Formation of covalent (disulfide) and noncovalent linkages, degradation of myosin heavy chain, decrease in α-helix content. Increase in hydrophobicity of myosin?
Water holding capacity	Good	Good
Microbial stability of gel	Poor	Good
Rheological characteristics	Viscoelastic nature	Viscoelastic nature so far reported only in shark and Alaska pollock
Influence of ionic compounds on the gel	Gel characteristics not affected	Adversely affect gel characteristics
Influence of heat on rheological properties	No significant change	Rapid fall in viscosity of gel giving free flowing dispersion. Solubility of proteins in dispersion not affected
Applications	Restructured products, edible films (but not a microbial barrier)	Restructured products, edible antibacterial film, spray dried protein powder, sauce etc.

Source: Reprinted from Venugopal, V., *Fish. Technol. (India)*, 40, 61, 2003. With permission from the Society of Fishery Technologists (India).

of the acid. Gelation of fish meat in the presence of 2–3% acetic acid significantly decreased the shrinkage and total expressible fluid and increased the firmness. In addition, acid-induced gels did not require starch for a firm texture. Rheological studies showed that there was no peak in the storage modulus in the gel in the temperature range of 32–43°C, suggesting that acid-induced gelation did not require setting unlike the conventional *surimi*. Incorporation of KCl and NaCl, however, weakened the acid-induced gel.[43]

Another modified acid process has also been developed for *surimi*, which is somewhat similar to that employed for producing soy protein isolate. In this, adding the acid first solubilizes the fish proteins. The unstable membrane lipid is largely removed after which the proteins are reprecipitated by adjusting the pH. The rigorous leaching process leads to a lighter color, so that the acid-aided process
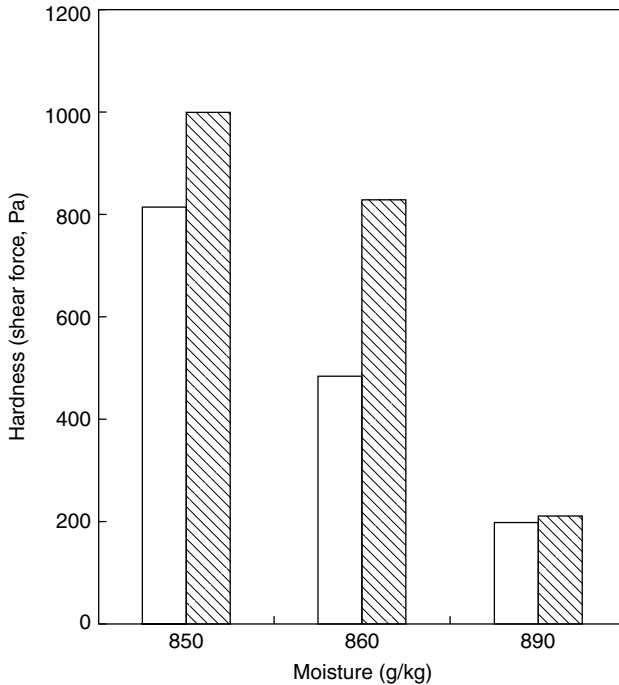
FIGURE 13.6 Influence of moisture content on hardness of acid-induced shark gel (Reprinted from Venugopal, V. et al., *Lebensm. Wiss. U. Technol.*, 35, 185, 2002. With permission from Elsevier)

makes it possible to use even fatty fish muscle for *surimi*. There is also less loss of protein and thus higher product yield.[44,45]

13.3.3 Application as Binder

The acid-induced gel can have potential as binder in food products. It has been used as supplement in patties and sausages, which enabled extended chilled storage for these products because of the antimicrobial properties of the acid-gel. A process for shelf-stable patties using hardhead catfish (*Aris felis*) has been reported recently, which employed acid-induced gel and dispersion as a binder and coating, respectively. In the process, a portion of the mechanically deboned meat mince was converted into acid-gel by the process described above. Some amount of the gel was also used to prepare dispersion having a viscosity of 0.60 Pa. s. The gel was incorporated in patties made from fresh catfish mince in which the meat was replaced by the gel at 10% level. The gel-incorporated patties were also given a coating with the gel dispersion. The process enhanced shelf life of the patties at 1 to 3°C, as compared with normal patties made from the fish. The gel-incorporated dispersion-coated product had a refrigerated shelf life of 3 weeks in comparison with a 2 week shelf life for the untreated patties, since incorporation

Whole shark
↓
Skin, remove bone, cut into small pieces (3–5 g each)
↓
Soak in excess of cold (<10°C) water, 18 h
↓
Drain and repeat washing twice
(hold meat in cold water for 1 h for each washing)
↓
Homogenize washed meat in equal amount of fresh cold water
↓
Lower pH of the slurry to 3.5 by acetic acid
↓
Gel
↓
Fill into suitable molds
↓
Steam for 15 min
↓
Cool, remove the heat-set gel
↓
Store at 0–3°C till use
↓
Salt, coat, and shallow fry

FIGURE 13.7 Process for development of restructured shark product (Reprinted from Venugopal, V. et al., *Lebensm. Wiss. U. Technol.*, 35, 185, 2002, with permission from Elsevier)

of gel together with dispersion coating suppressed microbial growth in the product during storage.[46] The process could be extended for value addition of other fish species also. Another novel use is as binder in aquafeeds to enhance their water stability (Venugopal, unpublished data, 2004).

13.4 APPLICATION OF PROTEIN DISPERSIONS

The free-flowing and thermostable nature of the acid-induced gel dispersion helps its application in a number of ways.

13.4.1 Edible Coating to Extend the Shelf Life of Chilled Fresh Fish

Environmental concerns with respect to waste disposal of synthetic packaging materials have led to interest in biodegradable films and coatings for food products.

Potential and innovative uses of edible films to improve food safety, extension of shelf life, and the possibility of cost reduction of packaging materials have been identified.[47,48] Edible coatings from proteins such as corn zein, wheat gluten, soy proteins, casein, collagen and gelatin, and polysaccharides including alginate, pectin, carrageenan, starch, dextrins, chitosan, and cellulose have been developed.[48,49] Some of the films, however, have limitations in their use. For example, cellulose-based films, besides being soluble in water, have a hydrophilic nature imparting it with poor moisture barrier property. Active packaging is another innovative concept that has been introduced to improve safety and delay of spoilage of foods. Incorporation of approved antibacterial compounds such as nisin in films can suppress growth of *Listeria monocytogenes* in the packaged food. A biofilm from hydroxypropyl methylcellulose cross-linked with citric acid film that contained adsorbed nisin has been reported to control *L. monocytogenes* in packaged foods.[50]

Development of edible coating from fish myofibrillar proteins serves dual functions of using low cost fish as an edible coating for improving the quality of high-value seafood.[51] However, much attention has not been paid in this aspect because of the inherent characteristics of fish structural proteins, such as poor water solubility and susceptibility to denaturation by heat. The thermostable fish meat dispersions have potential for use as edible films and coating to enhance the shelf life of fishery products. Acid-induced gel dispersion from freshwater fish rohu (*Labeo rohita*) meat could be used to enhance the shelf life of the fish fillets during chilled storage. For this, washed rohu meat was converted into gel dispersion, having a pH of 3.5, protein content of 3%, and apparent viscosity of 1 Pa s. Fresh rohu steaks were dipped for 1 h in this dispersion. The treated steaks were packaged in polyethylene pouches and stored under ice. It was observed that the dispersion-coated steaks gave a shelf life of 32 days as compared with 20 days for noncoated steaks stored under the same conditions.[16] The effect was due to acidic nature of the dispersion and presence of the antimicrobial acetic acid.[52] The shelf life of the dispersion-coated steaks could be further enhanced by exposure to gamma radiation at 1 kGy.[16] Although, the acidic nature of the dispersion could cause some bleaching of fish pigments in the fillets during prolonged chilled storage, this could be prevented by incorporating 0.5% butyl hydroxyanisole or ascorbic acid in the dispersion as antioxidant.[16] Comparable results were obtained with steaks of seer fish also. Coating of seer fish steaks with the dispersion prepared from the same fish enhanced the chilled storage life of seer steaks from 15 to 20 days.[53] Incorporation of antibacterial compounds in the protein dispersions could provide additional benefits of controling pathogens in the products, although this has not been examined.

Another use of fish-based edible coatings is to prevent quality loss in frozen fishery products during storage. The major quality losses in frozen fish are moisture loss, lipid oxidation, and discoloration (Chapter 4). Such changes are particularly significant during frozen storage of mince from fatty fish species. Recent experiments have shown that edible coatings from mackerel could be used as coating for blocks of mackerel mince to prevent weight loss and rancidity during prolonged frozen storage. Unglazed mackerel mince blocks lost 35% of its initial weight when
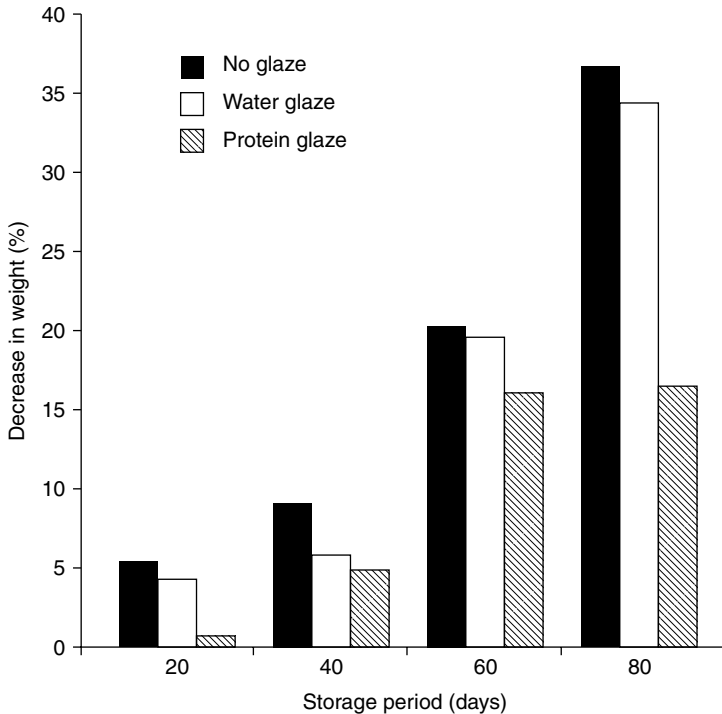
FIGURE 13.8 Influence of glazing of frozen mackerel mince blocks using dispersion prepared from acetic acid-induced mackerel mince gel (Reprinted from Kakatkar, A.S., Sherekar, S.V., and Venugopal, V., *Fish. Technol. (India)*, 41, 29, 2004. With permission from Society of Fishery Technologists [India])

stored at $-18°C$ for 80 days. Conventional water glazing could not completely prevent dehydration loss during frozen storage period. Glazing of the mince blocks with dispersion prepared from the same fish resulted in only 17% loss in weight, as shown in Figure 13.8. Furthermore, about 50% reduction in lipid oxidation was also observed in the dispersion-glazed mackerel mince blocks.

13.4.2 Protein Powder from Gel Dispersions

The poor solubility of fish structural proteins in water and their sensitivity to denaturation are problems in dehydration of aqueous solutions of fish meat to prepare functionally active protein powders. The problem was partially solved in the case of Alaska pollock by milling the fish *surimi* in water containing sorbitol and carbonic acid, the latter helping to reduce the high viscosity of the solution. The powder obtained by spray drying of the solution had 65% protein and as much as 24% carbohydrate. Apart from the sweet taste of the powder, the high content of carbohydrate was an impediment in its wider use as a food ingredient.[54] The possibility

TABLE 13.5
Proximate Composition and Other Characteristics of Spray-Dried Protein Powder from Threadfin Bream

Color	Colorless
Odor	Odorless
Density	0.13
Moisture	$5.4 \pm 0.3\%$
Protein	$93.1 \pm 0.7\%$
Lipid	$1.1 \pm 0.1\%$
Ash	$2.6 \pm 0.2\%$
Protein efficiency ratio	3.52 ± 0.27
Trypsin digestibility	$54.1 \pm 0.2\%$
Pepsin digestibility	$48.4 \pm 0.4\%$
Trypsin + pepsin digestibility	$81.9 \pm 0.6\%$

Source: Adapted from Venugopal, V., *Trends Food Sci. Technol.*, 14, 39, 1997. With permission.

of preparation of free-flowing thermostable water dispersions of fish meat rendered it suitable for spray drying without the use of additives such as carbohydrates. The solubility and stability of proteins in the dispersions at high temperatures were advantageously used for the development of functionally active protein powder by spray drying the dispersions. The resultant powder has more than 90% protein. Making use of this technique, protein powders having superior functional characteristics such as solubility and oil emulsification capacity have been prepared from fish species including capelin, threadfin bream, and shark.[28,55,56] The powder obtained from threadfin bream was colorless and odorless and had a protein efficiency ratio comparable with that of casein.[56] Its oil emulsification capacity and water solubility were 2 to 3 times higher than those of conventional protein powder prepared by drying and grinding fish meat. Table 13.5 shows the proximate composition and properties of spray-dried threadfin bream protein powder. It may be mentioned that apart from spray drying other methods could be examined to make protein powder, exploiting properties of dispersion such as instability of dissolved proteins in the presence of traces of salt.

13.4.3 Fish Sauces Using Gel Dispersions

Fermented fish sauces are considered delicacies in several countries, particularly in Southeast Asia (see Chapter 12). Traditionally sauces are prepared by fermenting whole fish using the enzymes of fish viscera. Fermentation can also be done using microorganisms, growth of which is facilitated by adding nutrients.[57,58] Hygienic and suitably flavored fish sauces could be prepared by fermenting weak acid-induced fish gel dispersions using lactic or propionic acid bacteria. A fish

sauce having a sour taste and negligible fishy odor was prepared by fermentation of shark gel with *Lactobacillus* spp. for 3 days at 30°C. Fermentation was indicated by fall in initial pH and utilization of glucose added in the dispersion. Since the shark gel has a refrigerated shelf life of 2 months, the process can help in the preparation of the sauce as per convenience.[59]

13.5 GELATION UNDER ALKALINE CONDITIONS

Most of the discussions above were on the gelation of fish muscle proteins under mild acidic conditions. Though not studied in detail, gelation of fish proteins has also been observed under alkaline conditions. Washed fish meat treated with ammonium hydroxide has been found to form gel. Gel of this type has been used to prepare edible coatings from fish myofibrillar proteins.[60]

In conclusion, a process to make novel weak acid-induced gel from washed fish muscle structural proteins offers potential for development of novel products from fish meat. The initial step of the process is conversion of the meat into gel by controlled modification of the pH using a weak organic acid. The gel could be used in the development of low sodium, restructured products incorporating suitable additives and as binder in sausages and aquafeeds. Functionally active protein powder, edible coatings, and glazes are some of the products that can be developed from thermostable gel dispersions of fish meat.

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14 Applications of Enzymes in Fish Processing and Quality Control

14.1 INTRODUCTION

Application of biotechnological methods in seafood processing has progressed at a much slower pace, except in the field of aquaculture. Application of biotechnology in the area of farming has resulted in production of transgenic fish species bigger in size with higher fat contents, facilitating economic gains.[1] Enzymological methods constitute a major area of application of biotechnology in food processing.[2–4] Enzymes, because of their appreciable activity at moderate temperatures, have the unique advantage of saving significant energy in food-processing operations. In addition, the specificity of enzymes can provide processes for making tailor-made products under environmental friendly conditions. Apart from their use as food-processing tools, enzymes can also be used in quality-control techniques. This chapter discusses the potential applications of enzymes in fish processing and quality control.

14.2 PROCESS DEVELOPMENTS USING PROTEASES

Proteases are hydrolytic enzymes that catalyze degradation of peptide bonds in protein molecules. Proteases may be endogenous (degrading peptide bonds within the polypeptide chains) or exogenous (degrading terminal amino acids of the chain). Applications of proteases in food processing include textural modification of bread, improvement of quality of dried egg products, tenderization of meat, protein recovery from bones, hydrolysis of blood proteins, soy sauce production, removal of off-flavor from soy milk, acceleration of cheese ripening, and clarification of wine. Nonfood applications include their use in detergents, reduction of tissue inflammation, and dissolution of blood clot, etc.[2,5,6] The application of proteases in the field of seafood processing, is detailed below.

14.2.1 Fish Protein Hydrolyzates

Preparation of fish protein hydrolyzate (FPH) has attracted attention of researchers during the last four decades. FPHs are prepared by treating fish meat with proteases for a few hours at appropriate temperature and pH conditions. The solubilized fraction is concentrated generally by spray drying to get the powdered protein

hydrolyzate. The product has better functional and nutritive properties as compared with whole fish meat or fish protein concentrate. FPHs have higher protein and lower fat and ash contents, as well as high solubility in water. The commercial aspects of production of FPH have been described.[2,5–9]

By using different fish species, enzymes, and digestion conditions, a wide range of hydrolyzates can be developed. The enzymes can be of plant (papain, ficin, etc.), animal (trypsin, pancreatin, etc.), or microbial (pronase, alcalase, etc.) origin. By careful control of hydrolysis, it is possible to suitably modify the functional properties such as water holding capacity, emulsification capacity, and foaming ability. Uncontrolled or prolonged proteolysis results in the formation of smaller and highly soluble peptides completely lacking these functional properties. The degree of hydrolysis is defined as the percentage of h/h_{tot}, where h is the number of free amino groups at the end of the reaction and h_{tot} is the total number of peptide bonds in the original protein.[7] This value is important to optimize the process parameters in order to get a product having ideal functional properties.

Twenty-three proteolytic enzymes have been screened for their use in hydrolysis of fish proteins.[10] It was observed that pancreatin, papain, and pepsin are suitable for the process. Studies on digestion of proteins using papain revealed that at pH 7.0 maximum solubilization occurred in the first few hours of treatment.[11] A temperature of 40°C instead of 65°C was advocated for obtaining longer peptides during proteolytic digestion. Fish hydrolyzates from fatty fish species give products containing significant amounts of lipids. The powder from lean fish, generally has 92% protein, 1.7% lipid, and 6.4% ash.[8,9] A process to make FPH from a low-cost fish, dhoma, consisted of treatment of mechanically deboned fish mince with papain at 55°C for 2 h. The powder obtained after spray drying of the hydrolyzate had a creamy color and 90% protein content. Polyphosphate improved the functional properties of the powder.[12] Protein hydrolyzates are generally bitter due to the formation of certain peptides during hydrolysis. It is important that the hydrolyzates are made less bitter in order to make them acceptable to the consumer. The debittering of hydrolyzates is achieved by plastein reaction, which is a reverse of the hydrolytic process. In this, a high concentration of the hydrolyzed proteins (up to 60% w/v) in water is treated with a protease. The removal of bitterness was originally thought to be due to the formation of a high molecular weight product through the condensation by enzymatic catalysis. However, it has now been shown that the product is formed by physical aggregation.[13]

Protein hydrolyzate from fresh and frozen shrimp using chymotrypsin or trypsin resulted in products with high levels of glycine, proline, arginine, and valine.[14] Optimum conditions for the process were 0.25–0.3% of enzyme concentration and 2.5–3 h of incubation period at 35–40°C. A number of microbial enzymes have been found to be superior to plant enzymes in their ability to cause solubilization of proteins from fish such as mullet.[15] Alkaline protease from *Bacillus subtilis* was more efficient than two neutral proteases from the same bacterium, giving more than 80% nitrogen solubilization. Treatment with microbial proteases for 50°C for 2 h with either neutrase or alcalase has been successfully employed to prepare FPH from Antarctic krill.[16] Microbial cells such as

those of *Bacillus megaterium* containing significant extracellular protease activity immobilized on calcium alginate, have been used to prepare FPH.[17]

Protein hydrolyzates are generally used for the modification of functional properties of foods and in dietetic foods as a source of small peptides and amino acids. As a result of high solubility and amino acid balance, FPH has obvious advantages over the traditional fish-protein concentrate. Its high dispersibility makes it suitable as a milk substitute for calves and as a protein supplement to cereal foods.[5,16] Fish-protein hydrolyzate has been successfully used as a nutrient in starter diets for Atlantic salmon (*Salmo salar*) fry, enhancing the growth rate and final weight of the fish.[18]

14.2.2   Protein Recovery from Fish Processing Waste

Filleting wastes are sources of high-quality proteins. Currently, wastes from filleting operations are discarded because of the difficulty in the extraction of the residual meat. Treatment with protease under mild treatment conditions extending for a few hours can result in the recovery of the proteins from fish-frame or shrimp-shell waste.[19] Cod frame treated with crude proteinase from tuna pyloric caeca for a period of 12 h at 50°C resulted in 80% of the protein.[19] Head and shell comprise approximately 60% of the total weight of shrimp. Chitin and its derivatives present in shrimp shell hold economic value because of their varied agrochemical applications. Recovery of chitin from these wastes necessitates removal of adhering proteinous matter. Traditionally, preparation of chitin involves demineralization and deproteinization with the use of strong acids or bases. However, the use of these chemicals may cause a partial deacetylation of chitin and hydrolysis of the polymer, resulting in a final product with lower molecular weight and inconsistent physiological properties. Use of proteases can eliminate these problems. Microbial protease from *Pseudomonas aeruginosa* can be employed to recover proteins up to 61% from shrimp and crab wastes through fermentation of the shellfish slurries. Solid-state fermentation of the shell wastes can enhance protein recovery up to 81%.[20]

14.2.3   Scaling of Fish

Currently, scaling of different fish species is done by manual or mechanical scrubbing and scrapping methods. The process is rather tedious particularly in the case of fish having large amounts of scales. Scaling of species like ocean perch, haddock, as well as many freshwater species, by mechanical means is harsh and results in tearing of the skin, incomplete removal of scales, partial damage of the skin associated with loss of color and shiny appearance and damage of muscle texture, and lower fillet yield. A need for scale-free fish fillets of species such as salmon, perch, ocean bream, and silver carp has resulted in the development of a new enzymatic method for the purpose. The process consists denaturation and loosening of the mucous layer and outer protein structures of the skin, enzymatic degradation of the outer skin structures, and washing off the scales by water jets. The fish is incubated

in a specially designed incubation tank filled with slightly acidified water solution of the enzyme, collagenase. After the treatment, the fish is lifted out of the incubation solution and washed well. The Norwegian technique can skin haddock at a rate of 1.3 t/h and has potential for extension to a number of other fish species including shark, rays, and skates.[21–23] In Iceland, experiments have been carried out on enzymatic skinning of skate wings. The skin is first denatured by warm water and subsequently treated with an enzyme mixture containing proteolytic and glycolytic enzymes for 4 h at 25°C.[23] Collagenase from crab hepatopancreas has been used for skinning of squid, production of caviar, and ripening of salted fish.[24]

14.2.4 Peeling and Deveining of Shrimp and Shucking Clams

A process for loosening the shell of shrimp and removal of visceral mass in clam by using a mixture of carbohydrases and cellulases derived from *Aspergillus niger* has been reported. In the process, 7.6 g ficin, 15.2 g amylase, and 7 g sodium bicarbonate were added to 2-gallon water. Thawed headless unpeeled shrimp weighing 2.5 kg were immersed in the solution maintained at pH 7.8 and 52°C. The water was circulated with a vacuum pump attached to the system to remove air from the shell. After 30 min, vacuum was broken and the shrimp removed from the enzyme solution. Jets of water and air were blown though the shellfish held on a wire mesh to remove the shell and the vein. The process has been successfully extended for shucking clam.[25,26]

14.2.5 Ripening of Salted Fish

Ripening is development of a characteristic flavor and soft texture in salted whole or gutted fish during storage. Ripening of salted herring involves action of endogenous proteases that hydrolyze the fish proteins causing an increase in soluble compounds such as peptides and amino acids, with associated changes in the tissue texture.[27] The ripening time, which normally extends up to a year in heavily salted herring, can be reduced by incorporation of exogenous proteases.[28] Curd formation in canned salmon can also be avoided by protease treatment.[29]

14.2.6 Production of Fish Sauce

In several Far East Asian countries, sauce is made by allowing fish to autolyze in the presence of high concentration (>25%) of salt, which takes about one to two years. The endogenous trypsin-like enzymes present in the muscle are responsible for this, since bacterial action is inhibited by high salt content. After the process, the clear liquid is decanted or filtered, which contains a high level of soluble nitrogenous compounds.[30,31] The product can be made within a shorter period of two months by the action of externally added pepsin under controlled conditions, such as a pH of 4.0 and lower salt concentration.[30,32,33] Capelin is one of the low-cost fish that has been examined for sauce production and is abundantly available in the

Atlantic Ocean. Addition of proteolytic enzymes such as fungal protease, pronase, trypsin, chymotrypsin, squid protease, or squid hepatopancrease to minced and salted capelin increased the rate of protein solubilization during the first month of fermentation.[34] Fermentation of squid meat with added proteolytic enzymes has also been reported.[35]

14.2.7 Roe Production

'Caviar' is riddled and cured roe (eggs) separated from the roe sack (ovary). Caviar production is a somewhat laborious process and is carried out either manually or mechanically. Proteases, including fish pepsins, have been used to hydrolyze the supportive tissue that envelops salmon and trout roe and roe sacks. Treatment of the roe for a few min at ambient temperature and subsequent washing in salt solution, result in a purified product with a yield of at least 80%.[36,37]

14.2.8 Production of Seafood Flavorings

Seafood flavors are in high demand for use in products such as artificial crab meat and fish sausage. Shrimp flavor is used as an additive in *surimi*-based products and cereal-based extrusion products. Proteolytic enzymes including those from bacteria can aid in the extraction of flavor compounds from shells and other materials, which are then concentrated up to 50 to 60% dry matter.[38] Recently, hydrolyzate from red hake (*Urophycis chuss*) mince and frame was prepared as a flavoring agent by treating the raw material with commercial proteases for 6 h. Addition of small amounts of sodium chloride and sodium tripolyphosphte improved the flavor of the hydrolyzate. The umami taste of the product was due to high content of glutamic acid.[39]

14.2.9 Tenderization of Squid

Many consumers express a negative opinion on the eating quality of squid due to its characteristic and rather tough texture. Squid meat can be tenderized by treatment with commercial bromelin or a crude lysosomal extract from bovine spleen. The squid rings obtained by transversally cutting the mantles are treated with one of the enzymes at a pH of 7.0 and temperature of 37°C for 30 min. The tenderization could be observed by textural changes and also by sensory evaluation.[40] Removal of squid skin required a pretreatment in 5% salt solution at 45°C for 10 min to soften the tough outer proteoglycans layer. At low temperature (3°C), a combination of 5% salt and an enzyme preparation from papaya latex can be used.[28]

14.2.10 Tenderized, Dehydrated Steaks from Freshwater Fish Meat

Dehydrated steaks of freshwater fish, rohu (*Labeo rohita*) were exceptionally tough and unacceptable to the consumers. A process has been developed recently for enzyme-tenderized, dehydrated steaks of rohu. The steaks from fresh rohu were

treated in an equal amount of 0.5% (w/v) papain for 1 h. The tenderized steaks were salted for 1 h by brining in an aqueous 10% solution of sodium chloride. The salted steaks were dried either in a solar or tunnel dryer at a maximum temperature of 60°C. Solar drying required longer drying time because of variations in the temperatures. The products prepared by both drying methods had a water activity of 0.66 and a proximate composition of 15–16% moisture, 59–60% protein, and 5.7% crude fat. Tenderization reduced hardness of the dried product and also enhanced its rehydration capacity. Deep fat frying of the dehydrated steaks gave a crispy product. Tenderization followed by solar drying was suggested as an economical process for value addition of the freshwater fish.[41]

14.2.11 Isolation of Pigments

Shellfish contain significant amounts of carotenoids bound to their exoskeleton. An enzymatic method has been developed to recover the carotenoids along with the proteins from shrimp and crab-shell waste. About 80% of the protein and 90% of the astaxanthin pigment from shrimp waste can be recovered as an aqueous dispersion after trypsin hydrolysis.[42] The extracted pigment has been found to be good to enhance the color of cultured fish species such as salmon.

14.2.12 Reduction in the Viscosity of Stickwater

Stickwater is a by-product obtained during production of fishmeal. It is high in protein and has excellent nutritional value. It can be sold as a feed as condensed or dried fish solubles. The protein content of stickwater presents a problem during evaporation. As the dissolved solids increase beyond 25%, viscosity increases causing inefficient water removal and leading to clogging of the evaporator. The problem could be solved by protease treatment. Commercial proteases such as alcalase or neutrase could be added to the stickwater at neutral pH at a temperature of 50°C, which significantly reduces viscosity enabling further evaporative concentration.[43] Table 14.1 summarizes the various applications of proteases for fish processing.

14.3 PRODUCTION OF POLYUNSATURATED FATTY ACIDS USING LIPASES

Long chain polyunsaturated fatty acids (PUFA), particularly, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are commonly found in marine animals and phytoplankton. Both EPA and DHA have therapeutic significance since their intake is associated with reduced risk of coronary heart diseases, tumor growth, thrombosis, and high blood pressure.[44] EPA contains 20 carbon atoms with five double bonds ($C_{20:5}$), and DHA contains 22 carbon atoms with six double bonds ($C_{22:6}$). The double bonds are arranged with the last one located on the third carbon atom from the end of the chain, that is, the methyl terminal, and hence, are referred

TABLE 14.1
Applications of Proteases for Fish Processing

Preparation of hydrolyzates from fish and shellfish
Debittering of hydrolyzate
Scaling of fish
Peeling and deveining of shrimp
Removal of clam viscera
Recovery of protein from filleting waste
Removal of protein from shellfish waste
Isolation of pigment from shellfish waste
Ripening of salted fish
Acceleration of fish sauce production
Roe production from fish
Membrane removal from cod liver
Seafood flavorings
Tenderization of squid
Tenderization of fish meat
Viscosity reduction of fish meal stickwater during drying
Prevention of curd formation in canned salmon

to as omega-3 fatty acids. Fish oils generally contain 20% PUFA. For instance, cod liver oil, a well-known food supplement, is a mixture of triglycerides containing more than fifty different fatty acids and has a content of 22 to 24% of PUFA (see Chapter 15).

Interests in requirements of EPA and DHA as nutritional supplements in foods for adults, infants, and patients have promoted research seeking novel lipid sources, and the development of chemical and physical modification methods for enrichment of fats and oils to meet these needs. Such efforts include production of structured lipids containing medium-chain and long-chain PUFA using processes such as molecular and fractional distillation, solvent and supercritical extraction, and chemical esterification. Other strategies are production of PUFA by microorganisms, generic modification of oilseed crops, modification of fats and oils involving blending, fractionation, and esterification by chemical and enzymatic techniques.[45–47] Inter-esterification based on exchanging the fatty acid components of a triglyceride or a mixture of triglycerides with either free fatty acids (acidolysis), or fatty acids of other triglycerides or monoesters (transesterification) have been used successfully. These may be chemical or enzyme-catalyzed reactions.

Long-chain fatty acids, being highly labile, procedures that involve extreme pH and high temperatures may partially destroy the natural all-*cis* configuration of PUFAs by oxidation or by *cis-trans* isomerization. These methods also make fish oils very heat sensitive and far more prone to oxidation than most other oil

types. Enzymatic treatment being mild has advantages over conventional chemical methods. Enzymatic fat modification is performed using lipases, mostly derived from microbial sources.[48] Lipases catalyze the hydrolysis of triglycerides, diglycerides, and monoglycerides in the presence of excess of water, however, under water-limiting conditions, ester synthesis can be achieved. Lipase-catalyzed acidolysis has been used to incorporate EPA and DHA into vegetable and fish oils to improve their nutritional properties. Fatty acid specificity of lipases is a crucial factor when considering the application of enzymes in processing marine oils.

A process has been developed for increasing the content of EPA and DHA esterified to triglycerides in fish oils to a level as high as 60%.[47,49] A 1,3-specific lipase from *Pseudomonas* spp. has been used to enrich the total EPA and DHA concentration in sardine oil from 29 to 44.5%.[50] The technique has also been used to enrich the PUFA content of tuna. A lipase from *Candida cylindracea* was the most effective in increasing the DHA content up to three times than that in the nonhydrolyzed fraction of the oil. Phospholipase A_2 obtained from porcine pancreatic gland selectively hydrolyzed phospholipid from cod roe at 20°C at pH 8.5 in the presence of calcium chloride and sodium deoxycholate within a period of 15 min.[51] The product was composed of 95% free fatty acids with EPA and DHA at 24 and 40%, respectively. This technique represents a process that could be used to produce PUFA concentrates from fish industry waste material such fish viscera.[47] A bacterium isolated from mackerel intestines has been found useful to concentrate EPA.[52] A dynamic model for the hydrolysis of triglycerides by lipase from *Candida rugosa* has been developed.[53] The enzyme immobilized on a hollow fiber membrane was highly stable. Menhaden oil was immobilized in a hollow-fiber reactor containing *Candida cylindracea* lipase. Using shell and tube reactors, saturated and monounsaturated fatty acid residues of the oil was selectively hydrolyzed at pH 7.0 and temperature 40°C. Lipase-assisted hydrolysis of menhaden and seal blubber oil has been carried out. A lipase from *C. cylindracea* increased EPA and DHA content in the oils. The increase of total PUFA in menhaden oil was 15%, the increase being essentially due to an increase of DHA from 10.1 to 25.6%. However, the content of EPA did not change significantly after 75 h of hydrolysis.[46]

14.4 TEXTURE MODIFICATIONS USING TRANSGLUTAMINASE

Texture of processed fishery products has a significant role in their consumer acceptance. While food additives such as starch and other carbohydrates have been used for modification of texture, the potential of transglutaminase (TGase) in textural improvement has been recognized recently. TGase (protein-glutamyl transferase, EC 2.3.2.13) catalyzes acyl-transfer reactions in which the γ-carboxamide group of peptide-bound glutamine serves as the acyl donor. When the ε-amino group of peptide-bound lysine acts as the acceptor, the ε-(γ-glutamyl)-lysine bond is formed between the proteins, resulting in cross-linking of proteins, as depicted in Figure 14.1. The enzyme is present in microorganisms, crustaceans, and finfish species including those from temperate waters.[54,56–58] TGase activity has been detected in the muscle of sardine, mackerel, red sea bream, carp, silver eel, white
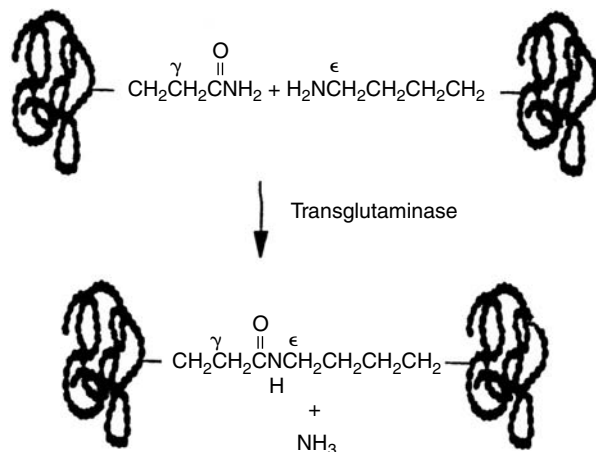
FIGURE 14.1 *Surimi* gelation and proteolysis of myofibrillar proteins (Reprinted from An et al., *Trends Food Sci. Technol.*, 7, 321, 1996. With permission from Elsevier)

croaker, walleye pollock, chum salmon, and rainbow trout as well as *surimi* from these species.[59]

The setting phenomenon of *surimi*, important in the development of seafood analogs, is as a result of cross-linking of the myofibrillar proteins, particularly myosin (see Chapter 8). The process requires some amount of salt to dissolve the proteins and thereby facilitate setting. The setting of *surimi* can be also induced by the addition of exogenous TGases in the absence of salt at 4 to 40°C.[59–61] Extent of TGase-mediated cross-linking reactions in actomyosin, however, depends upon the structural details of the protein, which varied among fish species and can occur even at low temperatures.[54–56,62,63] In view of the recent interests in low-sodium foods, the TGase-induced setting has been suggested as a method for salt-free restructured products from seafood such as Alaska pollock and sardine.[58] The gel strength, cross linking of myosin heavy chain and ε-(γ-glutamyl)-lysine content of Alaska pollock *surimi* markedly increased with extended setting time. Some of the other products examined include squid, mackerel, and big eye snapper.[64–66] Trypsin treatment can improve freeze-texturization of minced bream treated with TGase.[67]

14.4.1 Biodegradable Films

In view of environmental pollution associated with synthetic packaging materials, the prospects for biodegradable films are particularly bright in the food industry. Macromolecules such as alginate, pectin, carrageenan, starch, dextrins, and cellulose have shown potential for the development of edible protective films and coatings for packaging food products (see also Chapter 13). A number of proteins, both of plant and animal origin, have received attention for production

TABLE 14.2

**Some Potential Uses of Transglutaminases
in Fish Processing**

Surimi and *surimi*-based fish analogues
Fish meat paste formulations
Binding of ingredients such as soy bean,
  milk proteins, egg white, etc. with fish meat
Fish meat sheet formation
Raw and processed fish egg products
Modification of texture
Shark fin processing
Reduction of drip in thawed frozen fish
Freeze-texturization of fishery products

of edible films and coatings. Such edible films and coatings generally have excellent aroma retention and superior oxygen-barrier properties.[68] Biodegradable films from fish meat have been prepared.[69,70] The film prepared from Atlantic sardine had a water-vapor permeability of 3.91 mol, tensile strength of 17.1 MPa, and elongation of 22.7%, while the corresponding values for low-density polyethylene film were 0.0482, 12.9, and 500, respectively.[69] The cross-linking proteins for the development of edible films can be induced by TGases.[61] The applications of TGase in fish processing are summarized in Table 14.2.

14.5 EXTENSION OF SHELF LIFE AND QUALITY IMPROVEMENT

Enzymes may have applications in extension of shelf life of fishery products. Glucose oxidase, produced by molds such as *Penicillium notatum* and *Aspergillus niger*, has been shown to control spoilage of fishery products. The preservative action of the enzyme is due to the generation of gluconic acid, which lowers the surface pH, thereby retarding bacterial spoilage.[71] Furthermore, the hydrogen peroxide formed by the reduction of generated molecular oxygen also has antimicrobial activity.[72] Catalase or superoxide dismutase may be added to decompose the peroxide. Alternatively, glucose oxidase may be incorporated in ice or immobilized on an algin coating.[73] It is necessary to use a minimum quantity of glucose to avoid excessive formation of gluconic acid, which might precipitate fish proteins.[71] Glucose oxidase/catalase system for preservation of whole winter flounder and its fillets has been demonstrated. The shelf life was extended to 21 days against 15 days for untreated control samples, as observed by sensory and chemical parameters.[72] The enzyme treatment is beneficial for onboard extension of the shelf life of chilled shrimp.[74]

Lysozyme, which is found in almost all human and animal cells, is known to have antibacterial properties. The preservative effect of the enzyme is due to its action on the mucopeptide structure of bacterial cell walls. The enzyme could

be used for seafood preservation. An enzyme at concentration up to 150 μg/ml retarded the growth of microorganisms isolated from shrimp.[75] Lysozyme from Arctic scallop has been studied for antibacterial activity. At 4°C the enzyme exhibited about 55% of its activity at 37°C suggesting its potency at lower temperatures.[76] Lysozyme from clam shell (*Chlamys islandica*) has been examined as a bacteriostatic agent in specialty food and feed items.[24]

Off-odor including rancid odors and fishy taste pose problems in handling fishery products. Oxidative deteriorative reactions are a major reason for quality loss of flesh foods. The problem can be alleviated using glutathione peroxidase to prevent such deterioration processes in fish muscle during handling, storage, and processing. Other enzymes that may be of use to control endogenous enzyme activity in seafood include superoxide dismutase, protocatechuate-3,4-dioxygenase, and *o*-methyltransferase.[77] It is known that by treating fish portions with enzymes derived from *Aspergillus*, much of the off-odor could be eliminated.[78] Meat of elasmobranchs contains significant amounts of urea, which adversely influences the consumer acceptance of these products. Application of urease is useful for the removal of urea from their flesh. The enzyme has been isolated from *Cajanus cajan* seeds.[79] Alternately, the meat can be treated with soy flour, which is rich in urease.

Precooked frozen shrimp has a characteristic pink color due to their content of carotenoids. This color is easily oxidized to yellow, which can be prevented by giving the cooked shrimp a dip treatment in glucose oxidase. The enzyme may also be included in the glazing solution prior to freezing of shrimp. The packaged shrimp is held for 30 min at 6 to 10°C for the enzyme to function, before freezing at −35°C. The treatment is also good for color retention of cooked crab meat.[72] Glucose oxidase may also limit the availability of oxygen for oxidation and discoloration reactions catalyzed by enzymes like lipoxygenase and polyphenol oxidase.[73]

14.6 ENZYMES IN QUALITY EVALUATION OF FISHERY PRODUCTS

The quality of fish varies with species, sex, age, habitats, and type and level of microbial contamination. The current aquaculture practices lead to significant differences in the quality of farmed fish. Fishery products, whether marine, estuarine, or freshwater habitats, often function as carriers of health hazards such as disease-causing microorganisms, in addition to parasites, natural toxins, heavy metals, and other pollutants (see Chapter 2). A number of applications of enzymes for quality evaluation of fishery products have been recognized.[80] Many enzymes are also integral components of biosensors, which are finding importance as tools for the rapid evaluation of food quality. Uses of biosensors promise to revolutionize approaches to many analytical problems.

14.6.1 Direct Enzymatic Methods for Fresh Fish Quality

The *K*-value, which is a measure of concentrations of nucleotides present, at a given time, is a quality index for raw or chilled fish (see Chapter 2). A method

has been developed for determination of K-value of grass shrimp using a combination of three enzymes, namely, 5′-nucleotidase, nucleoside phosphorylase, and xanthine oxidase.[81] A simple colorimetric technique for the measurement of inosine monophosphate and hypoxanthine contents makes use of xanthine oxidase immobilized on a test strip and soaked in resazurin. When the enzyme test strip is moistened with fish extract and allowed to react for 5 min at room temperature, the color changes from blue to pink, the intensity of which can be correlated with a laboratory colorimeter.[3,82]

Histamine, responsible for scromboid poisoning, is formed though microbial decarboxylation of histidine (Chapter 2). A simple and quick method for histamine has also been developed.[83] The method is based on the formation of hydrogen peroxide by the action of a diamine oxidase on histamine. The addition of a second enzyme, a peroxidase, in the presence of hydrogen peroxide and a chromogen (leuco crystal violet) in a colorless reduced form facilitates its oxidation into crystal violet, which is measured colorimetrically. This method is rapid and suitable as a screening method for routine analysis.[84] An enzymatic method for measuring trimethylamine (TMA) levels in a perchloric acid extract of fish flesh has been reported.[85] This method uses a microbial TMA dehydrogenase which oxidises TMA accompanied with reduction of phenazine methosulfate (PMS), the latter converting p-iodonitrotetrazolium violet to red formazan, which is measured colorimetrically. The technique correlates well with the conventional picric-acid method. Ammonia has a potential as an objective-quality indicator for fish and shellfish, which can be determined enzymatically using glutamate dehydrogenase, reduced nicotinamide adenine dinucleotide (NADH), and α-ketoglutarate. The reduction of ammonia yields equimolar concentration of glutamic acid and NAD, which can be monitored spectrophotometrically.[86] Ethanol is derived from carbohydrates via anaerobic fermentation (glycolysis) and bacterial deamination and decarboxylation of amino acids such as alanine. The alcohol in fish can be determined enzymatically using alcohol dehydrogenase.[86] Table 14.3 gives some direct enzymatic assays of quality of fishery products.

14.6.2 Detection of Frozen Fishery Products

Fresh seafood usually sells at a higher price than frozen seafood and there is a need for analytical methods to differentiate frozen and thawed fish from their fresh counterparts. An enzymatic method to differentiate between frozen and fresh prawn has been reported. The method makes use of changes in β-hydroxyacyl-CoA-dehydrogenase (HADH) activity in the shellfish muscle due to freezing. The HADH activity is significantly higher in frozen-thawed prawns than in unfrozen samples.[87] Frozen-thawed trout tissue has shown a ten-fold increase in this enzyme activity when compared with unfrozen trout. Another method uses the difference in the levels of lysosomal enzymes, α-glucosidase, and β-N-acetyl glucosaminidase in the press juices and extracts from fresh and frozen fish. Freezing and thawing resulted in increased activities by several fold of the enzymes in the press juice of cod, saithe, red fish, and haddock.[88]

TABLE 14.3
Direct Enzymatic Assays of Quality of Fishery Products

Metabolite	Enzyme used	Product/remark
Hypoxanthine and inosine	Xanthine oxidase	Immobilized on test strip
Purine	5′-nucleotidase, nucleoside phosphorylase, xanthine oxidase	Grass shrimp
Ammonia	Glutamate dehydrogenase	Squid
Histamine	Diamine oxidase, Peroxidase	Scombroid fish
TMA	TMA dehydrogenase	Various fish
TMA	Enzymes from immobilized bacterial cells	Various fish
Ethanol	Alcohol dehydrogenase	Tuna, salmon, red fish, pollock, flounder, and cod
Urea	Urease	Shark, Ray

Source: Adapted from Lou, S., *J. Food Sci.*, 63, 442, 1998; Ehira, S., Saito, K., and Uchyama, H., *Bull. Tokai Reg. Fish. Lab.*, 120, 73, 1986; Huss, H.H., Quality and quality changes in fresh fish. Fisheries Technical Paper No. 348, Food and Agricultural Organization of the United Nations, Rome, Italy, 1995; and Sheppard, Jr., N.F., Means, D.J., and Ginseppi, E.A., *Biosens. Bioelectron.*, 11, 967, 1996.

14.6.3   Identification of Fish Species

Enzyme-linked immunosorbent assays (ELISA) are simple, rapid techniques that have been developed for identification of fish species in food products. An ELISA method to differentiate between the smoked meat of salmon, trout, and bream has been reported.[89] The method uses polyclonal antibodies that were raised in rabbits against soluble muscle proteins from the smoked fish species. Recognition of fish protein-bound antibodies is made with goat antirabbit immunoglobulins conjugated to horseradish peroxidase. The method allows clear, visual species identification.

14.6.4   Enzymes as Components of Biosensors

Biosensors are analytical devices composed of a biological recognition element such as an enzyme, antibody, receptor, or microbe coupled to a chemical or physical transducer. The biological component is normally immobilized at or near the transducer surface in order to maximize the response. The transducer converts the particular biochemical event into an electrical signal, providing a biochemically specific detector that can be easily interfaced to a computer or automated system. There are four major types of transducers: electrochemical (electrodes), mass (piezoelectric crystals or surface acoustic wave devices), optical (optrodes),
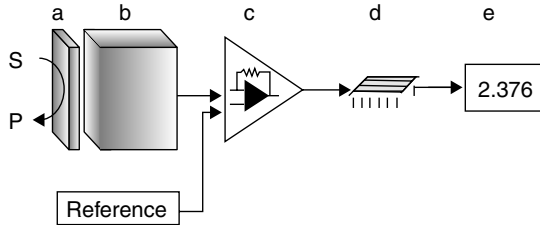
FIGURE 14.2 Schematic diagram showing the main components of a Biosensor. (a) Biocatalyst, (b) transducer, (c) amplifier, (d) processor, and (e) display unit (Reprinted from Bose, S., Khare, R.A., and Fegade, S.L., *Indian Food Ind.*, 23, 51, 2004. With permission from Association of Food Scientists and Technologists (India), Mysore)

and thermal (thermistors or heat-sensitive sensors). Of these, electrochemical detectors are the most commonly used. Many enzymes and cells consume and produce charged species, which can be monitored by potentiometric, amperometric, or conductometric techniques. These devices can be broadly divided into two groups, namely, enzyme sensors for food component analysis and immunosensors for detection of pathogenic bacteria. Biosensors use the specificity and sensitivity of biological systems and the computing capabilities of the microprocessor. They have several applications in food industries, which include proximate analysis, nutritional labeling, determination of pesticide residues, detection of processing changes, microbial contamination, enzymatic inactivation, and biological oxygen demand of wastes.[90] The details of construction of biosensors are described by several authors.[91–93] Enzymes remain, by far, the most commonly employed element in biosensors.[93] The characteristic properties of enzymes such as activity at moderate temperatures and substrate specificity make them ideal components of biosensors. In situations where enzymes have limitations such as nonavailability, requirement of cofactors, instability, etc., microbial, plant, or animal cells may be used. Figure 14.2 illustrates the schematic diagram showing the main components of a Biosensor.

Biosensors have been used to measure nucleotide concentrations to evaluate fish freshness.[94,95] An enzyme sensor specific for hypoxanthine (Hx) in fish was developed using an immobilized xanthine oxidase membrane and an oxygen probe. The enzyme was immobilized on a membrane prepared from cellulose triacetate, 1,8-diamino-4-amino methyl octane, and glutaraldehyde. Hx is oxidized to uric acid and hydrogen peroxide by the immobilized enzyme, the output and current of the oxygen probe decreasing due to oxygen consumption. A linear relationship was obtained between decrease in current and Hx concentration in the range of 0.06 to 1.5 m*M*. The sensor could be used for more than 100 assays and had a storage life of 30 days at 5°C.[94]

Another device was developed that used a xanthine oxidase electrode.[96] The enzyme was immobilized on a silk membrane, onto which a platinum disc and copper wire were attached. This device was highly sensitive to Hx levels in fish and therefore could be used to assess fish freshness. Another method using

an ammonia ion-selective electrode to measure volatile bases in fish has been proposed.[97] The probe could also be used to measure TMA. A polyamine biosensor has been developed using immobilized putrescine. The enzyme oxidized the putrescine and also cadaverine, spermidine, agmatine, and tyramine to produce the respective aldehyde. The consumption of oxygen during the oxidation process was measured by an oxygen electrode, which showed a linear response between putrescine oxidation and oxygen consumption within a range of 0.03 to 3 μM of the amine. The sensor when used in fish extracts responded linearly with storage time.[98] In another method, purified diamine oxidase from porcine kidney was immobilized onto a nylon membrane that was attached to an amperometric electrode. The biosensor was linear up to 6 mM with a limit of 25 μM for histamine, putrescine, and cadavarine. The enzyme membrane was stable for 2 months at 5°C and can be used for 60 assays.[99]

Formation of octopine from arginine and pyruvic acid is one of the major biochemical processes occurring in postmortem scallop muscle. About 1% (w/w) octopine is accumulated during storage of scallop in ice for 5 days. An octopine sensor for quality assessment of scallop freshness has been developed based on the immobilized enzymes octopine dehydrogenase and pyruvate oxidase. During the reaction, octopine is oxidized to pyruvic acid by the dehydrogenase enzyme, and the pyruvic acid is further oxidized by pyruvate oxidase to acetyl phosphate.[100] A good correlation was obtained between the octopine content in scallop adductor muscle as determined by the sensor and an HPLC method.[100] Biosensors containing urease can be used for determination of urea in the flesh of elasmobranches.[79,101]

Immunobiosensors have been developed for detection of pathogens including *Salmonella* spp., *Vibrio* spp., *Escherichia coli*, and other organisms using antibodies raised against these organisms, which are used as components of biosensors.[92] The use of antigens in combination with a transducer such as piezoelectric crystal detector offers an ability to develop group-specific immunosensors for the detection of an entire family of bacteria and also insecticides. The technique does not require the use of a label as in the case of radio-immunoassay techniques. A highly sensitive technique for the determination of mercury, silver, and copper at parts per billion levels based on the inhibition of urease is also available.[102] Detection of antibiotics in cultured fish is very important. Aquaculture operations, whether in freshwater, estuarine, or marine environments, requires control of salinity, biological oxygen demand (BOD), acidity, phytoplankton levels, water temperature, and feed quality. These parameters can be measured using biosensors. Conventionally, BOD tests take 5 days to complete and therefore are unsuitable for direct process control. Several biosensors have been developed for BOD measurements using microbial cells such as *Psuedomonas* spp. and *Bacillus* spp. as components of biosensors.[90] DNA hybridization is another rapid technique that offers the specificity and sensitivity required for detection of microorganisms in foods, which could be integrated with biosensors. The extent of hybridization between the specific DNA probe and the searched-for DNA could be coupled with the use of fluorescence, bioluminescence, electrical detection, and antibody-enzyme-based methods. The

TABLE 14.4
Enzymes-Based Biosensors for Fish Freshness Evaluation

Enzyme	Transducer	Metabolite	Fish	Detection range
Xanthine oxidase	O$_2$ electrode	Hypoxanthine	Tuna, seabream, yellowtail	0.06–1.5 mM
Xanthine oxidase, Nucleoside phosphorylase	O$_2$ electrode	Inosine, Hypoxanthine	Tuna, seabream, yellowtail	0.5–2 mM
Nucleoside phosphorylase, xanthine oxidase	Polarographic electrode	K-value (see text)	Rainbow trout, haddock, carp, sole	—
Immobilized bacterial cells	O$_2$ electrode	K-value (see text)	Bluefin tuna, yellow tail	5–26 μM
Xanthine oxidase	Amperometric electrode	Hypoxanthine		—
	Ammonia electrode	Ammonia, TMA	Cod, tuna, haddock, perch, flounder	10–60/5–30 mg ammonia/ TMA
Xanthine oxidase, nucleoside phoshorylase Nucleotidase	Amperometric electrode	K-value (see text)	Sockeye salmon, Pacific cod, Herring	—
Pyruvate oxidase, octopine dehydrogenase	O$_2$ electrode	Octopine and other amines	Scallop	0–40 mM octopine
Diamine oxidase	Amperometric electrode	Histidine, Cadaverine, Putrescine	Mackerel, sardine, tuna	25 μM to 6 mM
Putrescine oxidase	Amperometric electrode	Polyamines	Pollack	0.03–3 μM
Sulfite oxidase	Oxygen electrode	Sulfite	Different fish	5–550 μM

Source: Adapted from Venugopal, V., *Biosensors & Bioelectr.*, 17, 147, 2002. With permission from Elsevier.

potential areas of applications of biosensors in aquaculture operations are given in Table 14.4.

Despite extensive research during the past two decades and the clear demand for on-line measurement, biosensors have yet to be widely accepted for industrial bioprocess monitoring. The most successful biosensors available commercially are disposable glucose sensors for testing blood sugar of diabetic patients. A comparable amperometric biosensor system designed for the determination of nucleotide

TABLE 14.5
Extracellular Gastric Enzymes from Fish and Aquatic Invertebrates

Proteases	Class	Source
Pepsin	Aspartic protease	Sardine, capelin, Atlantic cod, Greenland cod, salmon, mackerel, orange roughy, blue fin tuna
Pepsinogen	Aspartic protease	Rainbow trout, blue fin tuna, shark
Gastricain	Aspartic protease	Hake, Atlantic cod
Trypsin	Serine protease	Sardine, capelin, Greenland cod, Atlantic cod, different salmon species, anchovy, Atlantic croaker, carp, hybrid tilapia, krill, crayfish, oyster
Alkaline proteinases	Serine	White croaker, chum salmon, tilapia
Neutral proteinases	Metalloproteinases	Crucian carp
Neutral proteinase (calpains)	Cysteine	Tilapia
Chymotrypsin	Serine protease	Capelin, herring, Atlantic cod, spiny dogfish, rainbow trout, scallop, abalone, white shrimp, grass carp
Collagenases		Fiddler crab, freshwater prawn, crayfish, Atlantic cod, king crab
Elastase		Carp, catfish, Atlantic cod

Source: Adapted from Shahidi, F. and Janak Kamil, Y.V.A., *Trends Food Sci. Technol.*, 12, 435, 2001. With permission from Elsevier.

concentrations as an index of fish freshness was made by Pegasus Biotechnology, Canada. More than a decade ago, the Oriental Electric Co. in Japan marketed the KV-101 freshness meter, a system containing soluble enzymes together with an oxygen electrode for quality assessment of fish in supermarkets.[103–105] An ammonia ion-selective electrode has the potential to replace conventional methods for volatile basic nitrogen measurement for rapid on-site screening of fish quality.[97] Test kits for ammonia based on glutamate dehydrogenase are now available from Sigma, USA, and Boehinger Mannheim, Germany.

14.7 FISH WASTES AS A SOURCE OF ENZYMES

During commercial processing of fish and shellfish, a large amount of offal, shell, and waste fish are accumulated. These are rich sources of enzymes and a number of valuable by-products.[106] Fish viscera are a rich source of digestive enzymes. Some of the enzymes that can be isolated from viscera include proteases, chitinase, alkaline phosphatase, hyaluronidase, and acetyl glycosaminidase. Table 14.5 indicates sources of extracellular gastric enzymes from fish and aquatic invertebrates. A large-scale process for the recovery of enzymes in

wastewater from the shrimp-processing industry has been reported. The water used in thawing frozen raw shrimp is flocculated by ferric chloride, concentrated by cross-flow ultrafiltration, and then freeze-dried. Alkaline phosphatase, hyaluronidase, β-*N*-acetylglucosaminidase, and chitinase have been recovered from shrimp shell waste in good yield.[107]

Processes are available for the isolation of both acidic and alkaline proteases from viscera of fish species such as salmon, mackerel, and cod.[108,109] Pepsins and gastricins have been isolated from fish gastric mucosa; trypsins and chymotrypsins from pyloric ceca; and trypsin like enzymes from hepatopancreas.[18] Trypsin and chymotrypsin, purified from cod viscera are available commercially.[23,31] Collagenase prepared from crab hepatopancreas has been used for skinning of squid (*Loligo* spp.). A method for isolation of a proteolytic enzyme from mackerel intestine has been reported.[110] The enzyme was used for recovery of fish bone from hoki (*Johnius belengeri*). At an optimum temperature of 40°C and pH of 9.0, treatment at an enzyme to frame ratio of 1:100 gave higher yield as compared with alcalase, trypsin, α-chymotrpysin, or neutrase treatment.[110] Lysozyme has been recovered from commercial processing wastes of Arctic scallop and clamshell, which has a potential for application as a preservative in refrigerated foods.[76] Cold-active fish pepsins from Atlantic cod and orange roughy have been used for caviar production from the roe of various species including orange roughy and salmon.[24]

Cold-active enzymes isolated from temperate-water fish have significant activities at low temperatures. From a thermodynamic point of view, these enzymes have lower free energies of activation.[111] The lower free energy is useful in various food-processing applications requiring low temperature treatment. These include low temperature proteolysis, caviar production, and extraction of carotenoprotein for use as colorants or flavorants in food or feed. Being heat labile, such cold-active enzymes can be inactivated when required by modest increases in temperature.[112] The industrial market for enzymes processing aids is steadily growing with world sales estimated at almost $1.5billion US.[111] Guidelines for the commercial applications of enzymes have been given.[113]

In conclusion, a number of enzymes have potential uses in fish processing and quality control. Their applications include modifications of proteins, enrichment of PUFA in lipids, shelf life extension, by-product recovery, and quality control. Protein processing using proteases includes preparation of protein hydrolyzates, debittering of protein hydrolyzates, removal of scales from some fish species, recovery of proteins from filleting wastes, roe processing, extraction of flavors, carotenoids, and other ingredients from fish waste and tenderization of fish meat. Glucose oxidase, catalase, and lysozyme have shown potential for preservation of fish. Apart from their role in process development, a number of enzymes may also be used as components of biosensor systems for rapid evaluation of fish quality. Several of these enzymes may be extracted from fish wastes generated during commercial fish processing. Recovery of such enzymes from the waste serves the dual purposes of waste disposal as well as providing environment friendly tools for fish processing. Integration of enzymatic techniques with seafood processing can help

in simplification of conventional technologies, diversification of the industry, and development of novel products. However, problems with enzymatic bioprocessing of foodstuffs need to be considered in developing the technologies.[114]

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15 Nutritional Value and Processing Effects

15.1 Introduction

The role of diet in maintenance of health has been well recognized. Educational campaigns undertaken in the last few decades have raised the awareness of the importance of both macro- and micronutrients in food and have made customers adopt a selective approach towards foods, preferring only those having low contents of saturated lipid, sugar, and sodium. In recent times, the importance of fishery products as a source of nutrients including high quality proteins, unsaturated lipids, a number of vitamins, and minerals has been realized. Several fishery items have attracted the attention of nutritionists and dieticians as a source of therapeutically important polyunsaturated fatty acids. This chapter is intended to discuss in brief the nutritional importance of fish muscle constituents and the effect of processing on them. At the onset, it is pertinent to discuss briefly the structure and composition of fish muscle before discussing the nutritional value of fishery products.

15.2 Structure of Fish Muscle

Finfish differs from terrestrial animals by the presence of red muscle or dark tissues. Fish (*teleost*) species comprise of the demersal (or bottom dwelling) and the pelagic (surface dwelling) fish. Demersal fish tend to drift with ocean currents, and therefore do not swim actively for long periods. Consequently, they do not possess much of the red or dark muscle required for prolonged aerobic metabolism of energy reserves. On the other hand, the subcutaneous muscles of pelagic fish contain significant amounts of dark muscle containing myoglobin. These fish swim actively for long periods and consequently their muscles are equipped for prolonged aerobic activity. Red muscle contains more mitochondria and less sarcoplasmic reticulum than white fibers. Red muscles also contain 2 to 5 times more of lipid, B-vitamins, glycogen, and nucleic acids than white muscles. The red muscles are also rich in enzymes of tricarboxylic acid cycle, pentose phosphate shunt, electron transport, glycogen synthesis, and liposis, whereas white muscles possess higher contents of Adenosine Triphosphatase (ATPase) activity, glycolytic acids, and water.

The muscles that form a fish fillet are the large lateral muscles, which run on both sides of the body. These are generally white to off-white in color. The fish muscles are divided into segments called "myotomes" by thin connective tissue membranes, known as "mycommata." Each myotome is composed of muscle fibers

running parallel to the long axis of the fish. The muscle fibers are generally less than 20 mm long and up to 1 mm in diameter. A membrane called "sarcolemma," surrounds bundles of these fibers. These fine fibrils merge with mycommata at the myotome–mycommata junction. In contrast, the muscle mass in terrestrial animals is elongated and is tapered into a tendon. A muscle fiber possesses all the components of a cell and contains 1000–2000 elongated, threadlike structures or bundles of myofibrils, each up to 5 μm in diameter. The myofibrils are segmented into sarcomeres, which are composed of thin and thick filaments, showing alternate arrangements of anisotropic (A) and isotropic (I) bands, bordered by Z-lines. The thick filaments, composed of myosin molecules, and thin filaments, consisting of double helical strings of actin, overlap and slide over one another during muscle contraction. The thick filaments of a vertebrate contain 200–400 molecules of myosin, which is the main protein in the contractile mechanism.

The muscle of crustacean shellfish abdomen is divided into segments and is only loosely attached to the shell. This muscle is developed in shellfish like lobsters and shrimp, however in crabs, the abdomen is much reduced and most of the edible flesh comes from the legs and claws. The muscle fibers in the legs and claws are in the form of sheet or bundles attached to the shell on either side of the joints. The adductor muscle, the main tissue of mollusks, varies in morphology and function among different species. In mussels, clams, and oysters, its function is to hold the valves closed against the spring of the shell. In cephalopods, the mantle that surrounds the viscera of the animal consists of muscle tissue, which in squid is stiffened in part by an endoskeleton, the cuttlebone or "pen." A large calcified internal shell, the cuttlebone and an ovoid body somewhat flattened dorsoventrally characterize cuttlefish. The fins are either terminal or marginal in position uniting at the apex of the mantle. The mantle is cylindrically elongated and tubular with almost parallel sides up to the point where the fins originate, then tapers to a blunt posterior point. The two types of muscle of the mantle, namely circular and radial, enable the mantle to contract and expand, and drive the animal along by a form of jet propulsion. The crustacean muscles are also striated with prominent A and I bands. The sarcomeres are delimited by Z-lines as in the case of the vertebrate muscle fibers. The tonic fibers have long (10 μm) sarcomeres while phasic fibers have 2 to 3 μm long sarcomeres. The muscle fibers of prawns are wrapped in bundles of endomysium and overlaid with perimysium. Tails of the crustacean shellfish are attached to the inner walls of their shells. During storage, these structures deteriorate through the disruption of Z-lines and H-zones, with separation of sarcomeres into two distinct sections. The structure and composition of muscles of fish and crustaceans have been discussed in detail.[1–4] Figure 15.1 shows structural hierarchy within a fish fillet.

15.3 COMPONENTS OF FISH MUSCLE

Nonintrusive measurements are being used in modern times instead of the conventional time-consuming chemical methods for on-line measurements of seafood composition and quality. Protein, water, and lipid can be easily detected using
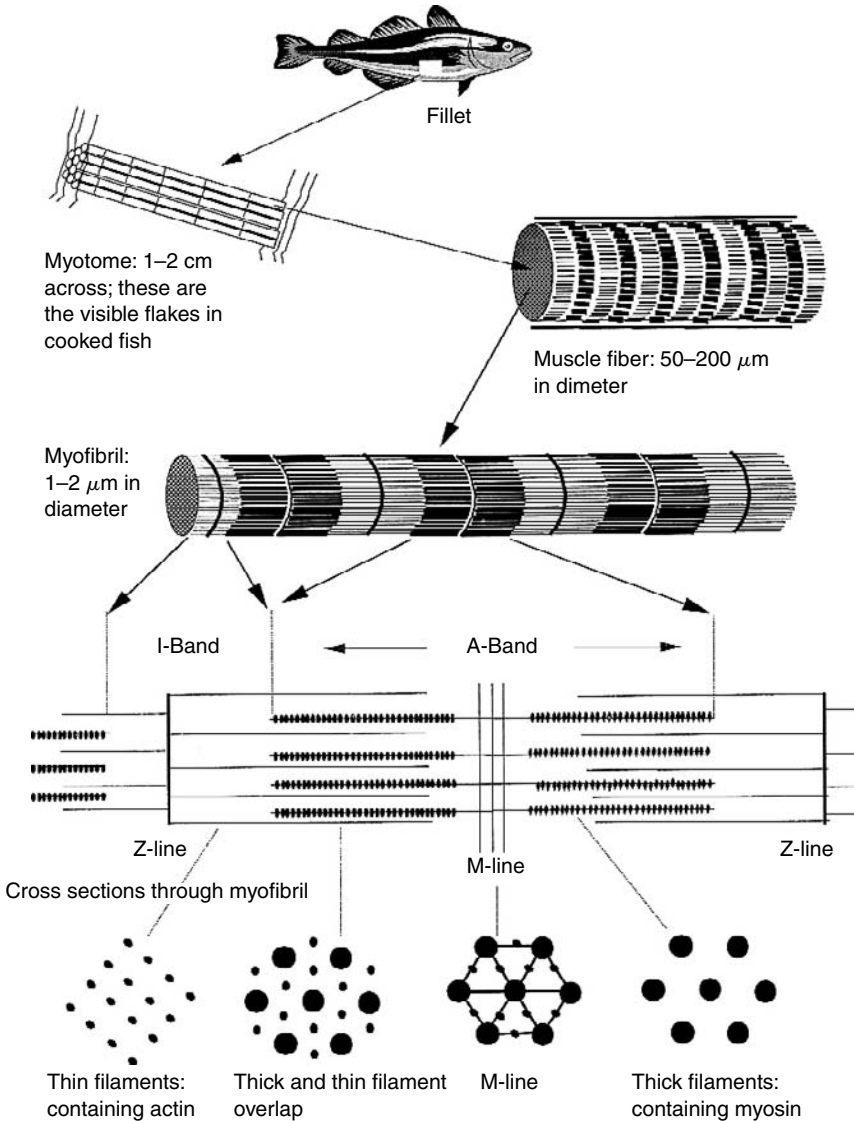
FIGURE 15.1 Structural hierarchy within a fish fillet (From Hedges, N., Maintenance of the quality of frozen fish, Russell Publishing, Brasted, Kent, U.K. With permission)

near infrared (NIR) spectroscopy. The sample material is exposed to light in the wavelength region from 700 to 2500 nm and the reflectance or the transmittance spectra are recorded. The chemical constituents such as protein, lipid, and water absorb NIR light at different wavelengths, and the amount of absorption depends on the concentration of the respective constituents.[5] Optical imaging or scanning techniques enable control of color and shape of the product, and rejection of samples

not fulfilling a given criterion, which could be the presence of blood stains, bones, or undesired color. Based on the information gathered in these imaging systems, fish fillets are portioned according to specifications. Table 15.1 gives proximate compositions of some marine fish species.

15.3.1 Proteins

The protein contents of most raw finfish flesh are in the range of 17–22%, with an average of 19/100 g, while the cooked portions may have as much as 35% protein. Freshwater fish have generally slightly higher moisture and lower protein contents than marine fish. Crustacean (crab, shrimp, and oysters) flesh is slightly higher in proteins. The contents of proteins in crustaceans and cephalopods are as follows: shrimp, 17.0–22.1%; scallop, 14.8–17.7%; squid, 13.2–19.6%; crab, 15.0–18.4%; lobster, 18.2–19.2%; krill, 12.0–13.0%; and mussel and oyster, 8.9–11.7%.[6] The myofibrillar protein contents of fish range between 65 and 75%, while sarcoplasmic (soluble proteins including enzymes) are in the range of 20–35%. Myosin constitutes 50–58% of the myofibrillar proteins of fish. Eventhough there is a slight difference in the composition of amino acids, the myosins of all vertebrates such a rabbit, chicken, cod, and tilapia are similar.[3,4] However, compared to carcass meat fish myosins are unstable, being more sensitive to denaturation, coagulation, degradation, or chemical changes. Nevertheless, some exceptions have been noted. Myosins from some species such as tuna, sea mullet, and tilapia have been reported to be almost as stable as rabbit or beef myosins.[7] Similar to mammalian myosins, fish myosins also possess ATPase activity, regulated by the presence of calcium and magnesium ions. The regulatory proteins, which are involved in the contractile mechanism, include actins (F and G types), tropomyosin, and troponin, are present in the thin filaments. The structural aspects of myosin have been discussed in Chapter 13. From a technological point of view, the structural proteins, namely, myosin, and the regulatory proteins determine the functionality of muscle as food, such as oil-emulsification capacity, whippability, gel-forming ability, and chewability among others.

Paramyosin is a protein found in striated muscles of invertebrates and is involved in the catch contraction of bivalves. The contents of paramyosin in scallop, squid, and oysters are 3, 14, and 19%, respectively. In the white adductor muscle of some oysters and clams, 38–48% of the myofibrils are paramyosins, which form a core with a surface layer of myosin. The protein has a molecular weight of 200–258 kDa, consisting of two subunits of 95–125 kDa with a glutamic acid content as high as 20–23%. The comparative biochemistry of paramyosins including those from mollusks has been reviewed.[8] As compared with red meat, fish meat contains only 3% stroma proteins, except sharks, rays, and skates, which may contain up to 10%.

The nonprotein nitrogen content of fish muscle is normally higher than that of terrestrial animals, and range between 10 and 40%, which contain amino acids, small peptides, trimethylamine oxide (TMAO), trimethylamine, creatine, creatinine, and nucleotides. Amino acid compositions of 60 commercially important

TABLE 15.1
Proximate Compositions (%) of Some Marine Fish Species

Fish	Size	Moisture	Protein	Fat
Anchovy (*Thrissocles kammalensis*)	15/50	72.4	16.3	8.2
Armed trevally (*Carangoides armatus*)	53/3500	73.1	22.2	3.6
Barracuda, Indian (*Sphyraena jello*)	33.5–85/290–3800	72.4	22.0	2.3
Bone fish (*Albula vulpes*)	60/3600	73.6	22.4	0.4
Captain fish (*Johnius heterolepis*)	30/500	78.7	18.0	1.2
Catfish (*Aarous gagoriodes*)	60/3200	79.8	18.1	0.8
Catfish (*Tachusurus filiceps*)	30/600	79.2	17.7	1.2
Cod, Atlantic (*Gadus morhua*, L)	NR/NR	82.5	19.4	0.3
Cod, Pacific (*Gadus callarias*)	93/14 kg	79.5	17.6	0.9
Croaker, silver (*Otolithus argenteus*)	34/350	77.6	19.7	1.7
Eel, american (*Macrozoarces americanus*)	63/1110	76.1	18.8	2.6
Eel, spotted (*Ilucoltes fimbriatus*)	36/260	81.1	15.8	0.6
Eel, sharp-toothed (*Muraenesox cinereus*)	150/5.1 kg	79.6	15.8	0.2
Eel (*Conger conger*)	125/4300	78.6	—	0.1
Emperor (*Lutjanus sebae*)	90/—	76.4	20.7	0.6
Goat fish (*Upeneus gracilis*)	14/65	76.7	19.8	0.3
Grouper (*Einephelus specilus*)	37/1300	78.0	19.1	1.8
Haddock (*Melanogrammus aeglefinus*)	45/1057	81.7	18.5	1.0
Hake, silver (*Merluccius bilinearis*)	80/NR	81.2	17.9	4.1
Herring, Atlantic (*Hippoglossus hippoglossus*)	200/30 kg	80.8	20.3	6.2
Herring, Atlantic (*Clupea harengus*)	18/168	69.0	17.3	11.3
Herring, Indian (*Ilisha indica*)	19/115	75.9	20.2	2.3
Jaw fish (*Johnius dussumieri*)	18/120	78.0	19.5	0.7
Lizard fish, Indian (*Saurida tumbil*)	31/455	76.9	20.2	1.2
Mackerel, Atlantic (*Scomber scombrus*)	39/850	74.1	22.8	11.4
Mackerel, horse (*Trachurus trachurus*)	18–39/120–730	73.8	19.4	5.6
Marlin, black (*Makaira mazara*)	—	77.4	20.3	0.3
Marlin, stripped (*Makaira mitzukurii*)	177/2.3 kg	79.5	19.8	0.1
Menhaden, Atlantic (*Brevoortia tyrannus*)	36/740	75.0	19.0	21.0
Ocean perch (*Sebastes marinus*)	90/1600	73.5	17.8	6.6
Pollock, walleye (*Theragra chalcogramma*)	55/1400	83.2	17.3	0.9

(Continued)

TABLE 15.1
(Continued)

Fish	Size	Moisture	Protein	Fat
Pomfret, black (*Parastromateus niger*)	518/854	75.2	18.9	1.9
Pomfret, silver (*Pampus argenteus*)	15.5–32/165–1240	69.5	17.7	10.0
Pony fish (*Leiognathus daurus*)	7/75	77.2	19.6	1.5
Queen fish (*Chorinemus lysan*)	60/3000	77.2	20.9	0.5
Ribbon fish (*Trichiurus haumela*)	55–102/225–885	75.9	17.5	0.6
Salmon, pink (*Salmo salar*)	62/1500	66.3	20.0	3.7
Scad, big eye (*Setar crumenophthalmus*)	14/150	73.5	21.9	2.8
Scad, Indian (*Decapterus kilche*)	18/110	71.9	21.5	5.0
Scad, round (*Decapterus russelli*)	30/400	77.5	20.0	0.8
Seer, Indian (Spanish mackerel) (*Scomberomorus guttatus*)	90/1200	75.8	19.7	2.2
Seer (*Scomberomorus commersoni*)	70/2500	76.2	21.1	1.2
Shark (*Scoliodon walbeehmi*)	50/110	78.0	18.0	0.8
Shark, black tip (*Carcharhinus limbatus*)	190/95 kg	74.0	22.0	0.3
Silver bream (*Sparus sarla*)	35/1700	73.8	19.0	4.0
Trevally, black-tipped (*Caranx melampygus*)	45/2400	76.8	21.0	1.4
Trevally, yellow-back (*Carangoides fulvoguttatus*)	45/2400	76.8	21.0	1.4
Trout, rainbow (*Salmo gairdneri*)	NR	66.3	21.5	11.4
Tuna, big eye (*Thunnus obesus*)	20/100 kg	74.6	23.0	0.6
Tuna, bluefin (*Thunnus thynnus*)	350/700 kg	71.2	20.0	10.5
Tuna, skipjack (*Ktsuwonus pelamis*)	60/5000	71.7	25.9	0.6
Turbot (*Rhombus maximus*)	57/8.7 kg	80.9	17.5	0.2

Source: Adapted from Bykov, V.P., *Marine Fishes: Chemical Composition and Processing Properties*, Amerind Publishers, New Delhi, 1983 and Venugopal, V. and Shahidi, F., *Food Rev. Int.*, 12, 175, 1996.

fish and shellfish have been summarized.[4] Shrimp, lobster, crab, squid, and other shellfish generally contain larger amounts of arginine, glutamic acid, glycine, and alanine than finfish.[4] The higher contents of these amino acids during the winter season make squids more palatable as compared with those harvested in summer. Shark extractives contain 100–150 mg betaine per 100 g meat. Demersal fish generally contain larger quantities of TMAO than pelagic fish, and its contents vary from 19 to 190 mg%. Elasmobranchs also contain high amounts of TMAO, while its content is small in mollusks and rather insignificant in freshwater fish species. Eventhough this compound is negligible in most freshwater fish, some species such as Nile perch and tilapia may contain 150–250 mg TMAO per 100 g.

15.3.1.1 Nutritive value of proteins

The nutritive value of fish proteins is comparatively high because of the favorable essential amino acid pattern.[7] Fish proteins are rich in all the essential amino acids (particularly methionine and lysine), in contrast with most proteins from plant sources, which lack adequate amounts of one or more essential amino acids. There are no significant differences in the amino acid composition of freshwater and marine fish. However, certain marine fish such as mackerel, tuna, etc. may be exceptionally rich in the amino acid, histidine (Chapter 2). As compared with red meat, fish proteins are considered nutritionally equivalent or slightly superior. They are highly sensitive to proteolytic digestion with a digestibility of more than 90%. The *in vivo* digestibility of proteins of raw fish meat is in the range of 90–98%, and that of shellfish, about 85%. The enhanced digestibility is mainly due to the absence of strong collagenous fibers and tendons in fish muscle, which are common in land animals. Protein efficiency ratio (PER, weight gained per g of protein consumed) of fish proteins, an index of protein quality, is slightly above that of casein, the major milk protein. The net protein utilization (NPU) of fish flesh is 83, as compared with values of 80 and 100 for red meat and egg, respectively.[3] A fish commercial collagen hydrolysate, *Peptan F*, has been found to enhance protein quality and flavor characteristics of beverages.[9] The nutritional values of proteins from different sources including fishery items have been compared.[10] The concentrated fish proteins or their hydrolyzates obtained by the action of proteolytic enzymes such as pepsin, trypsin, etc. have found applications as a protein supplement.[11,12] Nutritive value of cereal proteins could be increased when combined with a quality fish protein. Thus, addition of 3% of fish-protein concentrate to wheat flour (protein content, 10.4%) increased its protein content to 12.4% with an increase of NPU from 50 to 67. A recent animal feeding study conducted at Norway suggested the effect of fish protein hydrolyzate as a cardio-protective nutrient. The fish-protein treatment reduced plasma cholesterol level, gave higher content of high-density lipoprotein (HDL), and altered the fatty acid composition in liver, plasma, and triglycerol-rich lipoproteins in obese zucker rats.[12] In contrast to fish, cephalopods in general contain 20% more protein, 80% less ash, and 50–100% less lipid.[13] Cephalopods have a high biological value, *Octopus vulgaris* giving a value of 83.5 ± 1.[14,15] Table 15.2 gives comparison of essential amino acids in milk, fish meat, and Alaska pollock *surimi*.

15.3.2 Lipids

Marine fish are commonly classified according to the fat content of their fillets and grouped as lean (under 3% fat), medium (3–7% fat), and high fat (over 7% fat). Lipids in fatty fish are mostly subcutaneous in nature, whereas, in lean fish they are deposited in the liver, muscle tissue, and mature gonads. Lean fish such as sole are usually whitish in color, whereas, fish with higher fat content (e.g., cod, haddock, halibut, and pollock) are white to off-white. The flesh of high-fat fish (e.g., herring, sardine, anchovy, and salmon) is usually pigmented (e.g., yellow,

TABLE 15.2
Comparison of Essential Amino Acids in Milk and Fish

Amino acid	Fish[a]	Milk[a]	Surimi[b]
Lysine	8.8	8.1	11.7
Tryptophan	1.0	1.6	1.3
Phenylalanine	3.9	5.3	3.8
Histidine	2.0	2.6	2.5
Leucine	8.4	10.4	10.6
Isoleucine	6.0	7.2	6.2
Threonine	4.6	4.0	4.6
Methionine	4.0	4.3	3.1
Valine	6.0	7.6	4.9

Sources:
[a] Adapted from Huss, H.H., *Quality and Quality Changes in Fresh Fish*, FAO Fisheries Technical Paper No. 348, Food and Agriculture Organization of the United Nations, Rome, Italy, 1995.
[b] Adapted from Project Summary, Alaska Fisheries Development Foundation, *Surimi*, *It's American Now*, 1982–1987, Anchorage, AK.

pink, and greyish). In an individual fish, lipid content increases from tail to head, with higher level of fat deposition in the belly flap and dark muscle. The amount of lipids in fish may vary widely from 0.2 to 24%, depending upon anatomical position, sex, location in body, age, season, and diet. In many pelagic fish, lipid contents ranging from 12 to 20% are found during winter as compared with 3–5% levels during summer. The fat levels in some fish correlate with spawning cycles. For instance, anadromous fish store fat prior to migration to freshwater for spawning. A variety of seafood items including clams, cod, flounder, grouper, haddock, halibut, northern lobster, mahi-mahi, monk fish, perch, pike (Northern eye), pollock, orange roughy, scallop, shrimp, red snapper, sole, squid, tuna (skip jack), tuna (yellow fin), and whiting, contain less than 2.5 g total fat in 3 oz of cooked portions. On the other hand, the same amount of cooked portions of butterfish, herring, Spanish mackerel, salmon (Atlantic, coho, or sockeye), lake trout, tuna blue fin, and white fish provide 5–10 g of total fat.[16]

Marine lipids are composed of triacyl glycerols, phospholipids, sterols, wax esters, and some unusual lipids, such as glyceryl esters, glycolipids, sulfolipids, and hydrocarbons. Most of the variations in lipids are found in the triacylglycerol fraction while the phospholipids show much less variations. The phospholipids of tropical fish are more saturated than fish from temperate waters. The phospholipids of fish muscle contain generally more phosphatidylcholine than phosphatidylethanolamine. The marine steroids are composed of cholesterol, which is present at

50–90 mg per 100 g of fish meat. In some pelagic fish species such as anchovy, blue fin tuna, pilchards, and different mackerels, cholesterol may be up to 150 mg/100 g of meat, and may be as high as 250–650 mg in roe and liver. Shellfish tend to contain slightly higher amounts of cholesterol. Thus, crustaceans (crab, lobster, and shrimp) contain 69–100 mg per 100 g. Squid and octopus may contain 250 and 120 mg of the steroid per 100 g, respectively.[17–22]

The nature of fatty acids essentially determines the quality of lipids. The fatty acid composition of seafood is fundamentally different from meat, vegetable, and dairy products. In addition, the fatty acid composition shows marked variability within and between species. Fatty acids are formed from triglycerides by the action of muscle (also microbial) lipases. Fatty acids with chain lengths of 10 carbon atoms or less are referred as short-chain fatty acids and all of these are saturated. Fatty acids with 12 or 14 carbon atoms are medium-chain fatty acids and those with more than 14 carbon atoms are long-chain fatty acids, which may be saturated or unsaturated. As early as the 1920s, the nutritional significance of long-chain fatty acids, also known as essential fatty acids (EFAs), has been recognized. The important EFAs, linoleic acid (LA, $C_{18:2}$, n-6) and α-linolenic acid (ALNA, $C_{18:3}$, n-9) are present in vegetables and plants. The LAs and ALNAs are subjected to chain elongation and desaturation to give long-chain polyunsaturated fatty acids (PUFAs) containing 20 or more carbon atoms such as arachidonic acid (AA, $C_{20:4}$, n-6) and n-3 (also referred as omega-3) fatty acids, eicosapentaenoic acid (EPA, *cis*-5,8,11,14,17-eicosapentaenoic acid, $C_{20:5}$) and docosahexaenoic acid (DHA, *cis*-4,7,10,13,16,19-docosahexaenoic acid, $C_{22:6}$). (The letter "n" refers to the position of the first carbon atom where a point of unsaturation is found, starting from the methyl group of the fatty acid.) The fatty acids, which have attracted most are EPA and DHA attention. Fish lipids are different from those of terrestrial origin in that they contain significant amounts of EPA and DHA; however, the quantities vary among species and within a species according to environmental variables such as diet and their habitats.[3,19–21] Table 15.3 shows omega-3 fatty acid contents of some seafood.

15.3.2.1 Nutritional role of fish lipids

Lipids have important roles in human nutrition and disease management. A few decades ago, fats were viewed as providing only functionalities such as flavor and texture to foods. In recent times, the role of fats and oils in consumer health has been well understood. It has been recognized that an ideal fat to meet the nutritional requirements of an adult should aim at maintenance of health and prevention of diseases. Consumers have generally become aware of the ill effects of consumption of saturated fat and are turning to oils that are rich in unsaturated fatty acids. Since 1970s there has been an intense scientific interest in the health benefits of fish and fish oils, initiated with the studies on the longevity and coronary health of native Greenland Eskimos. These investigations led to the conclusion that longevity and coronary health of Eskimos was related to their diet, who consume

TABLE 15.3
Omega-3 Fatty Acid Contents of Some Seafood

Content (g/100 g meat)		
≤0.5	0.6–1.0	≥1.0
Atlantic cod	Atlantic mackerel	Anchovy
Atlantic pollock	Channel catfish	Atlantic herring
Catfish	Indian mackerel	Atlantic salmon
Haddock	Red snapper	Blue fin tuna
Oil sardine	Silver hake	Pacific mackerel
Pacific cod	Spiny dogfish	Pacific herring
Pacific halibut	Swordfish	Pink salmon
Rockfish	Torbot	Rainbow trout
Skipjack tuna	Trout	
Sole		
Yellow perch		

Source: Reproduced from Venugopal, V. and Shahidi, F., *Food Rev. Int.*, 12, 175, 1996. With permission from Marcel Dekker.

an average 450 g fatty fish per day.[23] Later, large scale epidemiological studies suggested that individuals at a risk of coronary heart diseases (CHD) benefited from the consumption of plant and marine-derived omega-3 fatty acids.[17,21,24–27] The major therapeutically important omega-3 fatty acids were later identified as EPA and DHA.

A beneficial effect of dietary omega-3 fatty acids on coronary heart events including nonfatal myocardial infarction and stroke has been observed in a recent study.[25] Consumption of cod oil helped to maintain the elasticity of artery walls, prevent blood clotting, reduce blood pressure, and stabilize heart rhythm. Studies conducted in India in experimental animals and men suggested that consumption of both oil sardine (*Sardinella longiceps*) and its oil lowered serum-total cholesterol, LDL-cholesterol, and triglycerides.[28] More evidence has shown that fish consumption favorably affects CHD mortality, especially nonsudden death from myocardial infarction.[29–33] Recent studies have shown that fish intake is associated with a reduced progression of coronary artery atherosclerosis in postmenopausal women with CHD.[31] Other studies have also reported an inverse relation between fish intake and CHD death.[32–35]

An amount of 4–6 g of EPA (20–30 ml fish oil or 300–400 g fatty fish per day) has been recommended to control CHD, although the ideal intake of omega-3 fatty acids for the therapeutic effect is not clear.[17] The effective dose could be reduced to 2–3 g EPA per day when fat intake was reduced to approximately 20% of calories as exemplified by Japanese diets.[17] The U.S. FDA recommends that consumers not

exceed more than 3 g of EPA and DHA per day from a dietary supplement.[21] The American Heart Association recommends that healthy individuals consume 2–3 oz servings of fatty fish per week and that the persons diagnosed with cardiovascular diseases consume 1 g each of the fatty acid per day.[34] Some of the fish species as sources of n-3 fatty acids include sardine, mackerel, anchovy, cod, Atlantic herring, salmon, bluefin tuna, red snapper, swordfish, and silver hake.[26] In case regular consumption of fatty fish is not possible, its oil may be administered in capsules, or other foods may be enriched with the oil. Aquacultured fish generally are not good sources of n-3 fatty acids. However, it is possible to rear fish such as salmon to have significant levels of PUFAs and the antioxidant, astaxanthin, by selective feeding techniques. A daily serving of 8 oz (227 g) of the fish provides 5 times the effective adequate intake of $C_{20:5}$ and $C_{22:6}$ (0.14 and 0.13 g/day, respectively) for pregnant or lactating women.[36]

The functional role of EPA and DHA in health is through their ability to give rise to a class of pharmacologically important groups of compounds such as prostaglandins, prostacyclins, thromboxanes, and leukotrienes (collectively called as eicosanoids), which can control the CHDs. The eicosanoids are formed from LA, arachidonic acid (AA), EPA, and DHA. Formation of eicosanoids from LA is through the intermediate formation of AA. The thromboxanes generated from EPA and DHA are weak in aggregation of platelets, unlike the thromboxanes produced from AA. In addition, AA is easily oxidized by the enzymes, lipoxygenase and cycloxygenase, to linear or cyclic peroxides, respectively, which participate in the form of hydroxy fatty acids to form leukotrienes. Unlike AA, EPA and DHA cannot be oxidized by cycloxygenase, and hence, their presence reduces the synthesis of leukotrienes. Therefore, availability of sufficient quantities of EPA and DHA help to alleviate the problems caused by AA-generated eicosanoids. Prostaglandins synthesized from omega-3 acids, as well have additional therapeutic uses including control of blood pressure and relieving of bronchial asthma.[37]

Essential fatty acids, especially, AA and DHA are essential for the development of fetal brain, central nervous system, and retinal growth in infants during pregnancy. DHA deficiency has been associated with visual impairment and delayed cognitive development. While both LA and ALNA can serve as precursors for the essential fatty acids, their ratio should be around 7:1, as generally found in human milk. Studies on human volunteers have shown that while conversion of ALNA to EPA can occur, conversion of ALNA to DHA is restricted, indicating a need for supplementation of DHA in diet. Mother's intake of fat has great influence on the health of the infant. Supplementation of mother's diet with sardines and other fish oils at a level of 2.6 g n-3 fatty acids per day resulted in an increase in DHA in maternal red blood cells from 4.6 to 7.2%, with a corresponding increase in maternal plasma. This subsequently enhanced DHA level in infant red blood cells. An amount of 500 to 600 mg of DHA per day has been recommended for expecting women.[38,39] Eating fish twice or thrice a week could provide the required DHA. Recent study showed the relationship between hostility and consumption of whole fish, omega-3 and -6 fatty acids. On the tests conducted using a sample of approximately 3600 adolescents, it was found that consuming DHA and whole

fish were independently related to lower hostility rates compared to those who had consumed no DHA or fish.[40]

In spite of the health benefits of fish lipids mentioned above, preliminary studies have indicated additional advantages of consumption of fatty fish.[41–44] Canadian researchers suggested that eating fish prevented neurological disorders such as Alzheimer's disease and dementia.[41] Finnish scientists showed that regular consumption of fish, at least twice a month, significantly reduced depression. As per a study conducted in Scotland, two thirds of children are likely to have fatty acid deficiency, which may be responsible for some of the behavioral patterns and symptoms of autism. Supplementation of diet of these children with fish oil can result in improvement in their behavior. Other studies have shown the linkage between DHA and incidence and duration of headaches, diabetes, arthritis, dry eye, and asthma among children. Benefits also included decreased chances of developing a type of lung cancer, arthritis, colitis, and inflammatory diseases, breast and ovarian cancer, and also control of age-related memory loss. Fish oil could be a significant tool in combating breast cancer.[43,44] An anticancer agent isolated from scallop has been shown to be effective at a dose of 200 μg. In Hyderabad, India, consumption of a specific local fish is recommended to cure asthma. These reports in general favor the beneficial role of consumption of marine fatty fish in health management, although some of the reported advantages may need further confirmation.

Figures 15.2 and 15.3 depict schematic diagrams on the role of fish in human nutrition.

In view of the recognized nutritional advantages, marketing campaigns have been launched for many fish products that tend to affirm that consumption of fish is an appropriate method of satisfying consumer's need for a variety nutritious, tasty, and healthy foods. These campaigns have resulted in positive changes in consumer attitude towards seafood.[45–47] According to a recent survey, 47% consumers associated omega-3 fatty acids with heart health and 32% considered themselves deficient in the compounds.[45] Omega-3 fatty acids are now being incorporated in a variety of products including frozen desserts, muffins, breads, sauces, margarine, pasta, cheese spreads, tuna burgers, yogurts, and salad dressings. These fatty acids are also finding use as nutrient supplement to flour in countries such as USA, Germany and Norway. Incorporation of the oil in encapsulated form prevents its oxidation.[39,48] Canada, perhaps, is the first country to recommend fortification of infant formula with omega-3 fatty acids. A number of countries including Australia, Canada, Japan, Sweden, and United Kingdom as well as World Health Organization and North Atlantic Treaty Organization have made formal population-based dietary recommendation for omega-3 fatty acid consumption. Typical recommendations are 0.3–0.5 g per day of EPA and DHA and 0.8–1.1 g per day of LA.[24,25,34,38] The U.S. Food and Drug Administration (FDA) has allowed a qualified health claim about EPA and DHA as dietary supplements to reduce risks of CHD.[48] Oil from menhaden fish (*Brevoortia* spp.) is a rich source of PUFAs. EPA and DHA make up approximately 20% by weight of the oil. The FDA has given the oil GRAS (generally recognized as safe) status in
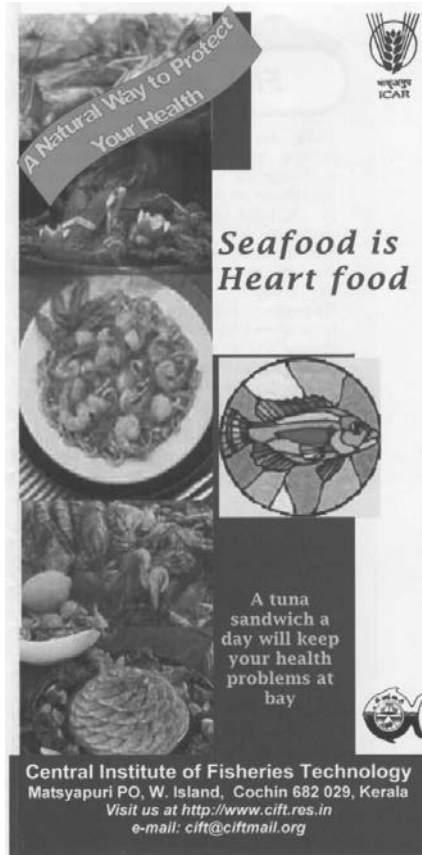
FIGURE 15.2   Schematic diagram on the role of fish in human nutrition. With courtesy from K. Devadasan, Director, Central Institute of Fisheries Technology, Cochin, India

1997, when it is not used in combination with other added oils that are significant sources of the omega-3 fatty acids. This allows use of the oil as a human food ingredient. Daily maximum intake of 3 g of EPA and DHA from the oil has been recommended.[48]

Recently, there has been a scare regarding the presence of higher levels of mercury in fish such as swordfish, shark, king mackerel, and tilefish.[49] The presence of copious amounts of mercury encouraged the U.S. FDA and the U.S. Environmental Protection Agency to issue advisory note to pregnant women and women of child-bearing age.[49,50] Avoiding consumption of potentially contaminated fish is a higher priority for these consumers. However, it has been concluded that for middle-aged and older men and postmenopausal women, the benefits of fish consumption far outweigh the risks.[51] Consumption of a wide variety of species in

FIGURE 15.3   Schematic diagram on the role and contribution of fish in human nutrition and diet. With courtesy from K. Devadasan, Director, Central Institute of Fisheries Technology, Cochin, India

accordance with recommended guidelines is the best approach to both minimizing mercury exposure and increasing omega-3 fatty acid intake.[36] Fish species such as Atlantic and Pacific herring, lake trout, Chinock, Atlantic and Sockeye salmon species, blue fish, and European anchovy can provide adequate amounts of omega-3 fatty acids in the range of 2.7 to7.5 g per meal to satisfy the nutritional recommendations as per the guidelines.[26,34]

15.3.3   Calorie

Fish contributes few calories to food. Even high fat fish will add fewer calories to the diet than an equal amount of most red meat.[52] Conventional finfish and seafood potentially provide 199–200 cal per 100 g, which is mainly attributed to the protein and fat contents of fish. Cooked low-fat white fish contains about 80–90 cal per 100 g raw edible portion, medium-fat fish about 100 cal, and high-fat

TABLE 15.4
Calorie and Fat Contents of Some Fish Preparations

Item	Serving	Calorie	Fat (g)
Fish steamed	150 g	167	6.3
Steamed fish with spring onions	150 g	212	10.0
Fish stew	150 g	263	15.1
Fish, fried	150 g		
Mackerel	2–3 pcs	273	21.7
Shark	2–3 pcs	273	24.5
Mullet	2–3 pcs	335	27.8
Sardine	2–3 pcs	281	29.9
Seer fish	2–3 pcs	306	24.1
Fish cutlet	2 pcs	455	32.0
Fish curry	150 g	485	35.0
Lobster, boiled	2–3 pcs	90	0.9
Lobster, fried	2–3 pcs	270	20.9
Prawn, fried	2–3 pcs	269	21.0
Shrimp rice	1 plate	544	14.0
Shrimp curry	150 g	651	34.1

Source: Adapted from Parekh, R., *The Saffola Food Check*. R.R. Publications, Mumbai, India, 2002.

fish contains between 150 and 225 cal per 100 g. Canned herring, salmon, and tuna have 208, 203, and 197 cal per 100 g, respectively. Chinook salmon may contribute as much as 222 cal, pink salmon can provide 119 cal, and mackerel as much as 191 cal/100 g. *Surimi*-based shrimp analog has an energy content of 116 cal, while cooked oyster has a value of only 76 cal/100 g.[53] Table 15.4 presents calorie and fat contents of some fish preparations.

15.3.4 Vitamins

Fish oils are rich sources of vitamins A, D, and E. Vitamin A is concentrated mostly in fish liver oils. Halibut and cod liver oils are rich sources of vitamin A and D. Sardine contain 100–4500 IU vitamin A and up to 500 IU vitamin D per 100 g meat, with an average of 125 μg/g oil. Herring, mackerel, salmon, and lake trout contain varying amounts of vitamin D in their tissues. A 3.5 OZ of salmon provides 90% of daily need of vitamin D Fish cannot synthesize vitamin E, and hence, the concentration of this vitamin, mainly α-tocopherol, is related to feed. Seafood provides moderate amounts of thiamin. However, much of thiamin is destroyed by heat and oxygen or is lost in cooking water or when exposed to ionizing radiation (Chapter 10). The average content of thiamin in 155 fish species is between 6 and 434 mg/100 g meat. Fish also contain modest amounts of biotin, folic acid, niacin, and panthothenic acid. The best sources of pyridoxine (vitamin B_6) are salmon

and tuna, and to some extent, shellfish. Modest amounts of riboflavin are present particularly in the dark flesh of some species like canned herring, mackerel, and pilchard. Pyridoxin is present in fish and shellfish in reasonable amounts, with tuna and salmon being rich in this vitamin. Fish and shellfish, particularly anchovies, clams, herring, oysters, pilchard, and sardines, are rich sources of vitamin B_{12}, containing 25–40 μg/100 g meat.[3,22,53]

15.3.5 Minerals

Seafood items are rich in important minerals and trace elements such as selenium and iodine, as compared with red meat. Shellfish contain nearly twice the amount as finfish. The total content of minerals such sodium, potassium, calcium, magnesium and phosphorus, and microelements such as selenium, fluorine, iodine, cobalt, manganese, and molybdenum in raw marine fish muscle and invertebrates are roughly in the range of 0.6 to 1.5% wet weight. Oysters are especially rich in zinc, iron, and copper. Oysters, clams, and shrimp contain more calcium than other fish and meat. Most fresh marine fish may be considered moderately low-sodium foods delivering approximately 140 mg sodium per serving. However, the sodium content of most processed fish and seafood products (frozen, canned, smoked, and cured) is substantially higher, ranging from 300 to 900 mg/100 g. The higher contents are the result of conventional onboard handling and processing treatments such as brining, storage in refrigerated seawater, etc. Battered and frozen seafood contain an average of 400 mg sodium per 100 g. The sodium content of fresh fish fillets ranges from 39 to 90 mg/100 g.[54,55] Selenium provides protection against mercury and cadmium toxicity. Seafood, especially tuna, is an important source of this nutrient. In general, shellfish tend to be richer sources than finfish.[22]

Seafood is also a source of calcium, its contents varying from 6 to 120 mg/100 g depending upon the species.[54] The calcium content in 100 g muscle may be as low as 15 mg in the case of mackerel, 15–50 mg in catfish, haddock, and oysters and above 100 mg in pollock, salmon, and trout.[20,54] Tuna bones were in use as a source of calcium in Japan in the mid-1980s in institutional feeding programs for elderly and in school meal programs. A fish processing company in Tokyo has successfully processed scales of sardine into an easily absorbable, edible food supplement containing calcium and collagen.[38] Fresh fish are a good source of potassium containing 250–320 mg of the element per 100 g. Shellfish (clams, oysters, scallops) and fish having dark-colored flesh such as bluefish, herring, mackerel, sardines, and smelt are reasonably good sources of iron, supplying 1–2 mg/100 g muscle. The iron content in 100 g meat may vary as 0.9 mg in cod, flounder, and pollock, 0.9–2.0 mg in carp, catfish, salmon, and trout and above 2 mg in clams, oysters, and shrimp. Consumption of sardines, whether raw or fried in olive oil, enhances dietary iron availability. Mollusks and crustaceans are good sources of zinc and copper, as well as iodine. Against a daily-recommended value of 1000 mg of phosphorus, 300 mg of the mineral could be obtained from 100 g meat of salmon or sardine. Its content is less than 200 mg in clams, flounder, and oyster. Catfish, cod, and pollock contain 200–300 mg of the mineral.[20,22,54,55]

Marine fish and shellfish are rich sources of iodine, being highest in oysters, followed by clams, lobster, shrimp, craw, and ocean fish. Copper, which is necessary for maintenance of blood vessels, tendons, and bone, is sufficiently available from crustaceans, especially lobsters. Oysters and clams provide between 45 and 60% of the daily requirement of the mineral. An average serve of fish or marine invertebrate can satisfy the total human requirements for essential microelements.[5,22,53]

A diverse array of bioactive compounds such as anticancer agents are present in marine organisms, particularly, jellyfish, corals, shark cartilage, shellfish, as well as in marine microorganisms.[56] A preliminary report from New Zealand has shown the potential of lyprinol oil found in mussel to prevent multiplication of cancer cells. The Pacific oyster contains a substance, which may alleviate diabetes by promoting the secretion of insulin. Shellfish is a rich source of chitin, a nonsoluble polymer of N-acetyl glucosamine units. Chitosan obtained by deacetylation of chitin is a versatile, water-soluble cationic compound. The pharmaceutical applications of chitosan include its uses as anticoagulant, artificial kidney membrane, sutures, immuno-stimulants, and antitumor agent. In addition, it has industrial importance for uses as flocculating agent for clarification of fruit juices, dehydrating agent for concentration of alcoholic beverages, and in biotechnology. Chitosan is also helpful in water purification, cosmetics, and to enhance the functional properties of packaging material. Chitosan coating has been shown to enhance shelf life of fresh fishery products. Algae, sponges, and marine microorganisms can also be sources of bioactive compounds. Marine microbial biotechnology and biodiversity have shown prospects for microbial processes for large-scale production of a variety of such compounds.

15.4 INFLUENCE OF PROCESSING ON NUTRITION

Processing has some effect on fish components depending upon the processing, packaging, and storage conditions. Most fishery products are subjected to cooking before consumption either at a centralized processing center or at the level of the consumer. Cooking, in general, enhances the digestibility of fish proteins. The loss of nutrients on cooking depends on the temperature, duration of cooking, and the type of nutrients. Mild cooking causes little loss of protein with only a slight loss in the available lysine, whereas drastic heating can significantly reduce the protein quality. Boiling has little effect in the composition of shellfish. Similarly, canning of fish and shellfish has little impact on proximate composition. Most cooking has a negligible effect on fatty acid composition of fish.[21,57,58] Canned fish, frequently packed in vegetable oil, not only increases calorie content but also may vitiate the beneficial effects of n-3 PUFA.[17] Cooking has negligible effect on cholesterol content, although the relative concentration may increase because of water loss.

The changes that take place in fats during heat processing greatly depend on the fatty acid composition. In the presence of oxygen, unsaturated fatty acids may be oxidized to highly reactive peroxides, which decompose to a wide range of compounds. These compounds, which include aldehydes, ketones, alcohols, small carboxylic acids, and alkanes, give rise to a very broad odor spectrum and

also yellowish discoloration to the product. These substances are formed slowly at normal frying temperatures in pure fats, but their formation is catalyzed by traces of metals such as iron and copper present in the fish. In addition, overheating or repeated heating of fats results in an accumulation of the oxidation products, making the fat potentially toxic. Therefore, care need to be taken to control frying conditions, particularly in the case of coated products from fatty fish species.

Chilling in ice of fish and shellfish can lead to leaching of some nutrients, while prolonged frozen storage is associated with changes in their texture and flavor (Chapter 4). The degree of sensory changes depends upon the nature of the product, freezing conditions, and frozen storage. Nevertheless, refrigeration and frozen storage have little effect on proximate composition of fish, although PUFAs can undergo autooxidation to fatty acid hydroperoxides catalyzed by heavy metal ions during extended frozen storage. The hydroperoxides may also be formed enzymatically by the action of lipoxygenases (Chapter 2). However, presence of tocopherols and as well as astaxanthins in some fish such as salmon might function as antioxidants to reduce rancidity development during storage.[21] In fresh prawns, stored for 6 months at $-18°C$, the content of LA decreased from 39 to 17% and of docosahexanoic acid from 10 to 2%. High hydrostatic-pressure treatment of fatty fish may lead to lipid oxidation and flavor changes (Chapter 11).

Sun drying of fish results in only minor changes in the contents of amino acids, sulfhydryl groups, and available lysine as well as digestibility. Lipid autooxidation in fish is much more rapid during drying than frozen samples.[21] Lipid oxidation is particularly significant in salted and dried fishery products, which could be controlled to some extent by vacuum drying, vacuum packaging, and treating the fish with antioxidants such as butyl hydroxyanisole, before drying. The hydroperoxides formed can interact with amino acids resulting in loss of nutritive value of the fish, such as available lysine. Further, increase in the intensity of brown color of dried products is due to Maillard reaction between carbonyl compounds generated during lipid oxidation with ε-amino groups present in the fish proteins.[59–61] Lamination of fish prior to dehydration has advantages such as increased surface area, resulting in lesser time for drying, uniform drying rate, and better product appearance.[62] Drying at temperatures above 60°C causes appreciable damages to proteins. Drying of threadfin bream (*Nemipterus japonicus*) at 50, 60, and 70°C for 12 h caused decrease in the sulfhydryl group contents from 27.3 to 20.3, 11.0, and 5.2 mol/g dry matter, respectively. The digestibility of the fish proteins decreased from 77.8 to 71.1 after drying at 60°C for 12 h.[63] The process of hot smoking may cause some health hazards to some fish depending upon the conditions employed, due to accumulation of carcinogenic compounds such as nitrosoamines and some polynuclear aromatic hydrocarbons, particularly 3–4 benzopyrene (Chapter 12).

The effect of processing conditions on the protein quality of *surimi* prepared from Alaska pollock was investigated.[57] The parameters studied were protein digestibility, protein efficiency ratio, trypsin inhibitor content, and protein solubility. Steamed kamaboko from Alaska pollock containing 5% starch and 33% water had good protein quality measured in terms of PER and protein digestibility. The protein digestibility of all the products ranged from 86 to 89%. Of the different

processing methods for kamaboko (Chapter 8), steaming did not give any significant advantage with respect to digestibility of proteins, although a higher PER was observed. A two-stage steaming process, the first at 40°C for 20 min followed by a second steaming for 10 min at 95°C was found to be the most effective for the protein quality. PER values of marketed Korean *surimi* products ranged from 2.8 to 2.9, which were superior to that of casein, having a PER value of 2.5.[64] The *surimi* prepared from Alaska pollock also showed a balance of essential amino acids, with an overall score of 101, as compared with beef, pork, chicken, and turkey, which had scores of 99, 104, 101, and 100, respectively.[57]

Vitamins are considered the most susceptible to loss during heat treatment, but the magnitude of loss depends on the specific vitamins and the conditions employed. The loss may be due to leaching of water-soluble vitamins into the cooking medium and destruction of unstable vitamins under certain treatment conditions. A combination of oxygen, light, and heat causes a greater loss of nutrients than any one of these factors individually. Folate and vitamin B_6 are susceptible to destruction due to oxidation. Riboflavin is reasonably stable during cooking, but is sensitive to light as it decomposes on exposure to ultraviolet rays. The fat-stable vitamin A and carotenes are relatively stable at normal cooking temperatures, but the high temperatures used in frying can produce oxidative loses and isomerization of the carotenes, with significant losses of biological activity. Vitamin E is slowly destroyed during frying and is decomposed by light.

In summary, seafood items have significant contribution in providing various nutrients. Some nutritional losses as a result of processing need not pose concerns and consumption of processed food including seafood need not necessarily amount to compromising with their nutritional value.[65] Nevertheless, utmost care in applications of the processing methods can prudently minimize the inevitable nutrient losses. It needs to be emphasized that apart from the advantages of value addition, processing, in general, has a number of nutritional benefits such as enhancing palatability of the product, digestibility of proteins, and bioavailability of nutrients including fat, vitamins and minerals, resulting in the overall increase in the health benefits.

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Appendix

A.1 INTERNATIONAL AND NATIONAL ORGANIZATIONS

Codex Alimentarius Commission, www.codexalimentarius.net

Food and Agriculture Organization www.fao.org

Food and Agriculture Organization Training and Reference Center for Food and Pesticide Control www.iaea.org/trc

International Food Contaminant and Residue Information System (INFOCRIS) www.infocris.iaea.org

International Database on Insect Disinfestation and Sterilization (IDIDAS) www.ididas.iaea.org

International Institute of Refrigeration, Paris www.iifiir.org

International Council of Food Irradiation www.icfi.org

World Fish Center www.worldfishcenter.org

World Health Organization, www.who.org

World Trade Organization www.wto.org

Australian fisheries www.affa.gov.au/fisherystatus

Center for Disease Control (USA) www.cdc.gov/

Department of Agriculture USA www.usda.gov

Environment Protection Agency, USA www.epa.gov/

Environment Protection Agency, Office of Science and Technology, USA Fish Consumption Advisory Site www.epa.gov/ost/fish

European Economic Commission, Food-Linked Agro-Industrial Research (FLAIR), Concerted Project on Harmonisation of safety criteria for minimally processed Foods, http://ins.info/EUII/Eurovoc/food_technology.htm

Fish.net www.newsdata.com/enernet/fishnet

Fishbase www.cgiar.org/ICLARM/fishbase.index.htm

Food and Drug Administration, USA http://fda.gov/oia/foodcosm.htm

Food Navigator www.foodnavigator.com

HACCP Training Program Resource database (USA) http://agricola.nal.usda.gov/

Health foods www.healthfocus.net

Institute for Aquaculture, University of Stirling, UK www.stir.ac.uk/aqua/

Joint Subcommittee, Aquaculture, Purdue University, USA http://ag/ansc/purdue.edu/aquatic/jsa

Lobster Institute,University of Maine, USA http://lobster.um.maine/

Marine Science Institute, University of Texas, USA www.utmsi.zo.utexas.edu

National Fisheries Institute, USA www.nfi.org

National Food Processors Association, USA www.fpa-food.org

National Marine Fisheries Service, USA www.nmfs.gov

National Science Foundation, USA www.nsf.gov

National Science Foundation, Seagrant program, University of Maryland, USA
www.mdsg.umd.edu/NSGO/mdsg/

North Atlantic Fisheries College www.nafc.ac.uk

Pacific States Marine Fisheries Commission www.vims.edu/

Quick Frozen Foods International www.quickfrozenfoods.com

Seafood data search www.seafood.com

Seafood Network Information Center, University of California, Davis, USA
www.seafood.ucdavis.edu/

Virginia Institute of Marine Center, USA www.vms.edu

A.2   TRADE ORGANIZATIONS

Astra Food Distribution System for Seafood www.astrainf.com

European Seafood Exposition www.euroseafood.com

International Boston Seafood Exhibition www.bostonseafood.com

International West Coast Seafood Show www.westcoastseafood.com

Marine Products Export Development Authority, Cochin, India www.mpeda.com

Maritech International, WiseFishTM www.wisefish.com

Mediterranean Seafood Exposition www.medseafood.it

Net.YieldTM Inventory Control Software for Weight based Products by Lan
Infosystems www.laninfo.com

ParityProTM Food Enterpris SystemTM www.paritycorp.com

Seafood business www.seafoodbusiness.com

Seafood Processing Europe www.europrocessing.com

Seafood Recipes www.seafood.allrecipes.com

ShrimpUSA http://www.shrimpusa.com

Singapore Seafood Exhibition/Seafood Processing Asia
www.singaporeseafood.com

A.3   FISH NETWORK

GLOBEFISH	INFOFISH
Fishery Industries Division	1st Floor, Wisma PKNS
FAO	Jalan Raja Laut
Viale delle Terme di Caracalla	P.O. Box 10899
1 00100 Rome	Kuala Lumpur 50728
Italy	Malaysia
E-mail: globefish@fao.org	E-mail: infish@po.jaring.my
Website: www.globefish.org	Website: www.infofish.org

EUROFISH
P.O. Box 0896
UN Centre, Midtermolen 3
DK-2100 Copenhagen
Denmark
E-mail: info@eurofish.dk
Website: www.eurofish.dk

INFOYU
Maizidian Street
Chaoyang District
Beijing 100026
P.R. China
E-mail: infoyu@agri.gov.cn

INFOSAMAK
71, Boulevard Rahal El Meskini
16243 Casablanca
Morocco
E-mail: infosamak@onp.co.ma
Website: www.infosamak.org

INFOPECHE
Tour C-19 etage
Cite Administrative Abidjan 01
Cote D'lvoire
E-mail: infopech@africaonline.co.ci

A.4 Technical Manuals and Guidelines

Selected Technical Manuals and Guidelines from Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire GL55 6LD, U.K.

Guidelines for the establishment of scheduled heat processes for low-acid foods

Guidelines on good manufacturing practice for sterilisable flexible packaging operations for low acid foods

Process control in hydrostatic cookers

Hygienic design of food processing equipment

Good manufacturing practice guidelines for the processing and aseptic packaging of low acid foods

Guidelines for the establishment of procedures for the inspection of frozen food factories

Guidelines for microbiological challenge testings

Guidelines for food pasteurisation treatment

Evaluation of shelf life for chilled foods

The shelf stable packaging of thermally processed foods in semi-rigid plastic barrier containers: A guideline to GMP

Guidelines for modified-atmosphere packaging (MAP)

Food industry specification for defining the technical standards and procedures for the evaluation of temperature and time-temperature indicators

HACCP — a practical guide-second edition

Guidelines for *sous-vide* processing

Manual of microbiological methods for the food and drink industry

Guidelines for the use of thermal simulation systems in the chilled food industry

A code of practice for the manufacture of vacuum and modified atmosphere packaged chilled foods

Guidelines for batch retort systems — full water immersion-raining water-steam/air

Guidelines for the Air Shipment of Fresh Fish and Seafood, 2nd ed., Air Transport Association of America and the National Fisheries Institute, 1525 Wilson Boulevard, Suite 500, Arlington VA, 23209, USA.

Freshness, Quality and Safety in Seafood, Technical Manual, Dalgaard, P., The National Food Center, Dublin, Ireland, 2000, http://ucdavis.ed/pub/qualitysafety.doc

A.5 RECOMMENDED CODE OF PRACTICES

Codex Alimentarius Commission, *Recommended Code of Practices, Principles of Food Hygiene*, Vol. 1; and *Recommended Code of Practices for Fish Products*, Vol. B. Joint FAO/WHO Food Standards Programme, Rome, Italy, 1969.

The Codex Alimentarius Commission in its 26th Session has endorsed the Draft Code of Practice with regard to Aquaculture and processing of Quick Frozen Coated Fish.

A.6 HANDBOOKS, MANUALS AND RELATED PUBLICATIONS

Handbook of Canning and Aseptic Packaging, 2000
   Ranganna, S. TATA McGraw Hill Publishing Co., 7, Weast Patel Nagar, New Delhi 110008, India

Handbook of Food Process Equipment
   Saravacos, G.D. and Kostaropoulos, A.E., Academic Press/Plenum Press, New York, 2002, 698 p

Food Safety in Shrimp Processing
   A Handbook for Shrimp Processors, Importers, Exporters and Retailers
   Kanduri, L. and Eckhardt, R.A.
   Publisher: Fishing News Books, 2002
   Source: Fishing News Books, Osney Mead, Oxford OX2 OEL, U.K.

Marine & Freshwater Products Handbook
   Martin, R.E., Carter, E.P., Flicker, G.J., and Davis, L.M., Eds. Technomic Publishing Co., Lancaster, PA, 2000

Directory of Fish Importers, Exporters and Producers, 2004
   Published by FAO GlOBEFISH, Rome, Italy
   Covers 1622 companies in 26 European countries

International Aquatic Animal Health Code — Fish, Molluscs, and Crustaceans,
   4th ed., 2001
   Publishers: Office International *Des Epizooties*, France
   112 rue de Prony, 75017 Paris, France
   Fax:330-1426. E-mail: oie@oie.int.

Handbook of Frozen Foods, Hui, Y.H. et al., (eds.), Marcel Dekker, 2004
    The Nutrition Handbook for Food Processors
    Henry, C.K. and Chapman, C.
    Woodhead Publishing Co. and CRC Press LLC, 2002

Guide to Traceability
    EUROFISH
    P.O. Box 0896
    UN Centre, Midtermolen 3
    DK-2100 Copenhagen
    E-mail: globefish@fao.org

Aquaculture
    Aquaculture in the Third Millennium
    Subasinghe, R.P., Bueno, F.B., Phillips, M.J., Hough, C., Mcgladdery, E., and Richard, A., Eds.
    Source: Network of Aquaculture Centers in Asia-Pacific, P.O. Box 1040, Kasetsart Port Office, Bangkok 10903, Thailand.
    E-mail: naca@inet.co.th.

Value Addition
    Studies on Seafood Value Addition, Moller, A.B.
    FAO/GLOBEFISH, Special Market Study, Rome, FAO, 2003, 93 p

Freshwater Fish Processing and Equipment in Small Plants
    Bykowski, P. and Dutkiewics, D.
    FAO Fisheries Circular No. 905, 1996
    Source: Publication Division, FAO, Viale delle Therme di Caracalla 00100 Rome, Italy

Market Potentials
    Markets for different species of marine and freshwater fish and shellfish in different parts of the world, markets for *surimi*, fish-based ready meals, canned and other processed products (individual publications)
    Publisher: Fish Marketing and Utilization Service (FIIU)
    Under GLOBEFISH market research programme
    Source: INFOFISH/INFOPESCA

A.6.1 Other Publications

More efficient Utilization of Fish and Fisheries Products
    Sakagnchi, M., 2004, Elsevier, Applied Science, London.

Risk Assessment and International Fish Trade
    Fisheries Technical Paper No. 442
    L. Ababouch, FIIU, FAO, Rome, Italy, 2004

Assessment and Management of Seafood Safety and Quality
Huss, H.H., Ababouch, L. and Gram, L.
Fisheries Technical Paper No. 444, FAO, Rome, Italy, 2003

Pre-Harvest and Post-Harvest Food Safety, 2004
Blackwell Publishing, 2121 St. Av., Ames, IA 50014

Control of Fish Quality, 4th ed., 1995
J.J. Connell
Fishing News Books
Martson Book Services Ltd.
P.O. Box 87
Oxford OX2 OST, U.K.

Fish Inspection, Quality Control and HACCP
R.E. Martin, 1998
CRC Press, Boca Raton, FL

Microbial Safety of Minimally Processed Foods
J.S. Novak, G.M. Sapers, and V.J. Juneja,
CRC Press, Boca Raton, FL, 33431

Product Development and Seafood Safety
Joseph, J., Mathew, P.T., Joseph, A.C., and Muralidharan, V., Eds., 2003.
Central Institute of Fisheries Technology
Cochin, India

Seafood Safety
Surendran, P.K. et al., Eds., 2003.
Central Institute of Fisheries Technology
Cochin, India

Food Authenticity and Traceability
Lees, M., Ed., 2003
Woodhead Publishing Co. Ltd.
Cambridge, U.K.

Post-Harvest Technology of Fish and Fish Products
Balachandran, K.K., 2002
Daya Publishing House, 1123/74 Deva Ram Park, Tri Nagar,
New Delhi 110 035, India

Sausage manufacture: Principles and Practice
Essien, E., 2003
CRC Press, Boca Raton, FL

Tropical Fishery Products
Gopakumar, K., 1997
Oxford and IBH Publishing Co., New Delhi, India

Fish Processing Technology, 2nd ed.
Hall, G.M., Ed.
Blackie Academic and Professional, London
Source: Blackie Academic and Professional, 2-6 Boundary Rowa, London SE1 8HN, U.K.

Surimi and Surimi Seafood, 2nd ed.
Park, J.W. 2005
CRC Press, Boca Raton, FL

Physical Principles of Food Preservation
Karel, M. and Lund, B., 2003, Marcel Dekker, New York

A.7 CONSULTANCY

Megapesca Lda
Rua Gago Coutinho
Valado Sta. Quiteria
2460-207 Alfeizerao
Portugal
E-mail: admin@megapesca.com

Center for Processed Foods
Sadashivnagar, P.O.
Bangalore 560 080 India
E-mail: cpfbang@bgl.vsnl.net

A.8 PLANT AND EQUIPMENT

Seafood process plants — sorting and grading conveyors, scaling and skin removing machines, steam cookers, shrimp processing systems, glazing lines, weighing and packing equipment, contact plate freezers, IQF brine freeze systems, air blast freezers, bandsaw machines for frozen fish, equipment for thawing, ice-making machines, RSW Refrigerated seawater systems, ice storage and delivery system, and others.

A.8.1 Information Sources

Seafood Business
P.O. Box 908, Rockland, ME 04841, USA
www.seafoodbusiness.com

INFOFISH (Contact address given above)
Website: www.infofish.org

A.8.2 Processing Plant Lay Out

Infofish Int., 2, 63, 1998.

Food Buyers Guide
See *Food Technol.*, 57 (12), 61, 2003

A.8.3 Containerized Processing Plant

Most fish processing equipment are now available in compact, mobile, and even containerized form containing flake ice-making machines, blast freezers, IQF freezers, cold rooms, etc. They provide an ideal package for remote, isolated coastal locations. A typical unit has five 5.0×2.5 m^2 containers. The assemblage is complete with a flake ice machine, after processing unit, tunnel freezer (2 mt/24 h), plate freezer (650 kg/3 h cycle) and a cold storage unit. *Source:* Anonymous, *Infofish Int.*, 2, 64, 1998.

A.8.4 Machinery, Equipment, Packaging and Products (Incomplete List)

Koppens/Alfa Laval Souath East Asia Pvt. Ltd.
11 Joo Koon Circle
Singapore 2262

Koppens Food Machinery Ltd.
Interchange Park
Newport Pagnell Bucks, MK16 9PS, U.K.

Koppens Food Processing Equipment
Golflinks 151
New Delhi 110 013
India

Lee Innovations Mecaniques Alimentaires (Lima)
99, Rue De La Gare
29114 Bannalec
France

Atlas Industries A/S
Baltorvej 160
DK-2750 Ballerup
Copenhagen
Denmark

Bilbum Machine Construction Co. Ltd.
418 Myojin-Cho
Fukuyama-Shi
Hiroshima-Ken
Japan

Daegwang Machine Co. Ltd.
338-3, Jangrim 2 Dong
Saha-ku
Busan, Korea

Griffith Laboratories
757 Pharmacy Avenue
Scarborough
Ontario MIL 318
Canada

IBIS Gutting Machines (trout, salmon, seabass, and catfish)
IBIS Gut Schnede, D-21444
www.IBIS-aquaculture.de

Turbo Marine Co.
2003 S. 50th St., Tampa
FL 33619, USA

Nan Rong International Corp.
ADD:22F-3, No. 110, ?SAN-TUO 4RD, Kaohsiug, Taiwan

Berg Chilling Systems Inc.
51 Nantucket Blvd.
Scarborough, Ontario
Canada MIP 2NR

Carnitech A/S,
Industrimarken 2
Serup
DK-9530 Stovring

Cryo-Quick Air Products and Chemicals
Tel.: (610)481-4911

Gregor Jonsson Inc.
1520 Berkeley Road
Highland Park, IL 60035, USA

Baader North America
Tel.: (941) 561-3600

First Victory Machinery Co. Ltd.
First Victory Trading Co., Ltd.
#254 Jen Ai Road
Ta-Liao Hsiang, Kaohsiung Hsien
Taiwan ROC
www.firstvictory.com.tw
Packaging/Brody Inc.
P.O. Box 956187
Duluth, GA 30095-9504, USA

Cox Technologies, USA
(temperature measurement of packaged seafood)
http://www.coxtechnologies.com/

A.8.5 Retort Machinery, Pouch, and Products

Tetra Pak
101 Corporate Woods, Pkwy
Vermont Hills, IL 60061, USA
www.tetrapak.usa.com

See Brody, A.L., *Food Technol.*, 57 (11), 73, 2003, for a list of suppliers of
retortable packaging and machinery

Forstar Instafoods
M52 MIDC Industrial Area
Taloja, Dist. Raigad
Maharashtra 410 208
India

A.8.6 High-Pressure Processing

ACB Pressure Systems
2, rue Leon Durache — 44945
Nantes cedex 9
France
www.acb-ps.com

Avure Technologies Inc.
23500 64th Ave. S.
Kent, WA 98032
www.avure.com

Elmhurst Res. Inc.
60 Loudonville Road
Albany, NY 12204
USA
www.elmhurstresearch.com

Motivatit Sea Foods Inc., Houma, La
Alstom, Boulevard Prarie au Duc
44945 Nantes Cedex 9
France

Flow International Corp.
23500 64th Ave. S.
Kent, Washington 98032, USA

Kobelco Steel Ltd.
9-12, Kita Shinagawa 50chrome
Shinagawa-ku
Tokyo 141-8688
Japan

Stork Food and Dairy Systems, BV
Ketelstraat 2
1021 IX Amsterdam
The Netherlands

Elmhurst Res. Inc.
60, Loudnville Road
Albany, NY 12204
www.elhurstresearch.com

Gregor Jonsson Inc.
1520 Berkeley Road
Highland Park, IL 60035, USA
www.jonsson.com

Scotsman Europe, /frimont S.P.A.
ViaPuccini. 22
20010 Pogliano Milan
Italy

A.8.7 Food Irradiation

Guardion
P.O. Box 17349
Memphis, TN 38187-0349
USA

A.8.8 Extrusion Cooking

Wenger Manufacturing Inc.
714 Main St.
Sabetha, KS 66534
USA

A.8.9   Coated Products

Nienstedt GmbH, Germany
www.nienstedt.com
Koppens Machinefabriek
BV Beebakker 11-pb 1
5760 AA Bakel
Holland

Stein Inc.
P.O. Box 5001
1622 First Street
Sandusky, Ohio 44871-8001, USA

Coat & Fry of Derby
Pontefract Street Ascot Drive
Industrial Estate Derby DE2 8JD

DCA Industries Limited
Gatehouse Road, Aylesbury
Bucks, HP 19 3DL, England

Newly Weds Foods Inc.
4140 West Fullerton Ave.
Chicago, IL 60639, USA

Thai Food Coatings
909 Moo 15, Teparak Road
T. Bangsaothong
King A, Banagsaothong
Samutprakan 10540
Thailand

New Food Coatings Pty. Ltd.
32 Davis Road,
NSW 2164 Australia

RHM Ingredients Ltd.
Owl Lane, Ossett
West Yorkshire, WF5 9AX, U.K.

EBI-Cremica Food Coatings (P) Ltd.
B-XXXIII-324 GT Road
Julender By pass
Ludhiana 141005, Punjab
India

AEW THURNE Inc
1148 ENSELL Road
LAKE ZURICH
IL 60047, USA

A.8.10 Modified-Atmosphere Packaging Machine

CVP Systems, USA

A.8.11 Novel Fish Products

Samolux Inc., USA, Tel.: (253) 874-2026
Icicle Seafoods, USA, Tel.: (206) 282-0988

A.8.12 *Surimi* Products

Viciunai Europe N.V., Belgium, www.vici.It

Aquascot and Mainstream, Scotland, U.K., www.aquascot.uk.com

Agifish Company, Vietnam, www.agifish.com

Regal Springs Tilapia, Switzerland, www.regalsprings.com

Southern Pride Catfish LCC, USA, www.southernpride.net

Hindustan Lever Ltd., Marine Division, India, www.hllexports.com/hllexports/marine/

Zaloom Marketing Corp.
31 Schreiffer St.
So. Hackensack, NJ 07606, http://www.surimiseafood

Alaska Fisheries Development Foundation, Inc.
508 West Second Ave., Suite 212,
Anchorage, Alaska 99501

A.8.13 Accelerated Freeze-Dried Products

Accelerated Freeze Drying Co. Ltd.
Amalgam House, Bristow Road
Wellington Island, Cochin 682 003
India

A.8.14 Ingredients Including Neutraceuticals

Food Ingredient Specialities (FIS)
Case Postale 55
1618 Chatel-ST-Denis
Switzerland

A.8.15 Software

FishMonger™ Seafood Software. M.T.
Morrissey, Seafood Laboratory, Oregon State University
2001 Marine Drive, Asatoria, ORR 97103-3427, USA
Wisefish, Maritime International www.wisefish.com
Netyield Law Infosystems Inc www.lawinfo.com
Qual Trace International www.qualtrace.com
(*Source:* Duxbury, D., *Food Technol.* 58 (1), 60, 2004)

Quality Index Method software: "QIM Eurofish", www.qim-eurofish.com

A.9 MICROBIOLOGICAL STANDARDS

TABLE A.1
Microbiological Criteria for Fish and Fishery Products as Required by European Countries

Microorganisms	Standard
Pathogen	
Salmonella spp.	Absent in 25 g ($n = 5, c = 0$). In addition, pathogens and toxins thereof, which are to be sought according to risk evaluation, must not be present in quantities such as to affect the health of consumers
Indicator of poor hygiene	
(a) *Staphylococcus aureus*	(a) $m = 100, M = 1,000, n = 5, c = 2$
(b) *Coliform*	(b) $m = 10, M = 100, n = 5, c = 2$
(Thermotolerant, 44°C) or	
(c) *Escherichia coli*	(c) $m = 10, M = 100, n = 5, c = 1$
Mesophilic bacteria (30°C)	
(a) Whole product	(a) $m = 0, M = 100,000, n = 5, c = 2$
(b) Shelled or shucked	
product except crab meat	(b) $m = 50,000, M = 500,000, n = 5, c = 2$
(c) Crab meat	(c) $m = 100,000, M = 1,000,000, n = 5, c = 2$

m, limit below which all results are considered satisfactory; M, acceptability limit beyond which the results are considered unsatisfactory; n, number of units comprising the sample, c, number of sampling units giving bacterial counts between m and M.

The quality of a batch is considered to be satisfactory when all the values observed are 3 times m or less; (b) acceptable when the values observed are between 3 m and 10 m (M) and where $c/n = 2/5$ or less.

The quality of a batch is considered to be unsatisfactory in all cases where values are above M, where c/n is greater than 2/5.

TABLE A.2

The Japanese Government Bacteriological Standard and the Tokyo Metropolitan Governmental Bacteriological Guidelines for Frozen Foods.

Product	Type of bacteria						
	1	2	3	4	5	6	7
Frozen foods for serving without cooking	$<10^5$/g	N			N	N	
Frozen foods for cooked serving (to be cooked before freezing)	$<10^5$/g	N			N	N	
Frozen foods for cooked serving (not cooked before freezing)	$<3 \times 10^6$/g	N			N	N	20
Frozen fish and shellfish for raw serving	$<10^5$/g	N		N			20
Frozen cooked octopus	$<10^5$/g	N					
Frozen oyster for raw serving	$<5 \times 10^6$/g		<230 MPN/100 g				
Frozen fish and shellfish for processing	$<5 \times 10^6$/g				N		25

N, negative; MPN, most probable number.

1. Standard Aerobic Plate Counts per g.

2. Coliform per 0.01 g.

3. *Escherichia coli* per 0.01 g.

4. *Vibrio parahaemolyticus* per 0.01 g.

5. *Salmonella* spp. per g.

6. *Staphylococcus aureus* per 0.01 g.

7. Volatile basic nitrogen mg per 100 g.

Source: MPEDA Newsletter, 4, 2001, 16, Marine Products Export Development Authority, Cochin, India. With permission.

TABLE A.3
Recommended Microbiological Limits for Seafoods (International Commission on Microbiological Specifications for Foods)

Product	Test	Limit[a] per g or per cm[b]
Fresh, cold-smoked, and frozen fish	APC	5×10^5
	E. coli	11
	Salmonella spp.	0
	V. parahaemolyticus	10^2
	S. aureus	10^3
Precooked breaded fish	APC	5×10^5
	E. coli	11
	S. aureus	10^3
Frozen, raw crustacea	APC	10^6
	E. coli	11
	Salmonella spp.	0
	V. parahaemolyticus	10^2
	S. aureus	10^3
Frozen, cooked crustacea	APC	5×10^5
	E. coli	11
	Salmonella spp.	0
	V. parahaemolyticus	10^2
	S. aureus	10^3
Cooked, chilled frozen crabmeat	APC	10^5
	E. coli	11
	V. parahaemolyticus	10^2
	S. aureus	10^3
Fresh or frozen bivalves	APC	5×10^5
	E. coli	16
	Salmonella spp.	0
	V. parahaemolyticus	10^2

[a] Acceptable or attainable in good commercial practice.
[b] Aerobic Plate Count (equivalent to Standard Plate Count).

TABLE A.4
US FDA Standards for seafood Microbiological Quality Parameters

Sl.No.	Products	TPC/g at 37°C	Coagulase Positive Staphylococci	E.coli/g	Salmonella	Listeria
1	Fresh, chilled or frozen fish/prawn/cuttle fish	5,00,000	100	20	Absent in 25g	-
2	-Do- cooked/boiled	1,00,000	100	Nil	Absent in 25g	Absent in 25g
3	Cuttle fish/Squid for raw consumption	2,00,000	100	20	Absent in 25g	-
4	Crab, Clam, Mussel, Scallop, Oyster	1,00,000	100	20	Absent in 25g	-

TABLE A.5
Capture Production of Some Principal Species in the Year 2002

Popular name	Scientific name	Capture, in tons
Anchoveta (Peruvian anchovy)	*Engraulis ringens*	9,702,614
Alaska pollock	*Theragra chalcogramma*	2,654,854
Skipjack tuna	*Katsuwonus pelamis*	2,030,646
Atlantic herring	*Clupea harengus*	1,872,013
Blue whiting	*Micromesistius poutassou*	1,750,078
Chilean jack mackerel	*Trachurus murphyl*	1,650,078
Large head hairtail	*Trichiurus lepturus*	1,452,209
Yellowfin tuna	*Thunnus albacares*	1,341,319
European pilchard	*Sardina pilchardus*	1,089,836
Atlantic cod	*Gadus morhua*	890,358
Atlantic mackerel	*Scomber scombrus*	769,068
California pilchard	*Sardinops caeruleus*	722,071
Gulf menhaden	*Brevoortia patronus*	585,647
Argentine shortfin squid	*Illex argentinus*	511,087
Bigeye tuna	*Thunnus obesus*	430,289
Indian oil sardine	*Sardinella longiceps*	409,894
Argentine hake	*Merluccius hubbsi*	409,488
Saithe	*Pollachius virens*	381,335
Northern prawn	*Pandalus borealis*	375,878
Pacific saury	*Cololabis saira*	335,473
Pacific cod	*Gadus macrocephalus*	332,692
American sea scallop	*Placopecten magellanicus*	280,572
Haddock	*Melanogrammus aeglefinus*	248,442
Nile perch	*Lates niloticus*	257,272
Blue grenadier	*Macruronus magellanicus*	248,442
Atlantic horse mackerel	*Trachurus trachurus*	216,622
Indian mackerel	*Rastrelliger kanagurta*	185,649
Atlantic surf clam	*Decapterus russelli*	176,269
Blue mussel	*Mytilus adulis*	156,341

Source: Adapted from FAO Yearbook, Fishery Statistics, Capture Production, Vol. 94/1, 2002. With permission from Food and Agriculture Organization, United Nations, Rome, Italy.

TABLE A.6

Current Processing and Quality of Selected Commercial Marine and Freshwater Fish Species

Fish	Family	Genus	Quality and major processing types
Marine			
Cod	Gadidae	*Gadus morhua*	Used fresh, filleted, salted, and frozen, oil is produced from liver
Haddock	Gadidae	*Melanogrammus aeglefinus*	Very fine in taste, processed as fresh, pickled, marinated, smoked, fried, roasted, or used for salad
Bluefish	Gadidae	*Pollachius virens, P. pollachius*	Meat is lightly tinted, grayish brown. It is filleted, smoked, sliced as cutlets or chops, and processed in oil
Whiting	Merlangius	*Merlangius merlawngius*	Good meat, easily digested. Fried, roasted, smoked, or used for sausage
Hake	Merlucidae	*Merluccius merluccius*	Fresh or frozen, all processing methods are used
Sturgeon	Acipenseridae	*Acipenser sturio*	Exceptionally delicate, when smoked. Caviar is made from the roe
Herring	Clupeidae	*Clupea harengus*	Fine white meat, fried, or grilled
Sprat	Clupeidae	*Sprattus sprattus*	Mostly cold or hot smoked or canned
Sardine	Clupeidae	*Sardina pilchardus*	Canned in oil, grilled, or fried
Ocean perch	Scorpaenidae	*Sebastes marinus*	Tasty meat, fattier than cod, filleted or smoked
Tuna	Scombridae	*Thunnus thynnus*	Tasty reddish meat. Fried, roasted, smoked or canned, or used for sausage
Catfish		*Tachusurus filiceps*	Fragrant meat, poached, grilled, crust-coated
Mackerel	Scombridae	*Scomber scombrus*	Tasty reddish meat, fried, grilled, smoked, or canned
Red mullet	Mullidae	*Mullus barbatus*	White fine and a piquant delicious meat, mostly grilled
Turbot (Flat fish)	Scophthalmidae	*Psetta maxima Rhombus maximum*	The highly valued flat fish has snow white colored meat, firm and piquant. Cooked, grilled, or poached
Halibut (Flat fish)	Pleuro-Nectidae	*Hippoglossus hippoglossus*	Tasty meat, it is poached, fried or smoked

(Continued)

TABLE A.6
(Continued)

Fish	Family	Genus	Quality and major processing types
Flounder (Flat fish)	Pleuro-Nectidae	*Pleuronectes platessa*	White meat, poached, fried, or smoked
Sole (Flat fish)	Soleidae	*Solea solea*	Finest flat fish, poached, fried, grilled, or roasted
Eel		*Nemichithys scolopaceus*	Long slender bodies
Freshwater fish			
Salmon	Salmonidae	*Salmo salar*	High quality fish (5–10 kg). Fillets used as *sushimi*, grilled, cured, or smoked
River trout	Salmonidae	*Salmo trutta*	High quality fish, bluish tinted when cooked, roasted, or smoked
Rainbow trout	Salmonidae	*Salmo gairdenerii*	Valuable fish. Meat is pale pinkish. Filleted, smoked, canned
Brook trout	Salmonidae	*Salvelinus fontinalis*	A worthy fish, meat is pale pinkish, filleted, fried, smoked
Whitefish	Salmonidae	*Coregonus* spp.	Processed as trout
Smelt	Osmeridae	*Osmerus eperlanus*	A fish bone rich meat, which is mostly deep fried
Pike (jackfish)	Esocidae	*Exox lucius*	Young pikes (best quality when 2–3 kg in wt.) are slender and tasty, cooked
Carp	Cyprinidae	*Cyprinus carpio*	Soft, digestible bluish meat.
Bream	Cyprinidae	*Tinca tinca*	Tender fatty meat, bluish when cooked
Perch	Percidae	*Perca fluviatilis*	Firm, white and tasty meat. Filleted, fried, or steam cooked
Tilapia		*Oreochromis* spp.	Second most cultivated fish in the world. Fillets chilled, frozen, or breaded
Channel catfish		*Ictalurus punctatus*	Tender meat, ivory in color. Average market size <1 kg

Source: Adapted from Belitz, H.D. and Grosch, W., *Food Chemistry*, 2nd ed. Springer-Verlag, Germany, 1999.

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