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Irradiation of fish, shellfish and frog legs

A compilation of technical data for authorization and control

International Consultative Group on Food Irradiation
established under the aegis of
FAO, IAEA, WHO

INTERNATIONAL ATOMIC ENERGY AGENCY IAEA

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IRRADIATION OF FISH, SHELLFISH AND FROG LEGS
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FOREWORD

The International Consultative Group on Food Irradiation (ICGFI) was established on 9 May 1984 under the aegis of FAO, IAEA and WHO. ICGFI is composed of experts and other representatives designated by governments which have accepted the terms of the "Declaration" establishing ICGFI and have pledged to make voluntary contributions, in cash or in kind, to carry out the activities of ICGFI.

The functions of ICGFI are as follows:

(a) To evaluate global developments in the field of food irradiation;
(b) To provide a focal point of advice on the application of food irradiation to Member States and the Organization; and
(c) To furnish information as required, through the Organization, to the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food, and to the Codex Alimentarius Commission.

As of June 1999, the following countries are members of ICGFI:

Argentina, Australia, Bangladesh, Belgium, Brazil, Bulgaria, Canada, Chile, China, Costa Rica, Côte d'Ivoire, Croatia, Cuba, Czech Republic, Ecuador, Egypt, France, Germany, Ghana, Greece, Hungary, India, Indonesia, Iraq, Israel, Italy, Malaysia, Mexico, Morocco, New Zealand, Pakistan, Peru, Philippines, Poland, Portugal, Republic of Korea, Syrian Arab Republic, Thailand, Tunisia, Turkey, Ukraine, United Kingdom, United States of America, Viet Nam and Yugoslavia.

This publication contains the most up to date data on irradiation of fish, shellfish and frog legs. It is intended to assist governments in considering the authorization of this particular application of radiation processing of food and in ensuring its control in the facility and the control of irradiated food products moving in trade. It was prepared at the request of the International Consultative Group on Food Irradiation (ICGFI) in response to the increasing acceptance and application of irradiation to ensure hygienic quality of food, especially those of animal origin.

It is suggested that the various guidelines, codes and other documents adopted by the ICGFI or prepared under ICGFI's auspices be also consulted (see bibliography). Up to date information including publications of ICGFI may be obtained from its Web Page: http://www.iaea.org/icgfi/.

The preparation of this publication was made on initial inputs of the late J.J. Licciardello of the US Marine Fisheries Service, Gloucester, Massachusetts, USA. Significant comments/contributions to this publication were made by G. Rodrick, Department of Food Science, University of Florida, Gainsville, Florida, USA. V. Venugopal, Bhabha Atomic Research Centre, Trombay, India, and R.M. Grodner, Department of Food Science, Louisiana State University, Baton Rouge, Louisiana, USA. D. Ehlermann, Federal Research Centre for Nutrition, Karlsruhe, Germany, undertook to finalize this publication on behalf of ICGFI. He was assisted by R. Molins and P. Thomas of ICGFI Secretariat in so doing. The IAEA officer responsible for this publication was. P. Loaharanu of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

EDITORIAL NOTE

The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

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1. INTRODUCTION

The late J.J. Licciardello of the US National Marine Fisheries Service at Gloucester, Massachusetts, prepared a Code of Practice for Irradiation of Seafood in 1984. At this institute a marine products development irradiator had been operated for some years and many publications originated from the extensive use of this facility. That code reviewed pertinent seafood irradiation research which had been mainly performed in USA and was prepared as a background material for the ICGFI Code of Good Irradiation Practice for the Control of Microflora in Fish, Frog legs and Shrimp (ICGFI Document No. 10). The monograph presented here is an update of Licciardello's early work, it has been amended with research from other sources and has been updated with research performed since its original compilation. In 1994, G.E. Rodrick (Department of Food Science, University of Florida) with input from V. Venugopal (Bhabha Atomic Research Centre, India) was asked to revise the document for publication as an ICGFI monograph. It was then sent to R.M. Grodner (Louisiana State University) for review. His response was incorporated in a draft which was circulated to ICGFI national contact points in 1996, for comments. A number of contact points responded including several comprehensive comments. At the request of the ICGFI secretariat, D. Ehlermann (Federal Research Centre for Nutrition, Karlsruhe, Germany) agreed to finalize the document. This concluding revision also incorporated substantial amendments and an update of the chapter on methods of quality assessment with the help of C. Meyer (Federal Research Centre for Fisheries, Hamburg, Germany). This publication is a comprehensive review of seafood irradiation research along with a review of factors that must be considered when selecting irradiation as a means of processing. It contains up to date data on the effects of irradiation on *Vibrio* spp., especially in connection with seafood products eaten raw. Ample literature references are given without being complete; for further information the ICGFI Web Page (http://www.iaea.org/icgfi/) and the Bibliography on Food Irradiation should be consulted (issued by the Federal Research Centre for Nutrition, Karlsruhe, Germany, containing more than 13 000 up to date entries; interactive http://www.dainet.de/bfe/common/irradiat.htm#english). Readers may also refer to the services of the National Agricultural Library, Beltsville, Maryland, USA.

To successfully irradiate seafood, it is imperative to understand and consider the importance of proper handling of seafood, packaging, etc., and many of the other various factors that influence seafood quality and perishability. It is also indispensable, when considering the practical implementation and commercial exploitation of radiation processing of seafood that the peculiarities of fishing operations and the limitations for such operations imposed by international regulations are taken into account. Finally, consumer demand for seafood products and delicacies develops with time and is different for regions; it will also determine commercial success of the utilization of seafood irradiation.

2. QUALITY CHANGES IN AQUATICS PRODUCTS

2.1. MEANING OF QUALITY

The majority of aquatic products are subject to very little environmental control prior to harvest from the open sea, unless they have been caged, penned or aquacultured. Therefore, such fishery products primarily are accepted or rejected onboard a vessel based upon a quick visual assessment by the fisherman, despite strict governmental regulations. While landing and before auctioning, fishery products usually are inspected and rated for acceptability by highly proficient experts mainly relying on experience and not on analytical criteria. In dealing with aquatic

products, the term, quality, covers a vast array of definitions to different people. In the simplest terms, quality relates to the overall freshness of the product, based primarily on the amount of chemical and/or microbial spoilage. Several countries in their food laws have provided objective criteria for quality. The extent of spoilage observed in a particular product is determined by physical and environmental factors to which the product has been exposed. Finally, quality is defined by consumers' willingness to buy.

Processing seafood by ionizing radiation aims at shelf-life extension as well as hygienization by eliminating pathogenic microorganisms of public health significance. The term shelf-life is ambiguous; it is related to the time interval elapsed from catch until a certain limiting quality is reached. Such limits can be: a) no noticeable difference from initial and highest possible quality, sometimes called 'high-quality life (HQL)'; b) some perceivable difference from optimum quality but still acceptable for sensory properties, sometimes called 'practical storage life (PSL)'; c) onset of spoilage where the product is to be rejected. In the literature when reporting shelf-life extension, such differing criteria have been used to define quality in many cases without specifying the relevant quality criteria and it is obvious that the reported 'shelf-life' will differ considerably depending on the quality criteria applied. This must be taken into account once the benefits of radiation processing applied to seafood are to be estimated from published data. Caution should be paid to the fact that shelf-life is sometimes reported as the time period until final spoilage occurs. To extend only the volume of low quality seafood close to spoilage on the market cannot be the goal of a high-quality operation such as radiation processing. Seafood is not only a basic source of protein supply but it is also a highly estimated delicacy. And only seafood of highest initial quality would be worth the effort and costs of radiation processing.

2.2. FACTORS THAT INFLUENCE QUALITY

There are many factors that can influence the quality of aquatic products and these include: (1) species; (2) size; (3) distance (travelling time) from fishing ground to the port; (4) diet effects (how fish feed); (5) fishing grounds; (6) sex and spawning effects; (7) parasites; (8) naturally toxic fish; (9) effects of pollution; and (10) other peculiarities in fish. The degree to which any one or combinations of these factors influence quality is variable.

2.2.1. Species effects

The rate of spoilage experienced by a product is species dependent. It is quite clear that fatty species, such as sardines and mackerel, spoil much more quickly than lean species like grouper whether in the chilled or frozen state. Such species are also rich in nutritious, poly-unsaturated fatty acids the oxidation of which causes conspicuous rancidity. Fat content in fish, especially pelagic fish, varies considerably over the course of a year, and it is these differences in fat composition that may also influence the quality of the fish. The glycogen contained in the fish flesh is converted to lactic acid after death. If there is a low lactic acid content, then the pH of the flesh will be higher. Spoilage bacteria are generally more active at higher pH, and thus spoilage can occur at a faster rate if less lactic acid is formed. Lean fish, with a lower glycogen content, stored under poor conditions can also cause an increase in the pH of the flesh. Low flesh pH is also quite undesirable in terms of the quality of the fish, and may result in a condition known as chalkiness. This is a condition that develops when the pH of the flesh drops below 6.0, and the raw fish fillets appear white and dull. The flesh will toughen more quickly at a low pH, and honeycombing also occurs in tuna flesh with a low pH.

2.2.2. Size effects

In general, large fish command higher prices mainly because some consumers feel that bigger is better. Large shrimp and lobsters are much more visually satisfying. However, there is little evidence to support claims that larger fish have better flavour or quality. Yet, processors will pay more for large products because the yield is higher, handling costs per unit weight are less, spoilage is slower, and more uniform products can be created from them. Immediately after harvest/catch fish carry microorganisms causing spoilage only on the surface and in the intestines; hence, microbial spoilage starts from these surfaces. It is well established that large fish have a longer shelf-life than small fish (Connell, 1980). Large fish have a smaller surface area to volume ratio than small fish, thus less of the interior portions of the larger fish is affected. Another size effect is again related to flesh pH. Smaller fish of a particular species tend to have a higher post-rigor pH than larger fish, thus bacterial actions can be enhanced (Connell, 1980).

2.2.3. Distance to port

The time required to eviscerate and properly ice fish products, i.e. the time elapsed between catch and final storage in the trawler's holds, determines the achievable shelf-life; the amount of icing to maximize shelf-life is directly related to how far the vessel has to travel between its fishing ground and port. This is of greater concern in warmer climates due to the effects that elevated temperatures have on the perishability of seafood products. Kordyl and Karnicki (1969) showed clearly that stored on deck mackerel (*Scomber colias*), horse mackerel (*Trachurus trachurus*) and cape hake (*Merluccius capensis*) spoiled within 2, 4, and 6 hours respectively at 27°C .

Furthermore, in long distance fishing travel from the fishing ground to the port might use a significant portion of the achievable shelf-life. Developments of international sea law, expansion of territorial waters, and over-fishing of many grounds has led to prolonged travel of trawlers, thus using periods as long as half of the achievable shelf-life. Such considerations, of course, do not apply to factory ships where the catch is directly converted into the final deep-frozen fishery product.

2.2.4. Diet effects

It is well documented that the diet of the fish, whether it be smaller fish or larger fish, can affect the quality, particularly the falvour of sea food products. This has been clearly demonstrated especially in aquacultured products, e.g. pond raised catfish take on whatever flavour that their diet consists of. Catfish fed turkey livers, developed a turkey liver flavour after 19 days on the diet. Similarly, an all cereal diet led to a cereal like flavour in catfish after 33 days on the diet. (Maligalig *et al.*, 1973). The identical effects occurs when 'wild' fish on the high sea are feeding on a 'blooming sea', i.e. on a fishing ground with explosive outgrowth of certain plankton; it is also well-known that shellfish can become toxic to man when feeding on such plankton ('red tide bloom') which by its physiology is producing some unwanted metabolic substances accumulated by the shellfish.

2.2.5. Fishing grounds

The area of the world in which fish are caught also influences the quality. Flavour of species varies seasonally with winds, tides, water quality and temperature, migration, pollution,

spawning status (as fish come to certain grounds only for certain phases of their life-cycle) and feed availability.

2.2.6. Sex and spawning effects

It is well known that male and females in the same species can command different values, based on their spawning status. The presence of eggs in a female may be highly undesirable in one species, yet considered a delicacy in another. Spawning tends to be very stressful to fish, and thus this stress leads to a poor health condition developing, and thus a less desirable fish. Flesh composition can change drastically with fat, protein and carbohydrate reserves being all used up, and thus the sensory characteristics of the fish are diminished.

2.2.7. Parasites

Many fish are in fact infected by parasites that may or may not be of concern for human consumption. Certain protozoa, flatworms and nematodes are of primary interest, but most are either located in the offal which is discarded anyway, or they are easily killed by cooking. The nematodes are of special interest because they can be encysted in the flesh of a fish. However, it is clear that these worms are also easily killed by freezing. It is obvious that the presence of visible worms or nematodes whether inactivated or not is unsavoury. Thus if raw consumption is the goal, freezing (for nematodes) or processing by ionizing radiation (for other parasites) prior to consumption is an effective means of killing these parasites. In other instances, despite inactivated or killed, the presence of the parasites in the cooked meat is not acceptable for aesthetic reasons.

2.2.8. Naturally toxic fish

Certain species of fish are naturally toxic and may cause illness or death if consumed. Most of these toxic fish are caught in the tropical and subtropical waters around the world. Thus, concerns with these species are only appropriate where they are indigenous. Naturally toxic fish are generally referred to as biotoxic. This should be contrasted with those fish and shellfish that may become toxic by contamination with chemicals and other pollutants dumped into the environment. Three main types of toxin occurring in fish or shellfish are: ciguatera, puffer fish poisoning, and paralytic shellfish poisoning.

2.2.8.1. Ciguatera

This condition affects several hundred species of coral reef dwelling fish and shellfish. Fish apparently acquire this toxin, but have the ability to lose their toxicity quickly. This is thought to occur as a result of changes in their diet (Connell, 1980). Humans can become poisoned with ciguatera by eating fish that are in the toxic state. Symptoms of the disease include nausea, diarrhoea, vomiting, and a tingling sensation in the hands. The toxin is heat stable and not easily degraded by cooking.

2.2.8.2. Puffer fish poisoning

Puffer fish are of the *Tetraodontidea* species, and consumption of this toxin is of greater concern than ciguatera. These fish are considered a delicacy and are consumed in only a few countries, including Japan. However, it is the viscera of these fish that are particularly toxic to

humans. The fish may be safely consumed as long as the flesh is not contaminated by contact with the viscera.

2.2.8.3. Paralytic shellfish poisoning

This poisoning results from consumption of a reddish dinoflagellate present in large concentrations in shellfish such as oysters, mussels, and clams. Molluscs feed on the dinoflagellates in seawater and concentrate the toxin produced by the organisms in their own bodies, without themselves being affected. The toxin is also heat stable, and not completely destroyed during cooking. This disease has been reported throughout the world, including the USA. For example, in 1973, five people near Sarasota, Florida, exhibited symptoms of paralytic shellfish poisoning after consuming clams caught in waters of the Gulf of Mexico where a red tide bloom had been reported (Anon. 1973).

2.2.9. Effects of pollution

The rivers and oceans of the world have been used as man's dumping grounds; the situation is only gradually improving. Thus, there are many potential sources of contamination which are capable of causing serious damage to the environment and health problems for humans. The main classes of pollutants are metals, chlorinated hydrocarbons, and microorganisms. Radiation processing of fish cannot contribute to alleviate this situation or to decompose some of the pollutants or residues; except for microorganisms which can be eliminated or at least reduced to a non-hazardous level. However, 'radicidation' is a perfect tool to reduce the microbial load which is already present without the additional man-made hazards and to minimize the pathogenic risk to the consumer.

2.2.9.1. Metals and other elements

Lead, mercury, cadmium, selenium, and arsenic receive the most attention. Yet, mercury is the only one to be linked to human disease caused by the consumption of contaminated fish. One of the most famous examples of mercury poisoning occurred in two cities in Japan in the 1950s: Minamata and Nigata. Wastewater discharges from local chemical plants were loaded with mercury which biologically transformed into methylated mercury. This assimilated and concentrated in fish in Minamata Bay, yielding 27 to 100 ppm of mercury on a dry weight basis in these fish. The population suffered 121 cases of mercury poisoning, 46 deaths, and 23 cases of a palsy-like affliction, now known as Minamata Disease. In Nigata, between 1964 and 1965, there were 6 deaths and 47 cases of mercury poisoning reported (Chanlett, 1979). The effects of cadmium, lead, selenium and similar pollutants on fish quality thus far has not been serious due to very small concentrations. Yet, filter feeding bivalves taken from in shore waters were found to contain high concentrations of cadmium, zinc, and lead (Chanlett, 1979).

2.2.9.2. Chlorinated hydrocarbons

Dichlordiphenyltrichloroethane (DDT) is a banned insecticide and an environmental residue which has been found in aquatic food chains, birds, and in many of our foods. Human illness from DDT has not been demonstrated, but birds have had documented health problems from feeding on insects and worms that have been treated with the chemical (Chanlett, 1979). Chlorinated hydrocarbons such as DDT, aldrin, dieldrin and polychlorinated biphenyls (PCBs) have all been linked to population decreases in birds, sea trout and salmon. Like DDT, PCBs persist in the open environment and accumulate in fish (Chanlett, 1979). Objectionable odours

or off-flavours in fish may also be the result of ingestion of pollutants or absorption of chemicals from the environment.

2.2.9.3. Pathogenic microorganisms

The introduction of raw or inadequately treated sewage into fishing areas is one way in which pathogenic microorganisms enter our food. Sewage almost always contains some bacteria and viruses which can lead to disease. Marine sediments are likewise loaded with such microorganisms. Two common bacteria of interest include *Salmonella* which cause nausea, vomiting and diarrhoea, as well as typhoid and paratyphoid, and *Shigella*, which is responsible for dysentery. *Vibrio* bacteria such as non-O1[1] *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are also a great concern especially in warm, temperate waters because they too concentrate in the digestive tract of shellfish. Since 1979, several hundred cases of *Vibrio* infections and over 60 deaths have been reported.

The Hepatitis A virus is connected with sewage pollution, and is responsible for causing infectious Hepatitis. These microorganisms are transferred through the water and faeces to mainly shellfish growing in sewage polluted waters. Bivalve shellfish, such as oysters, clams and mussels, tend to concentrate microorganisms and are thus a public health risk since they are frequently eaten raw. Oyster harvesting areas in Alabama, Louisiana, and Texas were closed when outbreaks of infectious Hepatitis and Norwalk were attributed to shellfish. Viruses can concentrate and accumulate in shellfish rapidly and can survive dry storage in shellstock for 1 month at 5°C (Chanlett, 1979).

2.2.10. Other peculiarities

There are many fish that are harvested which have serious defects. Colour changes are quite common, as are some physical abnormalities. Many times these can be attributed to prey-predator relationships at sea.

3. SPOILAGE OF MARINE AND FRESHWATER FOOD PRODUCTS

Spoilage commences with the death of the fish, and a complex series of reactions and interactions occur to spoil the fish. At the same time, early breakdown metabolites are responsible for flavour development and 'ripening'. There are many examples of reactions that may occur in spoiling fish; (1) the degradation of protein with the subsequent formation of hypoxanthine, trimethylamine and other products, (2) the gradual development of undesirable odours and flavours, (3) the softening of the flesh, and (4) the loss of cellular fluid and, hence, of juiciness. There are three basic mechanisms of spoilage in fish: microbial, enzymatic, and chemical.

[1] Serotype O1 and others are toxigenic strains of *Vibrio cholerae* which can cause cholera in humans, 'non-O1' designates a variety of other strain which do not cause cholera but milder forms of diarrhoea and infections.

3.1. MICROBIAL SPOILAGE

Microbial spoilage is by far the primary mechanism of spoilage of chilled fish and shellfish. Large numbers of bacteria are present in the surface slime of fish, on the gills, and in the intestines. The flesh initially is sterile. Bacteria can enter the flesh through any punctures or open wounds, and secrete enzymes that break down and dissolve tissue, causing spoilage. Such damages occurs, in particular, when fish is handled during *rigor mortis* which is unavoidable during certain fishing operations. The results of such penetration of microorganisms are odour and flavour changes which are described as sour, grassy, acidic, bitter, or sulphide and ammonia in the final stages. The slime on the skin and gills may change from a normal clear, watery appearance to cloudy or discoloured, which causes the skin to become dull and bleached looking; the gills especially develop early off-odours which serve as a first quality criterion during inspection upon landing. The stomach lining deteriorates and readily detaches from the internal body wall. The bacterial flora of fish (and its relative composition by species) is influenced by a number of factors such as season, environment, water quality, etc. After *rigor mortis*, bacterial spoilage begins and juices are released from muscle fibres. Thus, delaying rigor will extend the shelf-life. Low pH and proper cooling are the best methods, yet high temperature and low oxygen enhance rigor. The pH of the fish flesh is also important because lower flesh pH has slower bacterial decomposition (Connell, 1980). In the gills, the bacteria grow rapidly leading to off-odours and discolorations. Off-coloured, slimy gills are an indication of poor quality fish. Once in the gills, bacteria enter the vascular system, and into the flesh. Bacteria located on outer surfaces are closer to the fillets, than are the bacteria in the intestines. Fish may also become contaminated by chilling them using unsanitized ice. Fish can be contaminated by the deck of the vessel, the fishermen handling them, or by the storage pens or boxes. It must also not be overlooked that certain operations still onboard such as evisceration, filleting and removal of the skin from the fillets are all mechanical means to alleviating the intrusion of microorganisms into the flesh portion.

Bacteria causing spoilage vary greatly with temperature, but lower chilling temperatures are the greatest concern. *Pseudomonas* species predominate upon refrigeration and are mainly responsible for putrefaction (hence *Ps. putrefaciens*), followed by *Achromobacter* (now named *Moraxella*) and *Flavobacterium* species (Frazier, 1967). As the temperature rises, the genera *Micrococcus* and *Bacillus* seem to take over. Normally, the *Pseudomonas* species increase over time during refrigeration, while *Achromobacter* tend to decrease (being suppressed by the metabolic activity of *Pseudomonas*). The *Flavobacterium* levels increase temporarily with an eventual decrease (Frazier, 1967). *Ps. fragi* produce fruity, ammonia, and sulphide odours in cod (Shewan, 1962). Several of these organisms are capable of secreting proteases which can degrade fish muscle even at refrigerated storage (Venugopal, 1990). Fish flesh contains a large amount of non-protein nitrogen (NPN). Natural fish enzymes induce changes which increase the supply of amines, amino acids, and glucose for bacterial growth. Bacteria can convert these compounds to trimethylamine(TMA), ammonia, amines, and aldehydes. Hydrogen sulphide, mercaptans, and indole can be produced and they are clearly indicative of putrefaction. The odourless compound trimethylamine oxide (TMAO) found in salt water fish only, when reduced to TMA yields an ammonia odour, but can combine with other compounds to give off a fishy odour. The reduction of TMAO to TMA over time has been used as a chemical measure of spoilage in certain marine fish. Besides off-flavours and odours, a discoloration of the flesh may occur during spoilage such as with *Ps. fluorescens.* This is a yellow bacterium that causes flesh to turn a yellow to greenish-yellow colour (Frazier, 1967).

3.2. ENZYMATIC SPOILAGE

Enzymes are proteins present in the flesh and stomachs of fish and shellfish to catalyze chemical reactions. During life, enzymes control many body functions under a very complex control system; upon death, this coordinated control is no longer functioning. Upon harvest, fish and shellfish usually contain food in their gut, and thus enzymes are present. Upon death, these enzymes penetrate the gut wall, and the flesh may then be invaded by spoilage bacteria. Generally, such enzymes cause degradation. Involvement of hydrolytic enzymes in the spoilage of fishery products has been demonstrated by Warrier *et al.* (1985) and Sherekar *et al.* (1986).

Enzymes also are involved in the development of *rigor mortis*, a stiffening observed as a result of the coagulation of muscle protein. The degree of rigor depends upon the species, temperature, and condition of the fish. Rigor usually passes before bacteria invade the muscle, leaving the flesh soft and limp. *Rigor mortis* can have some effect on handling and processing. In some species, the muscle tends to contract under strain, resulting in broken tissues. Some crustaceans, such as shrimp, langoustines, lobsters, etc., suffer from an enzymatic change (polyphenoloxidase) resulting in 'black spot' or melanosis (Bramsnaes, 1969; Fieger and Novak, 1961). This is caused by a black pigment beneath the shell that starts in the membranes connecting the overlapping ends of the tails. Black spot does not affect the taste or quality of the shellfish, but is indicative of poor handling aboard the vessel, and the shellfish are deemed to be of poorer quality (Anon. 1980).

3.3. CHEMICAL SPOILAGE

Oils and unsaturated fatty acids (lipids) contained in fish flesh undergo changes that produce rancid odours, off-flavours, and colour changes; in the fresh state such fat compounds are responsible for desirable flavours. The combination of the oils and fats with oxygen, or autoxidation, competes with microorganisms and their enzymes in spoilage. The rate of rancidity is directly related to the species of fish. Fish with a high fat content and significant portion of multiple-unsaturated fatty acids have a short frozen shelf-life, because of oxidative rancidity reactions. Tuna, mackerel, herring, and salmon are examples of these types of species. Freshwater trout has a high fat content, and are also very susceptible to oxidative rancidity. Other factors also contribute to oxidative changes. Within the same species, small fish spoil more rapidly than large fish, due to surface bacteria effects (Merritt, 1969). Oxidative rates are affected by the state of the fish when harvested, diet, season, fishing ground, sexual development, as well as post-harvest techniques such as: bleeding, gutting, chilling, and storage (Merritt, 1969). The colour of the oils and fats varies from colourless in herring, to red in salmon (as the carotenoid pigments are soluble in fat). The colour also varies with the food eaten by the fish (Bramsnaes, 1969). Fish oil is highly unsaturated and will oxidize, become rancid, and turn yellow. Herring have a layer of oil just under the skin which turns rancid and yellow under poor conditions. The main colour pigments in fish flesh (except carotenoids in *Salmonidae*) are haemoglobin in the blood, and myoglobin in the cellular tissues, e.g. in tuna. Poor storage results in loss of the desirable fresh-red meat colour. Many quality changes occur in fish, individually or in combination, while stored on ice or frozen. These changes can be limited, or completely negated by utilizing proper handling and storage techniques. Irradiation of fish is a pasteurization treatment, hence eliminating the pathogenic and reducing the spoilage microflora. However, to prevent spoilage caused by chemical turnover, proper handling is indispensable.

3.4. SPOILAGE OF FRESHWATER FISH

Bacterial spoilage of freshwater fish is not as well understood as marine fish, however, there are certain similarities. Bacteria and their metabolic products lead to spoilage, as do enzymes in the fish muscle and intestines. The most common genera found are similar to those in seawater, plus species of *Aeromonas*, *Lactobacillus*, *Brevibacterium*, *Alcaligenes*, and *Streptococcus* (Frazier, 1967). The intestines of both marine and freshwater fish contain bacteria of the genera *Achromobacter*, *Pseudomonas*, *Flavobacterium*, *Vibrio*, *Bacillus*, *Clostridium*, and *Escherichia*.

3.5. SPOILAGE OF MARINE FISH

Shewan (1961) and Shewan and Hobbs (1967) combined the works of several researchers who studied the microflora of fresh marine fish (cod, haddock, hake, sole, and skate) caught in the north and mid-Atlantic. The most common genera isolated from the gills, intestines, and slime were *Pseudomonas* and *Achromobacter* , accounting for nearly 60 percent of the total bacteria isolates present. Comprising 20 percent of the isolates, were *Corynebacterium*, *Flavobacterium*, and *Micrococcus*. Fish do reflect the microflora of their environment, and thus fish from inland and coastal waters are subject to pollution. Microbial growth, i.e. multiplication and proliferation, starts with fish death, as the natural defense mechanisms are destroyed. Bacterial growth rates increase at temperatures above 4°C and can remain active up to approximately 60°C. This temperature range, where bacteria are most active, is referred to as the Danger Zone. Most bacteria are killed above 60°C, and growth is inhibited at or below 4°C (Davidson, 1975). However, a temperature below 4°C does not guarantee inhibition of bacteria. Some bacteria, psychrophiles, continue to grow at temperatures as low as 0°C to –2.7°C (Castell, 1973). When considering fish spoilage, it is important to note the psychrophilic nature of the indigenous microorganisms. Psychrophilic organisms, such as the genera *Pseudomonas*, *Achromobacter*, and *Flavobacterium*, those primarily associated with spoilage of fish, can reproduce at 0°C and higher (Adams *et al.*, 1964, Shewan *et al.*, 1960). The role of extracellular enzymes of these bacteria in fish spoilage has been discussed by Venugopal (1990). Two other important groups of bacteria are the mesophiles and thermophiles.

Mesophiles, which are microorganisms that do not grow below 10°C, include *Bacillus*, anaerobic types, and most human pathogens. Elasmobranchs (sharks, rays, and skates) contain high levels of urea in their flesh, which can be converted to ammonia. As microbial spoilage proceeds, the bacterial enzymes attack the flesh proteins, resulting in a weakening and softening of the flesh, and formation of urea, resulting in putrid odours.

3.6. SPOILAGE OF SHELLFISH

Shellfish are affected by microbial spoilage just as in fish. Shrimp, crabs, lobsters have species of *Achromobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, and *Proteus* present in their surface slime. On chilled shrimp, *Achromobacter* predominates, and causes the most spoilage (Frazier, 1967). Upon de-heading and peeling, stomach contents are inevitably spilled over the meat piece, thus spreading microbial contamination. Chilled crab meat is spoiled primarily by *Pseudomonas*, *Achromobacter*, and *Proteus* at higher temperatures (Frazier, 1967). Spoilage of raw lobsters is caused by species of *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and *Bacillus* (Frazier, 1967). Oysters, clams and other filter feeding shellfish, pick up sediment and water microorganisms, including pathogens. Oysters remain acceptable as long as they are chilled in the shell, but once they are shucked, they deteriorate rapidly. Near

freezing temperatures, *Pseudomonas* and *Achromobacter* species are chiefly responsible for spoilage, but *Flavobacterium* and *Micrococcus* species may also be present. Souring is the result of fermentation of the sugars by coliform bacteria, such as *Streptococcus,* and *Lactobacillus*, and yeasts that produce acids and sour odour. Growth of species of *Serratia* and *Clostridium* may also occur. Oysters can turn a pink colour which is caused by spoilage produced by an asporogenous yeast (Frazier, 1967). Bacterial counts in deheaded shrimp can be significantly lower in that the head (closely associated with the stomach) carries about 75 percent of the bacteria. The species found associated with shrimp are mainly *Achromobacter*, *Bacillus*, *Micrococcus*, and *Pseudomonas*, together making up 78 percent of the isolates. During iced storage, the flora on these same shrimp changed so that *Achromobacter* species made up 82 percent of the isolates after 16 days.

4. QUALITY LOSS AND ITS CONTROL

Three major avenues can be taken to slow down quality loss in fish: proper handling, cleanliness, and refrigeration. The importance of handling cannot be overstated, since spoilage bacteria enter through cuts and abrasions sustained during handling. Cleanliness is important for two reasons. First, the removal of slime and the guts will relieve the major sources of bacterial contamination. Second, sanitary handling helps block contamination from external sources. The primary way of slowing quality loss is through cool temperatures.

The appropriate handling procedures are difficult to codify; therefore, the principles of the approach to appropriate handling and of the control of any defined step or procedure have been laid down in writing. This systematic and scientific approach is now generally acknowledged as "Hazard Analysis and Critical Control Point (HACCP)" (Anon., 1996). It consists essentially of a well documented procedure to analyze any possible or imaginable hazard to fail to produce the required quality and the subsequent establishment of the most appropriate point to control this hazard during production. By itself, HACCP leads to protocols and sufficient documentation. The hazards analyzed include biological, chemical or physical contamination of the product. In the USA it is already regulated for meat and poultry production that HACCP is to be implemented. This model can easily be transferred to seafood and its radiation processing. As generic HACCP plans are general, they must finally be tailored to fit the product in a specific plant.

4.1. PREPARATION OF FISH PRIOR TO STORAGE

The chilling of fish and shellfish is accomplished by the use of ice or mechanical refrigeration. Fish begin spoiling at death, and poor quality results after only a few hours post harvest. Temperature is of paramount importance since bacterial growth and chemical changes depend on adequate temperature. Moreover, the preparation of fish prior to storage involves: (1) sorting; (2) gutting; (3) bleeding; and (4) washing. Some economically important species as red perch but also small fish such as sardines or sprat, however, are never gutted or bleeded.

4.1.1. Sorting

It is important that the catch be separated by species prior to processing. Soft-fleshed fish should be distinguished from harder fleshed fish, and smaller fish should be selected before larger ones.

4.1.2. Gutting

Gutting, or evisceration, consists of removing the intestines and gut cavity. The goal is to reduce the bacteria and enzymes present which contribute greatly to spoilage. The entrails should be removed and not allowed to contaminate the flesh.

4.1.3. Bleeding

After gutting, the fish is bled. Bleeding of lighter fleshed species produces a fillet that is more desirable. Any blood clots, or dark patches in the flesh affect the eye appeal and reduce product acceptability. Near freezing, fish blood remains fluid for about 30 minutes and clots rapidly after this time. After capture, fish should be cooled immediately, and bled. Overall colour improvement is obtained by proper bleeding. Bruises and other mechanical damage to the fish must be avoided as bloody patches remain.

4.1.4. Washing

Fish should be washed completely after gutting preferably using clean potable water. Washing is effective in removing blood and viscera from the fish. Poundboards, knives, shelves, and storage boxes should be regularly disinfected.

4.2. QUALITY CHANGES PRIOR TO FURTHER HANDLING/PROCESSING

Once gutted and washed, the temperature of the fish should be lowered quickly to just above freezing. Between −1°C and 4°C, the risk of spoilage is minimal. Between 4°C and 38°C, most bacteria are able to grow and flourish. From 38°C to 60°C, most food poisoning and spoilage bacteria grow very rapidly. Temperatures above 60°C kill most bacteria.

4.2.1. Chilling aboard fishing vessels

Good quality ice (drinking water quality) should be used to provide clean moisture, and aerated storage for the fish. Ice plays an important role in preventing dehydration of fish during storage, and serves to cool fish down to 0°C to 2°C. Ice adds protection in the form of the washing effect, in that as it melts, it washes away blood, bacteria, and slime. This is important in maintaining quality, however, good drainage must be employed so that the fish do not lie in the contaminated melted water. If there is great delay before cooling, bacterial spoilage will reduce the shelf-life. There are vast differences in the way different species spoil, so extra care may need to be taken depending on the catch. Haddock spoil more quickly than cod, and whiting spoil even faster. Elasmobranchs like sharks and rays produce significant ammonia during ice storage and usually become unacceptable after 8 days. These types should be clearly separated from others. Small fish spoil more rapidly than large ones, and fully fed fish spoil more rapidly than starved fish.

Each individual fish should be in complete contact with the ice, and should be arranged so that blood, bacteria, and slime can adequately drain. Fish should not be pressed down by the ice, so that shrinkage and loss of weight is kept to a minimum. Drip loss is another concern in that fluid is lost slowly from tissues, carrying away some flavour compounds, resulting in a general loss of flavour. Table I gives some observed storage times for a variety of species stored on ice.

TABLE I. ICED STORAGE TIME OF SOME MARINE SPECIES OF INTEREST

Species	**Days**
Cod	15–16
Hake	8–10
Redfish	over 15
Shark	8
Herring	4–5
Mackerel	5
Whiting	less than 15

FAO, 1975

There are three main methods of storage with ice: bulking, shelving, and boxing; the quality aspects of each method varies. Bulking and shelving are the main practice in long distance fishing, unless the product is immediately deep- frozen as on factory trawlers. Boxing is practiced in many fisheries because it results in the highest quality fish compared to other methods of ice storage. Boxing is most appropriate in small vessel fishing over shorter distances, up to about three days of voyage.

4.2.2. Chilled storage on shore

Once on shore, fish are kept on ice or in refrigerators before processing or sale. Icing is the primary means used to store fish in wholesale and retail outlets. There are significant markets in costal areas for fresh fish displayed in a bed of ice. Many factors dictate the shelf-life of the fish in the market such as the species, handling prior to reaching market, time on boat before icing, quality of the ice, and others. In general, the shelf-life of fish properly iced is about 7 days (ASHRAE, 1978). Shellfish are kept alive in walk-in coolers at 4°C, and oysters and clams have a 2 week shelf-life at this temperature. Crawfish, crabs, and lobsters may also be kept alive in large coolers, however, they may dehydrate and lose weight unless the humidity in the cooler is high.

4.3. QUALITY CHANGES DURING FREEZING AND FROZEN STORAGE

Fish, shellfish, and others are frozen to retard spoilage and extend shelf-life (Kreutzer, 1969). An increasing portion of seafood supply is produced applying this technology. Freezing really has no effect on the taste or nutritional value of the food, yet freezer storage does result in a gradual decrease in the quality of the product with some changes in taste, odour, texture, and colour. The degree of quality deterioration depends upon time in frozen storage, temperature, handling before and during storage, species, and others.

Freezing rates have a great influence on the quality of the frozen product. At low rates larger ice crystals are formed and protein denaturates resulting in impaired quality.

The overall quality and shelf-life of frozen seafood vary among species and the adequacy of handling. The fat content of a particular species has a significant affect on its suitability for freezing and maximum shelf-life. Species such as tuna, mackerel, salmon, and herring, have a relatively short frozen shelf-life, because the oils in the flesh are easily oxidized even in the frozen state. In lean species, such as cod and haddock, there is less oil, and oxidative rancidity is less severe. The problem of thaw-freeze burn must not be overlooked: Any slight fluctuations of temperature may cause ice/water to sublimate and re-crystallize, thus drying out the flesh and accumulating 'snow' in voids of the package.

The dehydration of the flesh causes the texture to become chalky and fibrous, the colour to change, and off-odours and off-flavours to develop. Contact with air enhances oxidation of the lipids in the flesh, and thus rancidity. The overall quality of fishery products can be preserved, and the shelf-life extended by controlling the environment immediately surrounding the product. Packages used for frozen fish products must have low oxygen permeability; it must fit tightly around the product to minimize air pockets. A coating glaze of ice functions by protecting against dehydration and oxidation.

5. METHODS OF QUALITY ASSESSMENT

Quality of fishery products comprises many aspects like freshness, sensory properties, nutritional relevant compounds and hygienic status. During storage quality is mainly influenced by a number of parameters which change in time leading to a loss of freshness. Assessment of the actual freshness of fish and fish products can be done by a number of methods belonging to the areas of sensory, physical, chemical and microbiological analysis (Connell *et al.,* 1976).

5.1. SENSORY METHODS

First of all, the quality of fish can be checked by use of human senses, which is the most important way to get information about quality of fish up to now. Sensory evaluation of food is a well established technology (Amerine *et al.*, 1965).

For raw products the appearance of the fish can be classified by use of defined categories, for example the EU scheme (Anon., 1996b). This is an example for a discriminative sensory analysis. Bright colours, clear slime, red and distinct gills, eyes with full turgor and elasticity of the flesh are preconditions for high quality. Another method for assessing quality of whole fish and even fillets is the quality index measurement (QIM) which is a descriptive sensory analysis (Hyldig and Nielsen,1998, Luten and Martinsdottir, 1998). QIM is based on the sensory parameters significant for raw fish. The scores (demerit-points) for all the characteristics of a given fish species are than added to give the so-called quality index. The quality of cooked samples can be described by sensory evaluation of the taste and smell, the texture and the appearance of the unsalted fish sample by expert panels (Paulus *et al.*, 1969). It must not be overlooked that due to shifts in the microbial flora irradiated fish products might develop new and uncommon odours (Pelroy, 1967) and sensory judgement might become more difficult.

5.2. PHYSICAL METHODS

Physical measurements described below give information on parameters related to fish freshness and quality but they are also influenced by other parameters and, therefore, not clear-cut. Several more physical measurements (like non-destructive near-infrared sprectroscopy) have

been proposed or studied in the laboratory. None of these methods however, gives a unique and unambiguous answer to whether the fish is fresh or not (Heya *et al.*, 1998).

Time and temperature dependent changes of electrical properties of the fish muscle like electrical resistance or impedance reflects storage time and give good impressions of the loss of quality. Thus, several instruments have been developed to judge fish quality from impedance measurements (Connell *et al.,* 1976, Ehlermann, 1972, Hennings, 1962, Jason and Richards, 1975, Karl, 1992). Measurement of electrical properties is rather more useful on whole fish than on fillets (Oehlenschläger and Nesvadba, 1998). The skin of the samples should not be injured or damaged. Freezing dramatically affects electrical properties due to tissue cell damage; estimation of elapsed storage period is not possible after re-thawing.

Mechanical properties like texture can be detected by using a wide range of instrumentation (Schubring, 1997). Simple texture meters (Messtorff, 1954) have been proven useful also for whole fish. In general, results of physical texture analysis show good correlations to results of sensory analysis for most fish species but not for all (Barroso *et al.*, 1998, Vincent, 1998).

Recent studies have indicated that instrumental colour measurement of fillets can be used as freshness indicator, but this is stated for only few species and more testing has to be done (Schubring, 1998).

5.3. CHEMICAL METHODS

Taste and smell of fish is strongly determined by a couple of volatile compounds, which are produced by fish enzymes or by bacteria and released during storage time. It has always been of concern whether traditional quality indices can also be used after radiation processing (Spinelli *et al.*, 1969). Commonly used tests for quantification of nitrogen from total volatile bases (TVB-N) and trimethylamine (TMA) as well as dimethylamine (DMA) can give informations about loss of freshness and the degree of spoilage (Rehbein and Oehlenschläger, 1982). However, TMA and DMA analysis does not apply for freshwater fish. In the same way other biogenic amines like putrescine or cadaverine can be measured by HPLC as spoilage indicators (Rawles *et al.*, 1996). A new method used in detecting volatile compounds is the use of “electronic noses”. These are multisensor systems, which can record profiles or fingerprints of selected volatiles simultaneously, showing good correlations to the loss of freshness (Olafsdottir *et al.*, 1998).

Besides the release of volatiles also changes take place in the activity of sarcoplasmic enzymes in the fish tissue; these activities can indicate whether fish samples has been stored frozen or not before testing (Rehbein, 1992).

Techniques for measuring the rate of lipid oxidation (Undeland, 1998) are mostly used in research, and very few are routinely applied in the fish industry. The same is true for measuring levels of nucleotides (Henehan *et al.*, 1998) but nevertheless these techniques give only valid results in the very beginning of shelf-life of fish and show strong variations between different fish species.

5.4. MICROBIOLOGICAL METHODS

In microbiological quality assessment the commonly used parameter is the aerobic total viable count (TVC). Much more reliable results can be obtained by measuring the numbers of specific spoilage organisms (SSO), which are *Shewanella putrefaciens, Photobacterium phosphoreum, Brochotrix thermosphacta* and lactic acid bacteria. *S. putrefaciens* is common on fresh fish from Atlantic and North and Baltic Sea (Gram *et al.*, 1989), whereas *P. phosphoreum* is identified as the main spoilage bacterium in processed, modified atmosphere-packed fillets from the same area (Dalgaard *et al.*, 1997). *B. thermosphacta* is described as the main spoiler from the mediterranean sea (Drosinos and Nychas, 1996). Cell numbers of all this bacteria correlate in a quite higher degree with sensory data and the remaining shelf-life of the fish samples than numbers of total viable counts.

Because of the relatively time consuming methods in microbiology a number of more rapid methods have been developed for quantification of bacteria (Ogden and Meyer, 1998). One of this methods is based on the measurement of changes of the impedance in growing cultures of *S. putrefaciens* or *P. phosphoreum* and gives results in about 24 h. Due to the very good correlations to sensory data this results allows the prediction of the remaining shelf-life, which is also a parameter of quality.

Only products of high initial microbiological quality should be submitted to radiation processing; radurization (extension of shelf-life by elimination of spoilage causing microorganisms) and radicidation (elimination of pathogenic microorganisms) requires that the general quality (as measured by certain standard counts of microorganisms) is not yet impaired (Anon., 1989).

5.5. SUMMARY

Quality of fish products is determined by investigating a number of factors in concert. Additional factors influencing the quality of fish are seasonal variations, method of harvest or post harvest handling practices. Quality changes can be minimized, if fish and shellfish are placed into cold storage quickly after harvest. Keeping in melting ice is the simplest and cheapest method because it is mostly available in industrialized countries and in many of the developing countries, and can be carried out by most fishing vessels. The ratio of fish to ice should be about 3 to 1. Ice is effective in preserving quality by cooling the product down to near freezing, and thus slowing down enzymatic and microbial activities. Melting ice is also useful because it washes away slime, blood, bacteria and products of ongoing deterioration.

The maximum shelf life for fish kept on ice varies with species, but is usually 2 to 3 weeks. Refrigerated storage rooms are viable alternatives to preserving fishery products without freezing. Freezing is a good method for the preservation of fish products, however, some products such as blue crab meat and fatty fish should not been stored frozen for long periods. The products must be frozen quickly to avoid quality losses and must be held at a storage temperature of −18 to −29°C to remain in prime conditions. The time between harvest and freezing, the speed of freezing and the storage temperature all affect the quality and shelf-life of the product. Many methods have been developed to asses the quality of fishery products, yet none seem to be any better than the use of the human senses – sight, touch, smell and taste.

6. PACKAGING

6.1. FUNCTIONS

The basic necessity of packaging is the need to case food for further handling including transportation, distribution and also radiation processing. In many instances of irradiation processes the necessity of packaging materials is to prevent recontamination or reinfestation of the food by containing the product and essentially protecting it from the surrounding environment. A very specific situation occurs in bulk storage of fish onboard a trawler where the hold and its constructive elements such as poundboards etc. make-up the packaging material. Packaging occurs before irradiation, Thus, the packaging material should be relatively puncture proof and impermeable to water, must not release any radiation-induced reaction products, and should also form a tight seal. Furthermore, the material should not result in any unacceptable sensory or visual changes. It can be stated, that most packaging materials which are suitable for food packaging in general are also quite durable under irradiation doses of 10 kGy or more. The package to be utilized has two other important functions including utility and communication. The package must be in a form that is easy to handle and transport long distances. Furthermore, it should also be remembered that a package, ie the label it carries, must convey the message the processor is trying to get across, while also stating all relevant or required nutritional information, brand name, size, ingredients etc.

6.2. PACKAGE SELECTION

Before ever selecting a package for use, each food type to be irradiated must be considered individually, with respect to fat content, fresh or frozen, etc. Irradiation can result in the oxidation of food products, and thus can have damaging effects on the flavour, odour, and sensory attributes of a food. Packaging of foods in modified atmospheres such as low oxygen etc., can be utilized to minimize these oxidative changes, as can freezing the product. The methods used during processing, the handling of the product, the distance that it has to be transported, and the length of storage time must all be considered when selecting a food package. Another concern with packaging deals with the development of an anaerobic environment which would be quite conducive to the growth of *Clostridium botulinum*. Irradiation doses that would be used as standard practices would be sufficient to kill spoilage and pathogenic microbes, however the spores of *Cl. botulinum* would not be killed. This situation is not at all different from conventional practices, for example vacuum-packaging smoked trout fillets and storing them at ambient temperatures; temperature abuse may lead to toxin production.

6.3. PACKAGING MATERIALS

There are two main types of reactions that can occur when packaging materials are utilized during irradiation: (1) degradation of the material, and (2) cross-linking of the material. With degrading polymers, they may suffer main-chain scission and a loss in its mechanical stability. These types include polyisobutylene, polymethacrylate, polytetrafluoroethylene. The cross-linking polymers are those that when exposed to irradiation have improved mechanical stability. These types include polyethylene, polypropylene, polystyrene, polysiloxane, and polyvinylchloride. Glass too can be used as a packaging material but it tends to change in colour to a yellowish brown; this must be considered because off-colours can make the product appear undesirable. Paper is another option but higher doses cause the cellulose (degrading polymer) to weaken, and thus the paper becomes brittle. Presently, the maximum allowed dose by the United

States FDA is 10. kGy (1 Megarad) with glassine paper and wax-coated cardboard, and 60 kGy for polymers like polyethylene (see Table II). Many countries have regulations on packaging materials similar to those followed in the USA; however, a great number of countries permitting food irradiation does not regulate packaging in the irradiation context. France has the requirement, that irradiation of materials intended for food packaging must be licensed, however, it has no regulation about packaging materials used for food to be radiation processed. It should also not be overlooked that many food-packaging materials are manufactured with the help of radiation (e.g. heat-shrinkable films or paper grafted on plastic materials); consequently, irradiating such material while serving to hold a food cannot be expected to generate a totally different reaction. In the literature several surveys of packaging for radiation processing and of chemical aspects of irradiating packaging materials are available (Buchalla *et al.*, 1992, Buchalla *el al.*, 1993, Kilcast, 1990, Thayer, 1988, Anon. 1997b).

TABLE II. IRRADIATION PRE-PACKAGING MATERIALS APPROVED BY UNITED STATES FDA

Material	**Maximum dose (kGy)**
Nitrocellulose—coated or vinylidene chloride copolymer coated Cellophane	10
Glassine paper	10
Wax-coated cardboard	10
Polyolefin film	10
Kraft paper (flour packaging only)	0.5
Polyethylene terephthalate film	10
Polystyrene film	10
Rubber hydrochloride film	10
Vinylidene chloride-vinyl chloride copolymer	10
Nylon-11	10
Ethylene-vinyl acetate copolymer film	30
Vegetable parchment	60
Polyethylene film	60
Polyethylene terephthalate film	60
Nylon-6 film	60
Vinyl chloride-vinyl acetate copolymer film	60
Acrylonitrile copolymers	60

Source: Anon., 1998

7. WHAT IS RADIATION?

The types of radiation used for processing of food are called 'ionizing'. By their energy, they are capable of removing electrons from molecules and atoms or to cleave molecules, thus converting them into 'ions'. Alternatively, they can displace electrons without causing ionization but 'unpaired' electrons on cleaved molecules or atoms. These are called 'free radicals' and are very reactive leading to many chemical reactions which finally cause the beneficial effects of food irradiation as well as the disadvantageous side-effects. There are two principle types of ionizing radiation, photons or electromagnetic waves and atomic corpuscles. Both types are capable of inducing radioactivity; for this reason only a few are suitable for food processing. The physical range of photons (electromagnetic waves) starts from low-frequency non-ionizing radio- and micro-waves, infrared, visible to ultra-violet light; hard ultra-violet light can already cause ionization, followed at shorter wave-lengths (higher frequency or photon energy) by X rays, gamma rays and cosmic radiation. Gamma radiation emitted from disintegrating radionuclides is also electromagnetic radiation. The corpuscular type of ionizing radiation physically includes all types of subatomic particles; alpha, beta, neutron etc. originating from disintegrating radionuclides as well as generated in high-energy machines (accelerators). Electrons from accelerators may be stopped in heavy metal targets and converted into X rays which are electromagnetic radiation. Of both physical kinds of radiation only the following are exploited for food processing (Anon., 1984):

— Gamma rays from the radionuclides ^{60}Co or ^{137}Cs
— Electrons generated from machine sources at or below an energy level of 10 MeV
— X rays (bremsstrahlung) from machine sources at or below an energy level of 5 MeV

As radiation interacts with matter, energy transfer occurs. This transfer may lead to heating, as observed with microwaves, or if the energy level is high enough, an electron may be removed from an atom or molecule. Depending on physical type and photon or particle energy transfer and penetration varies which leads to particular design of irradiation facilities. More detailed information can be found elsewhere (Josephson and Peterson, 1982, Urbain, 1986, Thorne, 1991, Murano, 1995, Diehl, 1995, Satin, 1996).

Processing by ionizing radiation has been proposed as a method of food preservation in that it can lead to DNA and cellular damage that is lethal to food spoilage microorganisms. It can lead to a destruction in the numbers of pathogenic and spoilage-causing bacteria, while not elevating the product temperature. Therefore, refrigerated and deep- frozen products may be processed by ionizing radiation without sensory or quality changes. The implementation of widespread seafood irradiation would be quite beneficial to both fisherman and consumers. Such a process would decrease spoilage in seafood products, namely bacterial deterioration, and thus would increase the shelf-life. There are many public health risks associated with the consumption of certain types of seafood and this technique could be employed to alleviate these health hazards, e.g. to eliminate *V. vulnificus* in raw oysters. A more stable and fresh supply of seafood could be provided to customers potentially year round, and this might lead to a decrease in prices if the seafood markets are provided with a large supply of high quality seafood.

8. HISTORY OF FOOD IRRADIATION

Food irradiation is a process in which food products are exposed to specific doses of ionizing radiation so that the product shelf-life and product safety are enhanced. Food irradiation is not considered a new processing technique. Roentgen discovered X rays in 1895, and

Becquerel discovered radioactivity in 1896. In these early days, available radiation sources were not strong or powerful enough to allow for industrial, commercial scale applications including food processing. The first patent ever on food irradiation was on the process itself (Appleby and Banks, 1905). The application of X rays was patented for insect elimination (Gillet, 1918) and proposed for trichina inactivation in pork (Schwartz, 1921). Also the beneficial application for disinfestation of cigarette beatles was discussed earlier (Runner, 1916). This idea was later expanded to the sterilization of canned food of any kind (Wuest, 1930). These early developments are described in several reviews (Diehl, 1995, Goresline, 1982, Goldblith, 1966). However, progress in the field of food irradiation was very slow to develop, and it was not until the early 1950s that any significant research in food irradiation was performed. The US Atomic Energy Commission launched a co-ordinated research programme and many other countries followed. At the same time powerful radiation sources became available either radionuclide or accelerators. International cooperation finally proved the wholesomeness of radiation processed food (see Section 14) and led to clearances of the process in many countries. The current status of clearances for sea food and frog legs is given in Table A.I in the Annex located at the end of this monograph.The actual status of clearances for irradiated foods can be found at the Web Page of the International Consultative Group on Food Irradiation (http://www.iaea.org/icgfi/).

9. PROCESS OF FOOD IRRADIATION

9.1. RADIATION SOURCES

The typical source of ionizing radiation used for radiation processing in general and for food irradiation in particular is the gamma ray emission from the radioisotopes. Cobalt-60 (^{60}Co) is the only radionuclide of commercial significance, and Caesium-137 (^{137}Cs) is only available in limited quantities at pilot plants. The ^{60}Co isotope emits two gamma rays of 1.17 and 1.33 million electron volts (MeV), whereas ^{137}Cs emits a 0.66 MeV gamma ray. The half-life of ^{60}Co is 5.3 years, and the half-life of ^{137}Cs is 30.2 years; thus determining the frequency of source replenishment. Energy levels of gamma rays from ^{60}Co (1.17 and 1.33 MeV) and ^{137}Cs (0.66 MeV) are far too low to induce radioactivity, and thus their use in a food irradiation technique cannot result in induced radioactivity in the food product. Gamma rays offer the advantage of maximum penetration into the food matter, however, they also have a significant disadvantage in that they are generated by a radioactive source.

Other sources of radiation that are currently allowed in food processing are (1) X rays generated from machine sources (linear accelerators) at energies of £ 5.0 MeV, and (2) electron beams generated from machine sources at energies of £ 10 MeV (Anon., 1984). These machine sources have the principal advantage over radionuclide sources in that they can be switched-off when unused. X rays have excellent penetrating power compared to electrons, while both of these sources have the advantage of being machine generated, and thus can be turned off and on. Electrons can be generated quite efficiently at very high dose rates allowing for short residence times of the product. This would be of advantage in processing bulk materials like grain. The conversion of electrons into X rays is a rather inefficient process; at present there is no industrial radiation processing using X rays.

A General Standard of Codex Alimentarius on food irradiation (Anon., 1984) has approved use of the above radiation sources for processing of foods.

9.2. RADIATION SOURCE SELECTION

The choice of the radiation source to be used is a critical decision (Chadwick *et al.*, 1977). Products should be well characterized prior to selection and many factors must be considered. The density or thickness of the product is important to know because if a product is too thick, electrons beams may not accomplish their goal due to their lack of penetrating power. Other considerations include the radiation dose needed to be delivered to the product and in which time period, or what kind of special packaging is needed to maintain product appeal. Product considerations also include the kind of geometrical arrangement for transport and irradiation treatment; dose distributions and homogeneity of dose throughout the product strongly depend on such factors and determine whether or not a given radiation source can be utilized. Also, the distance required to reach the irradiation facility is critical, because it may not be economically feasible to transport the product over great distances to be irradiated. Finally, the amenities of the irradiation facility such as forklifts, refrigeration etc., must be evaluated prior to use, as well as the scientific proficiency of the irradiation facility personnel (Anon., 1984).

9.3. RADIATION DOSE

One important parameter to consider in a food irradiation process is radiation dose; it is the sole quantity governing the achieved effect. Radiation dose is usually expressed in terms of how much radiation energy has been absorbed by a volume (mass) of a food product. The International System of Units (SI) uses the unit Gray (Gy). One Gray is equal to 1 joule of energy absorbed per kilogram of food mass. 1 Gy equals to 100 rad, now an obsolete unit but used in the older literature. Radiation dosimetry is the technique to determine and control dose (Chadwick *et al.*, 1977, McLaughlin *et al.*, 1989, Anon., 1997) and it is the central part of process control and documentation. The useful dose range for food processing is from 50 Gy in sprout inhibition of bulbs and tubers to 10 kGy as the highest dose needed for the majority of applications up to about 100 kGy to achieve complete sterility. Dose ranges useful in seafood applications are given in Section 10. 10 kGy is the thermal equivalent of 2.4 calories, which is essentially negligible; it corresponds to a temperature raise of only 2.4°C for water to which most foods are physically equivalent. Allowable sources for food irradiation cannot induce radioactivity regardless of how high the dose may be. A dose limit 'overall average dose' at 10 kGy has been set by international agreement (Anon., 1981, Anon., 1984); it covers the dose range of most technological effective applications. Utilization above this 10 kGy rule occurs with the irradiation of spices, in that they are approved for irradiation up to 30 kGy in the USA (Anon. 1994) and elsewhere. Furthermore, there are sterilization applications for hospital diets and some food items consumed by astronauts during space flights under special regulations up to 100 kGy.

9.4. RADIATION DOSE CATEGORIES

Depending on the dose that is chosen, irradiation can lead to a delay in the ripening of a food product, or it can lead to complete sterilization of a product. Dose categories may be termed low, medium and high (up to 1 kGy, up to 10 kGy, above 10 kGy resp.) According to the Joint Expert Committee on Food Irradiation (JECFI) (Anon., 1981); likewise, such categories might be termed by achievable effects. Below 1 kGy, food undergoes what is termed **radurization** ('rad' for radiation and 'dur' from Latin hard or durable). Radurization can prevent sprouting in vegetables, delay ripening in fruits, and inactivate parasites in meat and fish and kill or sterilize insects in grains or on dried fish and fruit (also called disinfestation). If the radiation dose

delivered falls in the range of 1–10 kGy, food undergoes what is termed **radicidation** ('rad' from radiation and 'cid' from Latin 'to kill'). At this level, parasites and insects are not only sterilized, but also killed. Furthermore, this level of radiation results in a significant reduction in the numbers of bacteria, yeasts, and moulds in a food product. The extent of reduction is high enough to practically eliminate pathogenic microorganisms in solid food which is comparable to heat-pasteurization of liquid food (also called 'radiation-pasteurization). If food is irradiated above 10 kGy, it undergoes what is called **radappertization** ('rad' from radiation and 'appert' after the French scientist Appert who invented sterile canning). Radappertization results in the complete sterilization of a food, as all bacteria are eliminated.

9.5. OPTIMUM DOSE SELECTION

The selection of a particular dose of irradiation (also called target, minimum effective dose) to be used for a seafood product is a highly variable decision. Different products have different requirements to ensure microbiological safety as well as shelf-life extension. Some products may need higher doses and others may only need a lower dose to generate the desired effect; some product may not tolerate a high dose. The selection of a dose should be based on all characteristics of the product and the detailed research data compiled in Section 10 can only be taken as a guide in selecting the optimum dose. The primary goal is to induce no sensory change in the product (which is crucial with some fatty fish species), while successfully eliminating any spoilage or pathogenic microorganisms that may be present. Secondly, it is desirable that irradiation yields a product that has an increased shelf-life as well as improved hygienic properties. The primary consideration for successful radiation processing is to use only a high quality product to begin with. If irradiation is tried to mask an inferior product, it is sure to fail (a spoiled, smelly fish remains putrid, only the health risk from pathogenic microorganisms is reduced by irradiation).

The state of the seafood product is very important to the irradiation process, because frozen products respond quite differently to irradiation than do products at refrigerated or room temperatures. This includes radiation sensitivity of microorganisms which are protected at frozen temperatures, hence, requiring a higher radiation dose in the deep- frozen state compared to iced or room temperatures. Other parameters that are generally considered prior to irradiation include ; initial bacterial load depending on fishing ground and previous handling or processing, packaging materials used and oxygen concentration, fat content of the seafood including relative composition and proportion of highly unsaturated fatty acids as well as its general biochemistry, and even the colour of the product. Fat content may be the most critical consideration due to the fact that lipid oxidation/oxidative rancidity are prominent spoilage mechanisms observed in fatty fish. Fish has relatively more free amino acids compared to other proteinaceous foods; consequently, even lean fish may spoil rather fast due to the action of proteolytic enzymes and bacteria. Furthermore, post-irradiation conditions such as storage temperature and proper handling must be controlled. The potential for recontamination is always great, if the product is not already in its final retail package.

9.6. PROCESS CONTROL AND LEGISLATION

In most instances, radiation processing of food is conducted at a commercial contract irradiation facility. This implies that the established engineering tools of process control are already implemented. These include for the irradiation process the strict control of the physical properties of the goods, i.e. size, weight, density, arrangement on trays or pallets, and the accurate measurement of the dose patterns for any configuration. Dose mapping studies are

conducted before commercial quantities are processed. For dosimetry, only traceable dose meters can be utilized, i.e. a link of calibrations in an unbroken line must be established and maintained to national and international standards. The dosimetry results are combined with the process parameters of the irradiation facility, most prominently conveyor speed or residence time of product units. All parameters are routinely recorded and documented; they are the best source for auditing and official control. In many situations, the expected positions of the maximum or the minimum dose are not accessible for routine dosimetry; instead measurements are taken at reference positions. Process control establishes a reliable link. Usually, the operator of an irradiation facility does not take the responsibility for the quality of the irradiated product. However, the Codex Alimentarius requires that (1) the food complies with the provisions of the Recommended International Code of Practice — General Principles of Food Hygiene, and (2) the irradiation of food should not be used as a substitute for good manufacturing practice. For this reason, clear conditions must be agreed upon between owner and irradiator of the food. Furthermore, the premises of the irradiation facility must also comply with any public health requirement affecting microbiological safety or nutritional adequacy and the general requirements of good hygienic practices in food processing must be met.

Regulations on food irradiation vary widely from country to country, this is also true for seafood (see Table A.I.). There is not yet commercially significant marketing of irradiated seafood and not at all any international trade. However, the maximum allowable doses listed allow for most applications as presented in Section 10.

10. SEAFOOD IRRADIATION RESEARCH

This section is a comprehensive review of seafood irradiation research. There is abundant published literature which may be retrieved elsewhere (Anon., 1997), and which is not completely reproduced by the references given here. The data compiled below should only be used as a guideline for choosing the appropriate treatment, some general guidelines for fish irradiation are found elsewhere (Anon., 1997, Licciardello and Ronsivalli, 1982, Nickerson *et al.*, 1983). This section includes irradiation research findings on marine finfish, freshwater fish, shellfish/molluscs, and others; common and scientific names of fish should be cross-checked (Anon., 1968) as fish names employed for certain species have not always the same meaning in different countries or regions where the same language is spoken. This section includes data on minimum necessary and on maximum acceptable doses, as well as shelf-life consequences and special considerations that must be made prior to carrying out such a procedure. 'Minimum necessary' dose is the effective dose needed to achieve the desired effect; it must be reached at any position within a consignment or lot. 'Maximum acceptable' dose is the highest dose which the product can tolerate without any detrimental effect on quality, especially sensory properties. Shelf-life is determined by the time interval elapsing between catch of the fish and final consumption; however, this is determined by the quality level taken for reference. There are two quality levels defined, 'high-quality life (HQL)' and 'practical storage life (PSL)'; in validating data from literature this reference quality needs to be carefully checked (Reinacher *et al.*, 1977). Caution should be paid to the fact that sometimes shelf-life is reported as the time period until final spoilage, whereas shelf-life extension should only be the prolongation of the period during which 'quality' is available. Otherwise, seafood irradiation would only contribute to having an increased amount of low quality product on the market (Reinacher *et al.*, 1977). Caution must also be paid to statements on 'maximum acceptable' dose as the criteria are not always clearly stated and in many instances not transferable to other regions with different eating habits or preferences. For example, with carp several panel members preferred samples treated with doses

above 5 kGy over the ones treated at lower doses because of their perception that detectable radiation off-flavour also enhanced the typical carp-flavour (Ehlermann and Muenzner, 1970c).

Also, in the Annex located at the end of this monograph, Tables A.II.–A.VI. show a compilation of the data in tabular form. These tables can be used as quick reference for data concerning different freshwater and salt water fish, as well as shellfish, bacteria, parasites, and viruses.

10.1. MARINE FINFISH

Anglerfish (*Lophius americanus*)

This fish is also known as monkfish, and only the tail portion is useable. The optimum dose was determined to be 1.5 kGy, while no maximum dose has been determined (Carver *et al.*, 1967).

Bombay duck (*Harpodon nehereus*)

This fish is not easily preserved by canning or freezing. Under refrigeration, shelf-life of fillets and dressed fish was observed to be about 5–7 days. Radiation doses of 1.0 to 2.5 kGy were effective in extending shelf-life to about 18–22 days. It was determined that 5.0 kGy is the maximum acceptable dose for this fish in that an off-flavour develops at this dose, but will subside after 4 days (Kumta and Sreenivasin, 1970; Sawant *et al.*, 1967; Bhadra *et al.*, 1973; Kumta *et al.*, 1973; Doke *et al.*, 1976b; Gore and Kumta, 1970).

A method for preparing Bombay duck laminates has been developed by Doke *et al.* (1976). The process consisted of compressing the fish fillets between two metal plates to remove tissue fluids. The fillets packaged in polyethylene bags and irradiated at 2.5 kGy were acceptable up to 20–22 days at 0–2 C as compared with non-irradiated samples which were acceptable only for 5 days. Dehydro-irradiated products could also be prepared from the fish by blanching the compressed laminates in steam for 15 min, dehydration at 55–60°C to reduce moisture to 35–40% and packaging in polycell bags. The dehydrated laminates were irradiated at 2.5 kGy to get products which were shelf stable up to 4 months at ambient temperature (Doke *et al.*, 1976a).

Butterfish (*Peprilus triacanthus*)

This fish has a high fat content, thus an oxygen scavenger of some kind must be added. The optimum dose for the fresh fish has been determined to be about 1.0 kGy. However, if irradiated in the frozen state, and under a vacuum, an optimum dose of 2.3 kGy has been observed, with maximum doses reaching 7.0 kGy (Carver *et al.*, 1967; Carver and Steinberg, 1959; Shewan, 1959).

Cod (*Gadus morhua*)

The form of this fish may be either dressed, filleted, or in steaks. Optimum doses were determined to be from 1.5–2.5 kGy, with a maximum dose of 4.5–5.0 kGy. There are however conflicting reports regarding this maximum dose in that off-flavours have been observed at doses above 3.0 kGy. Packaging under a vacuum did not appear to enhance irradiation effect; however, irradiation of the pre-rigor fish has been shown to give better sensory characteristics than the post-rigor irradiated fish. Also, the means by which cod are caught appear to affect

irradiation efficiency in that line caught fish perform better than those caught in a gill net or by trawling (Ronsivalli *et al.*, 1965; Carver and Steinberg, 1959; Slavin and Ronsivalli, 1964; Ampola *et al.*, 1969; Shewan and Liston, 1958; Rhodes, 1964; Bender *et al.*, 1958; Van Mameren *et al.*, 1969; Ronsivalli *et al.*, 1968; Carver *et al.*, 1969; Hannesson and Dagbjartsson, 1970; Slavin *et al.*, 1964).

Cod fillets that were 1 day or 3 days post-mortem, were irradiated at 1.0 kGy (maximum absorbed dose), and a shelf-life extension of 9 days was observed. If this irradiation treatment was combined with packaging at 60% CO_2 or with a 5% sodium sorbate dip, shelf-life was extended a few more days. No significant difference was detected in the one day old fish as compared to the three day old fish post-irradiation. Many parameters were evaluated in this study as measures of spoilage, including concentrations of TMA, DMA, hypoxanthine, as well as pH and aerobic plate count values. None of the parameters were significantly different when comparing irradiated and non-irradiated samples (Licciardello *et al.*, 1984b).

Atlantic cod fillets, *Gadus morhua,* both fresh and frozen were packed in polyethylene bags and irradiated at 1 to 5 kGy. The samples were stored at 4°C for 28 days and were analyzed in terms of odour, hypoxanthine, TMA, and TVBN. Results indicated that irradiation was quite effective in delaying deterioration in these cod fillets, and 2 to 3 kGy was determined to be the optimum dose (Thibault and Charbonneau, 1991).

Cod — Pacific (*Gadus macrocephalus*)

The optimum dose for on board irradiation was found to be 0.5–1.0 kGy. If fillets are irradiated, the optimum dose is less than 4.5 kGy, with a maximum acceptable dose of 7.0 kGy. This dose of irradiation extends the refrigerated shelf-life threefold (Teeny and Miyauchi, 1970; Miyauchi, 1960).

Dogfish (*Squalus acanthias*)

This is also a very fatty fish so oxygen scavengers should be incorporated. When these fillets were irradiated in nylon-PVDC-surlyn bags, under air, an optimum and maximum dose of 2.0 kGy was determined, yielding a 7 day shelf-life at 8°C (Licciardello *et al.*, 1984a).

Flounder — Blackback (*Pseudopleuronectes americanus*)

The optimum dose was found to be 4.5 kGy for dressed or filleted fish, with a maximum dose of about 9.0 kGy (Brooke and Steinberg, 1964; Carver and Steinberg, 1959).

Haddock (*Melanogrammus aeglefinus*)

The optimum dose for fillets is 1.5–2.5 kGy, yielding a shelf-life of 22–25 days at 5.6°C, and greater than 30–35 days at 0.6 C. A maximum acceptable dose was found to be 6.0–7.0 kGy (Steinberg, 1965; Coleby and Shewan, 1965; Slavin *et al.*, 1964; Ronsivalli and Slavin, 1965; Slavin *et al.*, 1963; Power *et al.*, 1964b; Ronsivalli *et al.*, 1970; Nickerson *et al.*, 1964; Ronsivalli *et al.*, 1968). This fish should be irradiated as soon as it reaches the shore. Also shipboard irradiation of whole eviscerated haddock was reported (Ehlermann *et al.*, 1977); however, stored under crushed ice, doses up to 1.3 kGy did not improve the shelf-life of 16 days at HQL.

Halibut (*Hippoglossus hippoglossus*)

The optimum dose for vacuum packed steaks is 2.0–3.0 kGy, yielding a shelf-life of 20 days at 5.6°C, and greater than 30 days at 0°C. A maximum acceptable dose was found to be 5.0 kGy. (Rhodes, 1964; Sieling, 1961; Eukel and Huber, 1960).

Halibut (*Paralichthys californicus*)

The optimum dose for vacuum packed steaks is 2.0 kGy, yielding a shelf-life of 14–21 days at 5.6°C, and greater than 21–42 days at 0.6°C. A maximum acceptable dose was found to be 5.0 kGy. (Slavin *et al.*, 1966; Steinberg, 1965; Miyauchi *et al.*, 1968; Dassow and Miyauchi, 1965).

Herring (*Clupea harengus*)

Herring is an extremely fatty fish, and thus oxidative rancidity is a large concern. Likewise, the effects of irradiation also contribute to rancidity, and thus fatty fish do not respond as well to irradiation as do lean fish. Thus, the use of vacuum packaging or oxygen scavengers is necessary for optimum results. The optimum dose has been determined to be 1.0–2.0 kGy, yielding a shelf-life of 10–14 days at 2°C. The maximum acceptable dose was shown to be less than 5.0 kGy, due to the loss of colour and natural flavour at 3.0 kGy and higher. Bismark herring was found to be optimally irradiated at 0.75 kGy. (Rhodes, 1964; Snauwert *et al*., 1977).

Herring smelt (*Argentina silus*)

The optimum dose was found to be between 0.5–1.0 kGy, which yielded an increase in shelf-life by 6 days at 0.6°C (Carver *et al.*, 1969).

Kembung Fish (*Rastrelliger neglectus)*

Kembung fish that is stored at 2–5°C for 12 days, has a significant increase in TVBN and hypoxanthine, with a decrease in the specific activity of SH-protease and acid phosphatase. Gamma radiation at doses of 1.0 to 2.0 kGy had no significant effect on the activity of SH-protease or acid phosphatase. There were however significantly lower TVBN values and hypoxanthine concentrations in samples irradiated at 2.0 kGy, compared to 1.0 kGy after 7 days of storage (Sofyan, 1978).

Mackerel (*Scomber scombrus*)

This too is a very fatty fish that needs to be either vacuum packed, or have oxygen scavengers incorporated. The optimum dose was determined to be 2.50 kGy, which yields a shelf-life of 30–35 days at 0.6°C. (Slavin *et al.*, 1966; Slavin and Ronsivalli, 1964).

Mackerel (*Rastrelliger kanagurta*)

This too is a very fatty fish that needs to be either vacuum packed, or have oxygen scavengers incorporated. The optimum dose was determined to be 1.5 kGy, which yields a shelf-life of 21–24 days at 0°C, 13–15 days at 5°C, and 7–11 days at 7.8°C. (Kumta *et al*., 1973; Hussain *et al.*, 1977; Ghadi *et al.*, 1978; Hussain, 1980).

Extensive studies on radiation preservation of Indian mackerel have been consolidated by Venugopal and Nair (1992). At an optimum dose of 1.5 kGy, the fish could remain in acceptable condition for a period of 25 days in ice. The treatment did not cause significant changes in flavour as judged by sensory evaluation. Adverse effects on the protein quality as judged by physico-chemical as well functional properties were also minimum. Air packaging of the fish in polyethylene pouches was recommended. The process was also scaled up for commercial conditions. Shelf-life of the fish could also be extended by irradiation under non-packaged conditions. This study showed that packaging was not necessary to sufficiently increase the shelf-life of irradiated iced fish. Packaged irradiated fish exhibited a shelf-life of around 25 days, whereas the iced stored fish were still quite acceptable at 20 days of storage. More importantly, the non-packaged, iced samples were more appealing in terms of odour, and had a visibly better appearance than the packaged fish. This deterioration in appearance of packaged fish was attributed to an accumulation of drip loss, such as blood and other fluids which lead to a browning of the fish. The only negative attribute associated with the unpackaged fish was a slight yellowing of the skin (Venugopal *et al.*, 1987, Venugopal *et al.*, 1982).

Indian mackerel (*Rastrelliger kanagurta*) was also irradiated at 0 to 3.0 kGy and was stored at 1–3°C. The bacterial load was 1×10^5 at day zero and raised to only 2.5×10^7 in 28 days. *Pseudomonas* and *Proteus* spp. were the predominant genera in the controls, whereas *Achromobacter*, *Flavobacterium*, *Bacillus*, and *Micrococcus* predominated in the irradiated fillets. Also, the moisture and protein contents decreased in the irradiated fillets, but it was determined that a pre-treatment with a dip in 10% sodium polyphosphate decreased the associated drip loss. Optimum conditions were maintained for 3 weeks at 1.5 kGy with a pre-dip in 10% sodium polyphosphate (Hussain *et al.*, 1985).

Another similar study examined mackerel fillets packed in polyethylene bags and irradiated at 1, 1.5, 2.0, and 3.0 kGy, followed by storage at 7–8°C. Optimum conditions were observed in the fillets irradiated at 1.5 kGy which stored well for 7 to 11 days (Haq and Hussain, 1986).

Mackerel, Pomfret, Seer and Rahu, tropical Shrimp etc.

Low dose gamma radiation was used to treat Indian mackerel (*Rastrelliger kanagurta*), white pomfret (*Scomberomorus guttatus*), and seer (*Stromateus cinerius*), which were then stored on ice for 3–4 weeks. The samples were evaluated with respect to fat oxidation in skin. Thiobarbituric acid (TBA) values increased in both irradiated and non-irradiated fish, particularly the in the mackerel and seer. However, it was observed that the TBA values in mackerel did drop later during storage. Only the pomfret skin exhibited skin oxidation after treatment, and this value continued to rise throughout storage (Ghadi and Venugopal, 1991, Doke *et al.*, 1992). Rahu (*Labeo rohita*) fillets were dipped into polyphosphate solutions and irradiated to 2 kGy; storage was at 1–3°C. Water holding capacity was improved, at the same time the phosphate dip sensitized bacteria like *Lactobacillus*, *Corynebacterium* and *Sarcina* (Haq *et al.*, 1984).

Sweetlip (*Lethrinus miniatus*), red emperor (*Lutjanus sebae*), mackerel (*Scomberomorus commerson*), whiting (*Sillago ciliata*), mullet (*Mugil cephalus*), barramundi (*Lates calcarifer*), sand crab (*Portunus pelagicus*), Moreton Bay prawns (*Metapenaeus spp.*), and king prawns (*Penaeus plubujus*) were irradiated by Poole *et al.* (1994) at 0, 1, 3, and 5 kGy at a constant temperature of 0–2°C.at a dose rate of 7.2 Gy per minute. The samples of each seafood were stored in crushed ice in insulated containers, and then irradiated. A microbiological, as well as

sensory analysis, was performed. It was observed that a 1.0 kGy dose resulted in a 1.5 to 4.0 log reduction in bacteria, compared to 3.7 to 5.7 log reduction at 5.0 kGy. All species, except the Moreton Bay and cooked king prawns, had acceptable flavour, texture, and odour after 5.0 kGy dose. The 3.0 kGy dose lead to adverse odours and flavours liberated in these two products (Poole *et al.*, 1994).

Nagli fish (*Sillago sihama)*

This is a tropical warm water fish found in the west coast of India. Air packed samples irradiated to 2 and 3 kGy doses and stored at 1–2 ^{o}C were found to be acceptable organoleptically up to 19 days while non-irradiated samples were unacceptable after 7–8 days. Dressing of the fish prior to irradiation had no additional advantage to shelf-life over whole fish. Total bacterial count, TVBN, TMA and sensory evaluation data revealed no significant differences between whole and dressed fish (Ahmed *et al.,*1997).

Ocean Perch (*Sebastes marinus*)

The optimum dose for the irradiation of ocean perch (redfish) has been determined to be 1.5–2.5 kGy, which yields a shelf-life of 30 days at 0.6°C, and 15 days at 7.8°C. If irradiated at sea with 1.0 kGy from an X ray machine source, the shelf-life was extended beyond the normal 16 days, yet the quality was markedly reduced (Steinberg, 1965; Ampola *et al.*, 1969; Ronsivalli and Slavin, 1965; Slavin and Ronsivalli, 1964; Reinacher and Ehlermann, 1978). However, due to the higher fat content and the associated specific fine flavour, higher doses than 1.0 kGy are not recommended because of sensory changes.

Ocean Perch (*Sebastodes alutus*)

The optimum dose for irradiating the whole fish at sea is 0.5–1.0 kGy. Again, elimination of oxygen from the surrounding environment is critical. The optimum dose for irradiating fillets of ocean perch is 1.0–2.0 kGy, which yields a shelf-life of 25–28 days at 0.6°C. It should be noted that the higher quality fish, respond much better to irradiation than do the poorer quality fish. (Miyauchi *et al.*, 1966; Teeny and Miyauchi, 1970; Miyauchi *et al.*, 1967).

Pollock (*Pollachius virens*)

The optimum dose for air packed fillets is 1.5 kGy, with an adjoining darkening of the flesh. At this dose the shelf-life is 28–30 days at 0.6°C and less than 20 days at 7.8°C. The maximum acceptable dose has not been clearly established, but reports place it at 2.3–2.5 kGy, or 5.0–8.0 kGy if blanched prior to irradiation (Ampola *et al.*, 1969; Slavin *et al.*, 1966; Steinberg, 1965; Carver and Steinberg, 1959; Coleby and Shewan, 1965; Slavin and Ronsivalli, 1964).

Pomfret (*Stomateus cinereus* = white : *Parastomatus niger* = black)

This is another species in which oxygen scavengers or vacuum packaging must be employed to maximum the sensory characteristics. The optimum dose for both species is 1.0 kGy, with a shelf-life extension at 0–2°C of 4 weeks for white pomfret and 10–16 days for black pomfret. The maximum acceptable dose for both pomfrets is 3.0 kGy (Kumta and Sreenivasin, 1970; Kumta *et al.*, 1973; Aiyar, 1976).

Rockfish (*Sebastodes spp.*)

These fish should be vacuum packed prior to irradiation. Optimum doses for vacuum packed rockfish were found to be between 1.25–2.5 kGy. The refrigerated shelf-life of rockfish irradiated at 2.5 kGy was 20 days. If black rockfish *(Sebastodes melanops*) is irradiated at sea, the optimum dose is 0.5 1.0 kGy (Miyauchi, 1970; Eukel and Huber, 1960).

Sablefish (*Anopoploma fimbria*)

The optimum dose for air packed sablefish is 3.0 kGy, with rancidity occurring after 7 days at 0.6°C. Similarly, vacuum packaging should be used to enhance the shelf-life of this fish. (Stansby and Kudo, 1964).

Sardines (*Sardinella melanura*)

The optimum irradiation dose for sardines is 0.23 kGy, which will triple the shelf-life at 1°C. No maximum acceptable dose has been determined (Hashish *et al.*, 1966).

Sole — Dover (*Microstomus pacificus*)

The optimum dose for onboard irradiation was found to be 0.50–1.0 kGy. This increased the iced shelf-life by twofold. Again, the pre-rigor irradiation was much more desirable than the post-rigor irradiation. It is also suggested that once on shore, these fish should be filleted as soon as possible. (Teeny and Miyauchi, 1970).

Sole — English (*Parophyrs vetulus*)

Fillets were reported to have an optimum irradiation dose of 2.0–3.0 kGy, yielding a 4–5 week shelf-life. Again, the limitation of oxygen in the environment is highly desirable in that rancidity reactions can be inhibited. If the whole fish is to be irradiated on board the vessel, then a dose of 0.50–1.0 kGy should be used for a pre-rigor fish (Teeny and Miyauchi, 1970; Miyauchi *et al.*, 1968; Slavin *et al.*, 1966).

Sole — Gray (*Glyptocephalus cynoglossus*)

The optimum dose for fillets of gray sole has been determined to be 1.0–2.0 kGy, which will yield a shelf-life of 29 days at 0.6°C or 10–11 days at 5.6°C (Miyauchi *et al.*, 1967; Ronsivalli *et al.*, 1968).

Sole — Lemon (*Microstomus kitt*)

Lemon sole if packed under nitrogen has an optimum dose of 2.50 kGy, but if frozen that doubles to 5.0 kGy. The maximum dose was reported to be 5.0–10.0 kGy. (Shewan and Liston, 1958; Coleby and Shewan, 1965).

Sole — Petrale (*Eopsetta jordani*)

The optimum dose for vacuum packed fillets was found to be 2.0–3.0 kGy, yielding a shelf-life of 28–49 days at 0.6°C and 14–21 days at 5.6°C. No maximum value has been clearly established; however, at 3.0 kGy significant odour and flavour changes have been detected. (Slavin *et al.*, 1966; Miyauchi *et al.*, 1964; Dassow and Miyauchi, 1965; Spinelli *et al.*, 1965).

Tuna (*Thunnus obesus*)

The research on this fish is limited, but it has been determined that a dose of 2.0 kGy will restore the bright red colour in previously oxidized tuna (Amano and Yamanaka, 1969).

Four days old frozen tuna loins, packed in polyethylene bags, were irradiated by a Phillips X ray machine at a dose of 2.2 kGy, at a dose rate of 2.60 grays per minute. Sensory panels determined the non-irradiated control samples to be acceptable for 15 days, as opposed to 25 days observed for the irradiated samples. Malonaldehyde content (TBA value) was monitored for the control and irradiated samples as a measure of oxidative rancidity, and it was found that a maximum value was observed for the control samples after 10 days, whereas the irradiated samples reached a maximum at 25 days, correlating well with the sensory panel results (Quaranta *et al.,* 1984).

Whiting (*Merluccius* spp.)

The optimum and maximum dose for vacuum packed silver hake (*Merluccius bilinearis*) was found to be 1.2 kGy, but this can be increased twofold with blanching prior to irradiation. Others have reported that maximum doses of 2.0 up to 4.5 kGy; however off odours have been reported at doses above 3.0 kGy (Carver and Steinberg, 1965; Brooke and Steinberg, 1964; Coleby and Shewan, 1965; Massa *et al.*, 1969).

European hake (*Merluccius merluccius*) can be irradiated optimally at 1.0–1.5 kGy, which will yield a shelf-life of 24–28 days at 0.5°C (Matutano Aranda and Alonso Rodriguez, 1970; de la Sierra Serrano, 1970). The maximum acceptable dose is 2.0 kGy. Argentine whiting (*Merluccius hubsi)* can be optimally irradiated at 5.0 kGy, with a corresponding shelf-life of 48 days at 4°C (Ritacco, 1976).

Hake fillets (*Merluccius merluccius hubsi)* were irradiated in a cobalt-60 irradiator at 2.0, 6.0, and 10.0 kGy, frozen and examined in terms of texture, elasticity, odour, colour, and drip loss. The irradiated samples at 2.0 kGy exhibited a 1 log cycle reduction in bacterial numbers versus the controls, whereas a 3 log cycle reduction was observed at 6.0–10.0 kGy. Nevertheless, the overall numbers always remained below 0.8×10^6. The products were organoleptically sound for 6 weeks, and a 6.0 kGy exposure was determined to be optimal (Valdes and Szeinfeld, 1989).

10.2. FRESHWATER FISH

Carp (*Cyprinus carpio*)

The optimum dose for vacuum packed samples stored at 0°C is 5.0 kGy, with a shelf-life of 35 days, compared to 15 days for non-irradiated (Ehlermann and Muenzner, 1970; Ehlermann

and Muenzner, 1970c, El-Nawawy, 1985). At a dose of 1.5 kGy, the period until spoilage at 0–2°C was extended from 16 to 31 days (Icekson *et al.*, 1996); this was based on sensory evaluation, as chemical freshness indices failed.

Channel catfish (*Ictalurus punctatus*)

The optimum dose level for catfish is 1.0–2.0 kGy, which yields a shelf-life of about 20 days at 0°C (16 day extension). The maximum dose was determined to be 4.0 kGy (Emerson *et al.*, 1966a, 1965, 1964).

Fresh iced catfish fillets were treated with 0.5–1.0 kGy, and subsequently packaged under 80:20 CO_2/air, 100% CO_2 and 100% air. These samples were evaluated in terms of microbial counts, colour by a Hunter Colour Difference Meter, and TBA values every 10 days for a 30 day storage period on ice. The irradiation was effective in reducing the microbial counts in all packages, yet no differences were detected in the different atmospheres used. Interestingly enough, an increase in aerobic plate count values was observed in all samples throughout the study. Hunter a values increased as a function of dose as did the TBA values which are associated with increased rancidity (Przybylski *et al.*, 1989).

Chubs (*Coregonus spp.*)

The optimum dose for smoked chubs is 1.0 kGy, which yields a shelf-life of about 42 days under refrigeration. The maximum acceptable dose is about 8.0 kGy. Frozen chubs can be irradiated at 8.0 kGy, and then smoked to yield a highly acceptable product (Emerson *et al.*, 1965a; Slavin *et al.*, 1966).

Gwyniad (*Coregonus Wartmanni* Bloch)

The optimum dose was 1.0 kGy for whole, eviscerated, scaled gwyniad packed under vacuum in plastic films impermeable to water and oxygen. Shelf-life of 9 days on ice was increased to 23 days. Higher doses effected even longer shelf-life, however, caused off-flavour (Ehlermann and Muenzner, 1969; Ehlermann and Muenzner, 1970).

Herring — Lake (*Coregonus artedii, Lesueur*)

The optimum and maximum doses have not been clearly established for lake herring fillets. However, at doses of 3.0 kGy, fillets were not organoleptically acceptable after 8 days at 0.6°C (Emerson *et al.*, 1965b).

Salmon

Salmon do not make viable candidates for irradiation. Not only is the fat content high, leading to oxidative rancidity, but the orange colour is easily bleached at doses of 1.0–2.0 kGy (Rhodes, 1964; Sieling, 1961). Vacuum packed silver or sockeye salmon irradiated at 3.0 kGy become rancid immediately after irradiation, whereas king and pink salmon irradiated at 3.0 kGy become rancid after 7 days at 0.6°C. Salmon treated with 1.5 kGy have a shelf-life of about 20 days when stored at 2.2–2.6°C. The optimum dose for salmon irradiation has been determined to

be less than 1.0 kGy, with a similar dose reported for smoked salmon (Eukel and Huber, 1960; Rhodes, 1964; Stansby and Kudo, 1964; Metlitskii *et al.*, 1968, Cornett and Vallet ,1989).

Smoked salmon fillets were irradiated at 2.0 and 4.0 kGy, stored at 2–3°C, and were subsequently analyzed in terms of shelf-life and microbiological content. The main quality attribute that was lost during the 4.0 kGy irradiation was the normal cherry red colour associated with smoked salmon. This loss of colour was not observed in the samples irradiated at 2.0 kGy. Regarding the microbiological assessment, both doses were very effective in reducing the number of microorganisms in the salmon, while the 4.0 kGy dose eliminated all coliforms, faecal streptococci, and *Staphylococcus aureus.* The unirradiated samples reached an unacceptable plate count value after 1 month of refrigerated storage, whereas the microbiological quality (plate count value) was maintained for 3 and 4 months at 2.0 and 4.0 kGy, respectively (Hammad and El-Mongy, 1992).

Tilapia and Silver Carp

Fillets of tilapia (*Oreochromis mossambicus*) and silver carp (*Hypophthalmichythys molitrix*) were irradiated at 1.0 kGy at 2.4°C. Thiamin content, as well as other chemical constituents were evaluated. It was determined that there was a significant loss of thiamin in silver carp, whereas all other chemical constituents in silver carp and tilapia remained unchanged. Furthermore, the nucleotide catabolite concentrations in the irradiated fish was not changed after irradiation. The bacterial level was markedly reduced in both fish, and these bacterial levels were maintained for 5 days post-irradiation at 1°C. The only sensory defect in post-irradiation quality was detected in the colour of the silver carp, which was redder in colour than usual (Liu *et al.*, 1991).

Threadfin (*Eleutheronesma tetradactylum*)

The optimum dose for threadfin has been determined to be about 1.0–2.5 kGy, with a three to fourfold increase in shelf-life (Aiyar, 1976).

Trout (*Salmo gairdneri*)

Trout do not respond very well to irradiation in that severe flavour changes are the result. Doses near or above 0.5 kGy have been shown to lead to flavour loss; however if pre-dipped in 0.2% ascorbic acid, doses of 2.0 kGy can be used without any loss of flavour. The shelf-life of vacuum packed samples irradiated at 1.0 kGy and stored at 0°C is 28 days compared to 14–17 days for the untreated trout (Ehlermann and Muenzner, 1970; Ehlermann and Muenzner, 1970b; Hansen and Jorgenson, 1967; Hussain *et al.*, 1976).

Trout — Lake (*Salvelinus namaycush*)

The optimum dose for lake trout has been reported as 3.0 kGy, which yields a shelf-life of 26 days at 0.6°C. This far exceeds the normal shelf-life of 8 days, and the pigment in these trout will not disappear until doses of 7.0 kGy are employed. (Graikowski *et al.*, 1968).

Whitefish (*Coregonus clupeaformis*)

Whitefish are fatty fish, requiring vacuum packaging to limit oxidative rancidity. The optimum dose for irradiation has been determined to be 1.5–3.0 kGy, yielding a shelf-life of 15–

29 days under refrigeration. Non-irradiated whitefish have a shelf-life of 12–15 days (Graikowski *et al.*, 1968; Ostovar *et al.*, 1967).

Yellow Perch (*Perca flavescens*)

The optimum dose has been established to be 3.0 kGy for perch fillets, with a maximum dose of about 5.0 kGy (Emerson *et al.*, 1964; Slavin *et al.*, 1966). The maximum dose has also been shown to be near 10 kGy, if the irradiated perch is deep fried (Emerson *et al.*, 1966b). If irradiated optimally, the shelf-life is 40–45 days at 0.6°C, and 21 days at 5.6°C (Emerson *et al.*, 1966b). At doses around 2.0 kGy, shelf-life is 38 days at 2.2°C and 14 days at 10°C (Graikowski *et al.*, 1970).

10.3. SHELLFISH

Clams — Meats

Clam meats have also been studied, and results by Nickerson (1963) indicate that there is no detectable organoleptic difference between non-irradiated controls, and clam meats irradiated at up to 8.0 kGy, after 40 days of storage at 6°C. Also, Nickerson (1963) showed that clam meats store well for 28 days after exposure to 4.5 kGy. Slavin *et al.* (1963) found that clam meats exposed up to 4.5 kGy, and stored at 6°C were of equal quality to those non-irradiated meats. No changes in nutritional quality were observed (Brooke *et al.*, 1964). Connors and Steinberg (1964) utilized a taste panel to sample clam meats irradiated from 2.5 to 5.5 kGy, and no significant difference was detected between the irradiated and non-irradiated meats.

Cockles have a specific red colour of the meat; a combination of CO_2 (50–100 %) atmosphere and irradiation (2 kGy, gamma), storage on melting ice was studied. The combination treatment extended shelf-life (borderline rejection) up to 18 days compared to 3 days of the untreated control (Ng, 1987).

Clams — Baby (*Venerupis semidecus sata*)

The optimum dose range has been determined to be 1.0–4.5 kGy, which yield a shelf-life of 4 weeks at 0–2 °C (Yamada and Amano, 1965).

Clams — Soft Shell (*Mya arenaria*)

Shucked clam meats, air packed in a metal can have been reported to have an optimum dose of 4.5 kGy, compared to 3.5 kGy for those that are vacuum packed. The shelf-life of the clams irradiated at the optimum dose is 30 days at 0.6°C and 18–25 days at 5.6°C, compared to 5–7 days at 0.6°C for untreated clams. (Nickerson and Goldblith, 1964; Ronsivalli and Slavin, 1965; Slavin *et al.*, 1963)

Clams — Surf (*Spisula solidissima)*

Shucked clam meats air packed in plastic pouches have been reported to have an optimum dose of 4.5 kGy, yielding a shelf-life of 50 days at 0.6°C, compared to 10 days for untreated surf clams. Doses of 1.0–2.0 kGy result in a shelf-life of 40 days (Carver *et al.*, 1967).

Crabs — Dungeness (*Cancer magister*)

Pre-cooked crabmeat packed in a can or pouches can be optimally irradiated at 2.0–2.5 kGy, yielding a shelf-life of 28–42 days at 0.6°C, or 14–21 days at 5.6°C. Non-irradiated dungeness crabmeat has a shelf-life of 6–14 days (Miyauchi *et al.*, 1964; Scholz *et al.*, 1962). Eviscerated, in-shell dungeness crab, when treated at 2.0–2.5 kGy, has a shelf-life of 24 days compared to 10 days for the nonirradiated.

Crabs — King (*Paralithodes camtschatica*)

The optimum and maximum acceptable dose for cooked, vacuum packed king crabmeat is 2.0 kGy, which yields a shelf-life of 35 days at 0.6 °C and 14 days at 5.6°C, compared to 5–9 days at 0.6°C for the non-irradiated samples (Miyauchi *et al.*, 1964; Miyauchi *et al.*, 1966). Above this dose, off odours and flavours predominate.

Crabs — Swimming (*Portunus pelagicus*)

Precooked crabmeat, air packed in polyethylene bags, has been reported to have an optimum dose of 2.0 kGy, which yields a shelf-life of 28 days at 3°C compared to 7 days for non-irradiated crabmeat. A dose of 0.75 kGy yields a product with a shelf-life of 14 days (Guevara *et al.*, 1965; Loaharanu *et al.*, 1972).

Lobsters — American (*Homarus americanus*)

Pre-cooked lobster meat, air packed in plastic bags is optimally irradiated at 0.75 kGy, yields a shelf-life extension of 14 days. Higher doses result in significant off flavours and odours (Power *et al.*, 1967; Dagbjartsson and Solberg, 1973).

Lobsters — European (*Homarus gammarus*)

The optimum dose for European lobster has been determined to be 1.0–3.0 kGy. However, this is based only on appearance and odour (Rhodes, 1964).

Lobsters — Norwegian (*Nephrops norvegicus*)

The optimum dose for blanched tails is 2.0–3.0 kGy, which yields a shelf-life of 5–6 weeks at 0–1°C, compared to 5–6 days for totally untreated tails, and 4 weeks for blanched and non-irradiated tails (Hannesson and Dagbjartsson, 1970).

Mussels (*Mytilus smaragsinus*)

Shucked mussel meats, air packed, has been reported to have an optimum dose of 1.5–2.5 kGy, which yields a shelf-life of 6 weeks at 3°C compared to 3 weeks for the non-irradiated mussels (Loaharanu *et al.*, 1972).

Oysters (*Crassostrea virginica, Crassostrea pacificus*)

Gardner and Watts (1957) treated oyster meats at 0.63, 0.83 and 3.5 kGy of ionizing radiation and observed the development of undesirable odours. This odour was described as a

"grassy" odour for the raw irradiated oyster meats. An "oxidized" odour was detected for the cooked irradiated oyster meats. Gardner and Watts (1957) further concluded that ionizing radiation would not be effective for preservation, in that enzyme action continues even at 3.5 kGy, and 5°C.

Shucked oyster meats can be optimally irradiated at 2.0 kGy, resulting in a shelf-life of 21–28 days at 0.6°C, compared to 15 days for non-irradiated oyster meats. A yellow colour develops in oyster meats treated at doses above 2.0 kGy, and a grassy odour develops at doses above 8.0 kGy (Novak *et al.*, 1966; Gardner and Watts, 1957; Slavin *et al.*, 1966; Miyauchi *et al.*, 1967).

Novak *et al.* (1966) irradiated oyster meats in cans in air at 2.0 kGy. The irradiated samples, along with the non-irradiated controls were stored on ice for 0, 7, 14, 21 and 23 days, and subsequently sampled by an experienced taste panel. The irradiated samples were found to be acceptable throughout the 23 day period, whereas the non-irradiated controls were found to be spoiled by day 7, as well as each subsequent day. Day 7 exhibited the first noticeable organoleptic (sensory) change, as the non-irradiated controls were scored lower than the irradiated oyster meats (Novak *et al.*, 1966). Slavin *et al.* (1966) concluded that the optimum dosage for irradiation of oyster meats is 2.0 kGy, which when subsequently stored at 0.6°C, resulted in a storage time of 21 to 28 days. Metlitskii *et al.* (1968) followed up this conclusion showing that the shelf-life of oyster meats irradiated at 5.0 kGy, and stored at 2°C is 60 days.

Liuzzo *et al.* (1970) investigated the optimum dose of radiation for shucked oyster meats, that would result in maximum shelf-life, and minimal alteration in food components. They found that a maximum dose of 2.5 kGy could be utilized to extend the shelf-life of oyster meats by some 7 days. The organoleptic quality was not lowered, as determined by a taste panel, until after 7 days of storage on ice. Furthermore, it was observed that doses above 1.0 kGy altered the retention of the B-vitamins, the percentage moisture, the percentage ash, glycogen content, and the soluble sugar content of oyster meats (Liuzzo *et al.*, 1970).

Mallett *et al.* (1991) irradiated Massachusetts's shellstock oysters at several different doses and found no significant difference in 6 day survival times at doses up to 2.5 kGy. Also, the median post-irradiation survival time for shellstock oysters was in excess of 25 days for doses of 2.5 kGy and lower. Furthermore, a professional taste panel determined that oysters irradiated below 3.0 kGy were of fair and acceptable quality.

Dixon (1992) irradiated Apalachicola Bay, Florida shellstock oysters at Food TECHnology Service Inc. (formerly Vindicator, Inc.) in Mulberry, Florida. A two to three log cycle reduction in bacterial numbers was observed at all doses immediately after irradiation. The shelf-life of these oysters was limited in that D_{50} (number of days at which 50% of the oysters were dead) values were calculated to be 30, 25, 7, and 6 days at 0.5, 1.0, 2.0, and 3.0 kGy, respectively. A value with greater economic implications is the D_{20} (20% death) value, which were calculated to be 17, 8, 4, and 4 days at 0.5, 1.0, 2.0, and 3.0 kGy, respectively. Even though bacterial numbers were significantly reduced in irradiated oysters and *V. vulnificus* demonstrates a high radiosensitivity, the shelf-life of the irradiated shellfish was markedly reduced at doses higher than 1.0 kGy.

Scallops (*Placopecten magellanicus*)

Shucked scallop meats, air packed, can be optimally irradiated at 0.75 kGy, yielding a shelf-life of 28 days (raw) and 43 days (cooked) at 0°C, compared to 13–17 days for the non-irradiated meats. Doses on the order of 1.5 kGy or higher result in a soft, spongy, mushy texture (Power *et al.*, 1964a).

Chilled saucer scallops (*Amusium balloti*) were treated with 0.5, 1.5, and 3.0 kGy of radiation in polyethylene bags. Shelf-life analyses were performed with storage times being 13, 18, 23, and 42 days at 0, 0.5, 1.5, and 3.0 kGy. A 2 to 4 log reduction in bacterial numbers was observed, and the natural garlic flavour of these scallops was eliminated by this irradiation (Poole *et al.*, 1990).

Bis-(methylthio)-methane is an off-odour component in the scallop, *Amusium balloti*. This odour is garlic like, and has a threshold of 0.3 mg/kg in water. Scallops were irradiated in a 60-Cobalt source at 3.0 kGy, after which a trained taste panel could not detect any presence of bis-(methylthio)-methane (Freeman and Shaw, 1986).

Shrimp — White, Pink, Brown (*Penaeus setiferus, aztecus, duarum*)

The optimum dose for both raw and cooked Gulf of Mexico shrimp is 1.5–2.0 kGy, which yields a shelf-life of 21–30 days at 0–1°C, compared to 14 days for the non-treated shrimp. At 5.6°C, the shelf-life is 16 days for the irradiated, and 7 days for the non-irradiated shrimp. At doses of 5.0 kGy, a noticeable irradiation flavour develops, and it should be stressed that shrimp should be irradiated as soon after harvest as possible (Novak and Rao, 1973; Learson *et al.*, 1970; Kaylor *et al.*, 1970; Dassow and Miyauchi, 1965).

Shrimp — European Brown (*Crangon vulgaris* and *Crangon crangon*)

The optimum dose for peeled European brown shrimp is 1.5 kGy, which yields a shelf-life of 23 at 2°C, compared to 9–16 days for non-irradiated shrimp (Vyncke *et al.*, 1976). The removal of oxygen from the environment also help maximize the radiation effects. The hand-peeling of this rather small species (0.5 g or less per tail piece) is the main limiting problem, including hygiene, shelf-life and necessary transports (Ehlermann, 1976; Ehlermann and Muenzner, 1976; Ehlermann and Diehl, 1977).

Shrimp — Deep-sea (*Pandalus borealis*)

The optimum dose for deep-sea shrimp is less than 2.0 kGy. The development of black spot or melanosis is enhanced by irradiation; however, if blanching is employed prior to irradiation it can be controlled. A 2 minute blanch plus 2.0 kGy results in an acceptable product with a shelf-life of 34 days at 0–1°C. A 5 minute blanch plus 1.0 kGy also resulted in an acceptable product that had a shelf-life of 41 days at 0–1°C (Hannesson and Dagbjartsson, 1970, Morais, 1984).

Shrimp — Pacific (*Pandalus jordani*)

No optimum/maximum doses were clearly established, but rather a threshold dose of 5.0 kGy was set for frozen Pacific shrimp. This dose yields a shelf-life of 3 weeks at 3°C with no noticeable off-flavours (Scholz *et al.*, 1962).

Shrimp — Tropical (*Penaeus* spp.)

The optimum dose for raw, beheaded, tropical shrimp has been established at 1.5–2.0 kGy, yielding a shelf-life of about 42 days at 3°C. If a 4 minute blanching is employed prior to 1.5–2.0 kGy, the shelf-life is extended to 130 days. (Guevara *et al.*, 1965; Kumta *et al.*, 1970). At terminal spoilage, combination treatment of blanching (80°C, 4 min) and irradiation (2.5 kGy) of shrimp resulted in predominance of *Bacillus* spp.. (Kamat and Kumta, 1974, Debevere *et al.*, 1981, Wills, 1981, El-Fouly, 1987, Lacroix *et al.*, 1995).

A dehydro-irradiation process for shrimp has been developed by Gore *et al.* (1970).

Shrimp

Freshwater prawns (*Macrobrachium rosenbergii*) were irradiated at 1.45 and 2.3 kGy and analyzed in terms of shelf-life and bacteriological quality. A 1 to 2 log cycle reduction in total numbers of bacteria was observed across the doses. After 28 days of storage, total counts increased about 2 to 3 log cycles. Furthermore, total volatile base nitrogen (TVBN) was increased after 8 days of storage in the 2.3 kGy exposure (Angel *et al.*, 1986).

Squid

Combined treatments with irradiation were utilized on dried cuttle fish (DCF) and roasted dried cuttle fish (RDCF) to reduce the microbial contamination. The DCF samples used had aerobic plate counts of 10^6–10^8 colony forming units (CFU)/g with the organisms *E. coli*, *Cl. perfringens*, *Staph. aureus* and moulds being detected. A radiation dose of 3.0 kGy was sufficient to completely eliminate all pathogens and molds in the DCF. The RDCF had an aerobic plate count of 10^3 CFU/g with low levels of *Cl. perfringens*, and *Staph. aureus.* Molds were also detected at a level of 10^2–10^5 CFU/g. With this sample, a dose of 2.0 kGy was effective in eliminating all pathogens and molds. The shelf-life evaluations were really based on initial water content of the samples. In samples with 21% moisture, spoilage occurred by rapid mould growth in 1–1.5 months at 20–24°C or 30–32°C. If the moisture level was below 19%, spoilage did not occur for more than 9 months at 20–24°C or 30–32°C. Furthermore, polyethylene bags and double polycell bags were used as packaging materials and no difference in radiation efficiency was detected among the two packages (Bui-Thi and Yen).

10.4. OTHERS

Red hake fish mince

Washed red hake (*Urophycis chuss*) fish mince was irradiated at 0, 0.66 and 1.31 kGy and then stored aerobically at 3.3°C. Total aerobic plate counts were monitored and found to be less than 10^6 CFU/g for 4, 10, and 17 days after irradiation at all doses. The gel-forming properties of the hake was also evaluated, and it was observed that there is a dose dependent decrease in the gel-forming capacity. Sensory evaluation showed that irradiated samples were superior for 12–18 days longer than the non-irradiated controls, as well as microbiologically for 6–13 days longer than the nonirradiated samples (Dymsza *et al.*, 1990).

Cod fish mince

Deep frozen blocks of cod fillets were used as raw material; after thawing the fillets were minced, salt added and portions of about 70 g formed. These were individually packed under vacuum in plastic pouches impermeable to water and oxygen. At 3.0 kGy the shelf-life was extended from 7 to 21 days (Ehlermann and Muenzner, 1969). Skinned fillets from a local market were minced, hydrocolloids added and frozen in 1 kg-portions; irradiation was at –20°C to a dose of 3.0 kGy and subsequent storage was at −18°C. Chemical and physical indices showed no significant differences between irradiated and non-irradiated sample over 3 months of storage (da Ponte *et al.*, 1986).

Kamaboko

'Kamaboko' is a pâté (fish paste) made of fish mince, starch (potato, etc.), other binders, spices and food colorants which is a favourite in Japan; it may be successfully preserved by use of ionizing radiation (Kashiwagi and Brewbaker, 1966, Kume, 1975, Oku, 1984a/1984b, Oku, 1982a/1982b, Oku, 1981, Oku, 1976, Oku, 1975, Sasayama, 1977, Sasayama, 1973, Sasayama, 1972). Fish varieties used were sardine and pollock, doses ranged up to 5.0 kGy, refrigerated storage was at 1–4°C and at 10–12°C. Shelf-life extension was from about 5 days (unirradiated) up to 34 days (5.0 kGy); with the highest dose only genus *Bacillus* survived the irradiation treatment. However, slight effects on flavour were observed and it was concluded that 3.0 kGy is the optimum dose; at 10–12°C the shelf-life extension is reported from 14 days to 42 days. Several chemical indices were studied, especially amino acids and lipids: no significant changes were observed, except for the fat moiety where a slight decrease in polyenes and an increase in saturates and monoenes was observed. Also sterilization at doses of 12.0 and 26.0 kGy was successfully studied. In all experiments the ratio between maximum and minimum dose in the product was as low as 1.2.

Fish powder

Dried fish powders were inoculated with mesophilic aerobic bacteria, molds, and coliforms at 10^3–10^7 CFU/g, 10^2–10^3 CFU/g and 10^2–10^6 CFU/g, respectively. It was found that if the samples were irradiated at 5.0–10.0 kGy, all molds and coliforms were eliminated, but the mesophilic plate counts were reduced only to $<10^3$ CFU/g. This radiation dose did not change the amino acid levels, TBA value, trimethylamine-nitrogen, or the colour of the samples. The sensory quality had greater acceptability than the controls for 3 months post-irradiation (Cho *et al.*, 1992). Also, dried fish were irradiated at 1.0 kGy which proved effective for disinfestation and reduction in microbial populations (Matin *et al.*, 1992).

Frog legs

Frog legs were inoculated at 6.9×10^7 CFU/mL with *V. cholera* O1 serotype, Inaba serotype, ElT or biotype. The organism remained viable for more than 28 and 2 days when stored at –20°C and 4°C, respectively. The fresh and frozen inoculated frog leg samples were irradiated at 0.50 and 1.0 kGy. This study showed that *V. cholera* will survive frozen storage for 28 days, yet when irradiated all CFU/mL could be eliminated at 0.50 kGy or above (Sang *et al.*, 1987). This is very useful information in that *Vibrio* spp. can be eliminated completely with low doses of gamma radiation.

Frog legs are inherently loaded with *Salmonella* spp. on their skin, of which there is a zero tolerance level for acceptance. Basically for good practice, frog- legs should be washed well and not cross-contaminated. It has been determined that the D_{10} for *S. typhi* in the fresh state on frog- legs is 0.85 kGy, compared to 1.25 kGy in the frozen state (Tambunan, 1984). It was determined that a dose of at least 3.0 kGy was necessary to completely eradicate *Salmonella* from the fresh frog- legs, compared to 4.0 kGy in the frozen state (Tambunan, 1984). It was established than an initial chlorination wash at 50–200 ppm, combined with a minimum of 2.0 kGy or a maximum of 3.0 kGy would be the most effective treatment in eliminating and keeping out *Salmonella* from frog legs (Tambunan, 1984).

10.5. CONTROL OF PATHOGENIC MICROORGANISMS

It should be noted that there is ample literature on fish microbiology, the effect of ionizing radiation on the flora found on fish, the environmental factors such as substrate, pH and temperature as well as the type of the radiation source which may influence the radiation sensitivity of particular species or strains (Anon., 1970, Teufel, 1983, Gould, 1986, Thayer *et al.*, 1990). Besides reducing spoilage-causing microorganisms, radiation processing of food is an especially effective means of controlling pathogenic microorganisms (Mayer-Miebach, 1993). Below, pertinent examples are given for the most common microorganisms on seafood and for the effect of radiation processing. When validating such data, it must be taken into account, that irradiation of food introduces no special microbiological problems (Anon., 1981). That is to say, such irradiated food and especially seafood needs to be handled in the same way as the non-irradiated product and as the product treated by some other method. Finally, it requires that good manufacturing practices are adhered to; for seafood this is mainly temperature control.

The radioresistance (D_{10} value) of *E. coli*, *S. typhimurium*, *S. flexneri*, *Strep. faecalis*, and *Staph. aureus* were determined in soft-shell clams and mussels (Licciardello *et al.,*1989). The D_{10} values were 0.4, 0.64, 0.35, 0.85 and 1.0 kGy for *E. coli*, *S. typhimurium*, *S. flexneri*, *Strep. faecalis*, and *Staph. aureus*, respectively. The culture media chosen and temperature of incubation were critical in determining these D_{10} values (Radomyski *et al.*, 1994).

Grass prawns (*Penaeus monodon*), surface inoculated with *V. cholera*, *Staph. aureus*, *E. coli* and *S. enteritidis*, were irradiated at -10 °C ± 2 °C at a wide variety of doses from 0–10 kGy, and stored for 48 days. Irradiation flavour threshold dose was found to be exhibited at 4.5 kGy. A 2–3 log reduction in bacterial numbers was observed up to 7.5 kGy, and D_{10} values were found to be 0.11, 0.29, 0.39 and 0.48 kGy for *V. cholera*, *Staph. aureus*, *E. coli* and *S. enteritidis*, respectively. Nutrient damage in the prawns was also assessed and it was observed that $C_{20:5}$, $C_{22:6}$ and thiamin levels were reduced by 22, 25, and 32%, respectively. Interestingly enough, amino acids, saturated fatty acids, riboflavin and niacin were resistant to the doses employed (Hau *et al.*, 1992, Hau and Liew, 1993).

S. typhimurium and *S. enteritidis* were irradiated in 0.1M phosphate buffer and D_{10} values were found to be between 0.225 and 0.250 kGy. These same bacteria were inoculated at 1×10^8 cells/mL, then irradiated in shrimp homogenate, and the radioresistance (D_{10}) increased from 0.30 to 0.45 kGy. Finally, it was determined that a dose of 4.0 kGy could be used to completely eliminate *Salmonella* in frozen prepackaged shrimp (Nerkar, 1990, Kampelmacher, 1983). It was also found that *Salmonella*, *Vibrio* and *Aeromonas* spp. can be easily eliminated from fresh or frozen blocks of seafood at 4–5 kGy (Venugopal and Nerkar, 1987). In oysters, doses lower than 3.0 kGy may achieve reasonable safety levels even against *Salmonella*

enteritidis, assuming the number of potential *S. enteritidis* viable cells is as low as it should be if good primary production practices and HACCP are observed (Gelli *et al.*, 1999).

Oysters are passive feeders that filter large volumes of sea water to procure their nutrients. Pathogenic bacteria may be among the many nutrients and other particles suspended in the water, taken up and thus concentrated by the oysters. These microorganisms may include *Salmonella* spp. and other human enteric pathogenic bacteria contaminating the water as a result of sewage discharges from urban areas, as well as other bacteria whose natural habitat is sea water, such as the *Vibrionaceae*. This bacterial genus includes well-known pathogens such as *Vibrio cholerae, V. Vulnificus and V. parahaemolyticus*. The hazard that this poses to human health lies in the fact that oysters, whether in the shell or shucked, are usually consumed raw. Irradiation at relatively low doses (1.0–2.0 kGy) has been shown to effectively eliminate these contaminants (Matches and Liston, 1971; Cisneros *et al.*, 1999; Kilgen *et al.*, 1999) and allow for continued popular enjoyment of such raw fish and seafood dishes as sushi or the popular South American "ceviche", as well as fresh, live oysters and clams.

V. cholerae has been implicated in the cholera pandemic that afflicted many South and Central American countries from 1991 to 1993, with a toll of more than 200,000 cases and thousands of deaths (Albert *et al.*, 1997). Other members of this troublesome group of bacteria are *V. vulnificus* and *V. parahaemolyticus*, frequently found in raw shellfish, especially those harvested in warm waters. Cisneros *et al.*, (1999) isolated halophilic strains of *V. parahaemolyticus* and *V. alginolyticus* from oysters collected in the north coast of Cuba, near Havana. During the summer months, oysters were found to contain up to 10^6–10^7 CFU/g *V. parahaemolyticus. V. vulnificus* was also isolated in 3 of 48 samples tested, whereas *V. cholerae* non-O1 was present in 50% of samples. Several species of these potentially pathogenic *Vibrio* were occasionally found simultaneously in some of the samples, but no *V. cholerae* biotype O1 was isolated.

In vitro experiments were conducted by Gelli *et al.* (1999) to evaluate the effect of irradiation on various biotypes and serotypes of *V. cholerae* group O1 and one strain belonging to group O139, on *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *Aeromonas hydrophila*, *Plesiomonas shigelloides* (18–24 hr culture in alkaline peptone water); *Salmonella typhi*, *S. enteritidis*, *S. typhymurium*, *Shigella flexneri* and *Escherichia coli* O157:H7 (18–24 hr culture in buffered water). *In vivo* studies were also conducted using oysters that were inoculated with pure cultures of *V. cholerae* and *S. enteritidis* through the natural feeding mechanism of the molluscs. For this purpose, oysters were kept in a tank containing sea water inoculated with the microorganisms of interest at levels of 10^6 CFU/mL and allowed to feed naturally for 24h The process resulted in oysters containing 10^6–10^{10} colony forming units (CFU)/g after only 2 hours in the tank. Radiation doses applied were within the range 0.50–3.0 kGy in 0.50 kGy increments. Viable cell counts in oysters decreased 4–10 $\log_{10}$ units depending on the radiation dose absorbed. The *Vibrionaceae* and other cultures tested were considerably more radiation sensitive than *Salmonella* spp. Thus, a dose of 1.50 kGy was enough to eliminate an initial population as high as 10^{10} CFU/mL *Vibrio* spp., *A.hydrophila*, *P. shigelloides, Shigella flexneri and E. coli* O157:H7 *in vitro*, whereas 2.50 kGy were needed to achieve a similar reduction in cultures of salmonellae. *S.enteritidis* and *S.typhimurium* proved to be the most radiation-resistant cultures tested, while reduction in bacterial numbers as a function of radiation dose was similar for all other cultures and strains.

Parallel experiments with non-inoculated, irradiated oysters conducted by Gelli *et al.* (1999) indicated that the irradiation process at doses up to 3.0 kGy is not lethal to the oyster variety used in the study, *Crassostrea brasiliana*. Oysters irradiated at 3.0 kGy survived up to 10 days post-irradiation. Similar results were obtained by Cisneros *et al.*, (1999) in oysters of the species *Crassostrea virginica* collected from the north coast of the Havana province, and by Kilgen *et al.* (1999) in similar oysters from the Gulf of Mexico coast in Louisiana. This may be of particular importance in such countries as Chile and Uruguay, where marketing of fresh shellfish in the shell requires that the molluscs be alive.

The efficacy of treatment with ionizing radiation to eliminate potential contamination with *Vibrio cholerae* O1 biotype El Tor in various shellfish, molluscs and fish native to the Peruvian Pacific coast was studied by Torres *et al.* (1999). The species studied included "abanico" clam (*Argopecten purpuratus*); "choro" clam (*Aulacomya ater*); a third type of clam (*Gari solida*); the snail *Thais chocolata*; a species of shrimp (*Penaeus vannamei*), and the popular fish locally known as "jurel" (*Trachurus picturatus murphyi*) and "lisa" (*Mugil cephalus*). The fish and some of the other products examined are frequently consumed raw, sometimes as a local dish called "ceviche." A D_{10} value of 0.14 kGy for *Vibrio cholerae* biotype El Tor serotype O1 inoculated on the shellfish was consistently calculated, whereas such value was 0.11 kGy in shrimp, 0.12 kGy in jurel and 0.13 kGy in lisa. The results were used to determine the radiation dose required to render the products under study *V. cholerae*-safe. This was accomplished by irradiating samples of all products after inoculating them with 10^6–10^8 CFU/g of the *V. cholerae* pure culture, and ascertaining the dose needed for extinction of all viable cells of the pathogen. It was concluded that a dose in the range 1.0–1.2 kGy would ensure the absence of *Vibrio cholerae* in these products.

Cisneros *et al.* (1999) had similar results concerning the radiation doses required for extinction of populations of *Vibrionaceae* isolated from Cuban oysters, cultured and inoculated back into similar, shucked oysters at the level previously found to be naturally present in the summer months (ca. 10^7 CFU/g). A dose of 1.2 kGy was sufficient to render the shucked oysters safe to eat raw from the standpoint of *Vibrio* spp.

Mesophilic aerobic bacterial counts in mussels (*Mytilus* sp.) collected monthly at sea during the southern winter months (May through August) or bi-weekly thereafter, throughout the entire year, in commercial fishery areas on the Uruguayan Atlantic coast fluctuated between 10^3 and 10^5 colony forming units (CFU)/g and total coliform counts, in turn, varied between 3–93/g as determined by the most probable number (MPN) technique, depending on the time of the year (Lopez, 1999). In all cases, highest counts coincided with the summer months. This represents a hazard to consumers because mussels are often eaten in raw form in Uruguay. More than one half of the samples (51%) presented contamination with some potential pathogen: *Vibrio* spp. in 32%, and *Salmonella* spp. in 19%. *Vibrio* species isolated from 22% of the samples were identified as *V. cholerae* non-O1, whereas 10% of mussels had other *Vibrionaceae*. *V. parahaemolyticus* was not isolated in any mussel tested. The D_{10} determined for pure cultures of the isolates were in the range 0.11–0.19 kGy. This indicated that a dose of 1.0 kGy would be sufficient to ensure the safety of the raw mussels in terms of *Vibrio* spp. contaminants. Subsequent studies using aquarium-contaminated mussels confirmed the efficacy of the treatment. In addition, mussels irradiated at 1.0 kGy survived for at least 48 hours post-irradiation, thus allowing compliance with Uruguayan law in that mussels must be alive during marketing.

Radiation sensitivity of *V. parahaemolyticus* was evaluated in phosphate buffered saline, shrimp homogenate and frozen shrimp. D_{10} values were found to be 0.03 to 0.05 kGy in phosphate buffered saline, and 0.04 to 0.06 kGy in the frozen shrimp homogenate. The data indicated that 0.90 kGy would completely eliminate the bacteria from the frozen whole shrimp. Furthermore, no significant quality or sensory changes were observed in the shrimp (Bandekar *et al.*, 1987). Ito *et al.* (1989), in turn, confirmed the D_{10} value of *V. parahaemolyticus* isolated from frozen shrimp imported into Japan to be 0.03 kGy in 1% NaCl + 0.067 M phosphate buffer; however, the equivalent value in raw and cooked shrimp was 0.38 kGy. In separate studies, Rashid *et al.* (1992) and Ito *et al.* (1993) reported that a dose of 3.0 kGy was enough to reduce *Vibrio* spp. and *Aeromonas hydrophila* in frozen shrimp by 4 log cycles, whereas 3.50 kGy were needed to achieve similar reduction in numbers of *Listeria monocytogenes* or *Salmonella* spp.

V. vulnificus, strain 1008H, was inoculated into shrimp and crabmeat at 10^6 CFU/mL. Irradiation doses of 0.15 and 0.25 kGy resulted in a four log cycle reduction, while 0.35 kGy completely eliminated all *V. vulnificus* from the samples (Watson, 1987). *V. vulnificus* was also inoculated into crabmeat homogenate at 1.7×10^6 CFU/g, and 0.15 kGy and 0.25 kGy resulted in a 4 and 5 log reduction in numbers, respectively. A dose of 0.35 kGy completely eliminated all *V. vulnificus* (Grodner and Watson, 1990). *V. cholera* was inoculated into crabmeat homogenate (*Callinectes sapidus*) 10^7 CFU/g. A dose of 0.25 kGy was effective in reducing *V. cholera* greater than three log cycles, while 0.5 and 1.0 kGy completely eliminated all *V. cholera* from the samples (Grodner and Hinton, 1986).

The influence of fat levels in fish on radiation inactivation of four food-borne pathogens was studied by Kamat and Thomas (1998). Cells of *Listeria monocytogenes* 036, *Yersinia enterocolitica* F5692, *Bacillus cereus* and *Salmonella typhimurium* at logarithmic phase were inoculated in 10% fish homogenates of low (0.39–1.1%), medium (4.25%) and high (7.1–32.5%) fat levels and subjected to gamma irradiation at ice temperature (0–1°C) with doses ranging from 0.05 to 0.8 kGy. The radiation survival curves of *L. monocytogenes* and *B.cereus* were characterized by shoulders, while a tailing effect was depicted by cells of *Y. enterocolitica* and *B. cereus.* The D_{10} values ranged from 0.1 to 0.3, 0.15 to 0.25, 0.1 to 0.15 and 0.09 to 0.1 for *L. monocytogenes* 036, *B. cereus, Salm. Typhimurium* and *Y. enterocolitica* F 5692, respectively. This order (D_{10}) of radiation resistance of each organism was not affected by the fat content of the fish. Inoculated pack studies with each pathogen in fatty (Indian sardine, 7.1%) and lean (Golden anchovy, 0.39%) fish showed no difference in their survival after exposure to 1 and 3 kGy doses which corraborated the above observation. Further, the number of viable organisms for each monitored for two weeks at 2–4°C on appropriate selectve media revealed that their recovery and subsequent growth was not influenced by the fat levels of fish. No increased growth of any of the pathogens tested was evidenced in irradiated fish, when compared with non-irradiaed one (Kamat and Thomas, 1999).

Clostridium botulinum is a spore forming bacterium that may produce severe toxins. Contradictory results on the effects of irradiation on *C. botulinum* in a wide variety of food substrates have been published; however, seafood as substrate has caused particular concern. There is a risk that applying radiation doses that would eliminate a sizable proportion of the spoilage microflora, while allowing botulinal spores to survive, followed by subsequent temperature abuse, the product may sustain growth and result in toxin production (Shewan and Hobbs, 1970, Eklund and Poysky, 1970). Type E-toxin is one of the strongest natural toxins and is formed under anaerobic conditions which almost ever prevail in packaged fish due to the oxygen consumption by the initial microflora. More exactly, the question is whether 'total

rejection spoilage time (untrained panel)' is significantly shorter than the period until toxin is formed (mouse test) (Leone, 1970; Ehlermann, 1971). Many inoculated-pack studies have been conducted on a wide variety of fish and a range of inoculum (Goldblith and Nickerson, 1968, Eklund, 1982). In order to observe any effect the inoculum must be high, usually 10^4 spores /gram or more which does not reflect natural contamination. Several reports proved toxin development before unanimous panel rejection was reached at doses of 2.0 kGy stored under temperature abuse condition (10–20°C). However such a situation is not unique to radiation processing; it has already been observed with vacuum-packaged smoked trout fillets which had been stored too long at too high temperatures; consumers did not notice the spoilage and fatalities occurred. This fundamental problem caused a range of research studies (Hussain *et al.*, 1977b, Ehlermann, 1971).

Validating the research findings has to take into account the microbiological fundamentals: *Cl. botulinum* is able to grow only at temperatures of 10°C and higher; the most hazardous toxin, type E, is only formed at temperatures above 3°C and under anaerobic conditions. The latter must always be assumed as microbial activity in packaged fish will consume any oxygen present at a very early stage. Validating the research findings has also to take into account that many of the early studies were not conducted using experimental designs as are the agreed practice today. In particular dose distribution in samples was not strictly controlled; this resulted in portion of samples receiving much higher doses than the nominal value. Consequently, the risk of toxin formation before spoilage occurs was overestimated in such studies.

Cl. botulinum type E was inoculated into haddock fillets at 10^4 spores/g and irradiated at 1.0 and 2.0 kGy. Those irradiated at 1.0 kGy were acceptable because spoilage occurred before toxin production was noticed. However, the 2.0 kGy exposure resulted in haddock fillets that had toxin present prior to spoilage developing (Eklund, 1982). The same result was not observed with petrale sole fillets, in that at both doses, spoilage occurred prior to toxin production. This indicates that different species of fish are more or less conducive to *Cl. botulinum* (Eklund, 1982, Siddiqui et al., 1979, Shamsuzzama, 1988, Shamsuzzama *et al.*, 1990). Non-inoculated, naturally contaminated cod and haddock did not form toxins at doses as high as 2.0 kGy and stored at 10°C (Nickerson and Goldblith, 1969/1971). Deheaded shrimp and chucked oysters were inoculated by *Cl. botulinum* and stored on ice over 30 days; no toxin was observed regardless of dose up to 5.0 kGy (Jimes, 1967). When inoculated shrimp were stored at 5°C toxin was observed in all samples after 7 days, except samples treated with 5.0 kGy where toxin was detected after 30 days. When chucked oysters were stored at 5°C toxin was observed in all samples regardless of dose up to 5.0 kGy after 30 days. Generally, it is recommended to keep fish at the temperature of melting ice or below 3°C; this would not allow for toxin production by *Cl. botulinum*. The possibility that a specific botulism public health hazard exists for irradiated seafood can be questioned on the basis of such studies which more closely resemble practical situations; thus, the problem of *Cl. botulinum* is more of a theoretical one. Nevertheless, it has hampered the clearance of processing fish with ionizing radiation until today.

Total volatile acids (TVA) and TVBN were evaluated after the irradiation of Bombay duck, Indian mackerel, white pomfret, seer, and shrimp at 0 to 5.0 kGy under ice. These volatiles are produced primarily by target organisms such as *A. hydrophila*, *S. typhimurium, B. megaterium* and *Ps. marinoglutinosa* and other mixed flora. The total volatiles were reduced 50 to 70% in the irradiated samples as compared to the controls, suggesting a microbiological method for detection of irradiated fish (Alur *et al.*, 1992).

Irradiation D_{10} values were determined for *A. hydrophila* in growth broth, potassium phosphate buffer, and in ground bluefish using ^{137}Cs source with a dose rate of 0.1 kGy/minute. The D_{10} values calculated were found to be between 0.14 and 0.22 kGy, with increased radiation resistance being exhibited in frozen samples. It was determined from this study that doses in the area of 1.5 kGy are sufficient to eliminate *A. hydrophila* in retail fresh foods (Palumbo *et al.*, 1986).

Parasites

The radioresistance of some important parasites infecting either freshwater or marine fish have been studied. The minimum effective dose (MED) for *Clonorchis spp.* larvae in rat bioassays was determined to be 0.15 kGy. Using a hamster bioassay, for *Opisthorchis viverrini* MED in fish and in solution was found to be 0.10 kGy. The MED for *Gnathostoma spinigerum* was found to be approximately 7.0 kGy in mice. *Anisakis simplex* was found to be quite resistant to irradiation, with doses of 2.0–10.0 kGy required to inhibit larvae motility. Bioassays in mice and rat with *An. cantonensis* showed it to have a MED of 2.0 kGy, versus 4.0 kGy for *A. costaricensis*. Finally, *Paragonimus westermani* larvae in crabs had a MED of 0.1 kGy, using the cat as a final host (Anon., 1992).

10.6. SHIPBOARD IRRADIATION

Shipboard irradiation was a challenging topic in the 1960 and 1970s; the USA, USSR and Germany operated research vessels carrying irradiation facilities. The basic idea was that fish after catch have a very low load of microorganisms compared to the considerable increase until unloading at the harbour. Consequently, irradiation as early as possible would further reduce that microbial load resulting in much less spoilage until reaching the harbour (Slavin *et al.*, 1966; Carver *et al.*, 1968; Novak and Rao, 1973; Teeny and Miyauchi, 1970; Champion, 1970; Ehlermann and Reinacher, 1978, Rogachev *et al.*, 1972, Anon., 1971, Feldt, 1973, Gruenewald and Ehlermann, 1979). Some of the results from these experiments were rather promising. Unfortunately, changes in international laws and the expansion of territorial waters did cut-off many fishing nations from their traditional fishing grounds and the whole economy and industry in high-sea fishing underwent tremendous changes. The need for on-board irradiation did no longer exist.

11. DETECTION OF IRRADIATED SEAFOOD

Processing of food by ionizing radiation does not change the identity of food. Non-irradiated and irradiated food is visually and sensorially identical. There are only very few exceptions when at elevated radiation doses the product quality is impaired and detrimental changes occur in colour, flavour, and texture. However, such an impaired product will not reach the market, as there is no economic incentive to offer products the consumer will not demand. The problem of identifying a processing treatment is not unique to food irradiation; it applies likewise to organic food, to kosher or 'hallal' food, and to fruit and vegetables stored under modified atmospheres. However, this fact has caused great concern among consumer organizations and government regulatory agencies only with regard to food irradiation. Therefore, at the International Conference on The Acceptance, Control of, and Trade in Irradiated Food in 1988, it was recommended, that ... *governments should encourage research into methods of detection of irradiated food ...* (Anon., 1989). The draft regulation on food irradiation for the European Union requires that the market is regularly observed and that standardized detection methods be used.

A follow-up question is: Has this product received the appropriate dose within the allowable limits, or how much radiation dose has this product received? The availability of analytical methods to detect the radiation treatment in the food itself will contribute to check compliance with existing regulations, e.g. enforcement of labelling, control of prohibition, and to facilitate international food trade. In addition, consumer confidence in the correct application of radiation processing and its control by authorities will be enhanced.

The last 10 years have seen an enormous development in methods which are suitable to identify irradiated food (Delincée, 1991, Delincée, 1993, Delincée, 1998, Anon., 1993, Haire *et al.*, 1997), not at least due to international programmes, e.g. the joint FAO/IAEA worldwide Analytical Detection Methods for Irradiation Treatment of Foods(ADMIT) programme (McMurray *et al.*, 1996c) and the European Community Bureau of Reference (BCR) programme on methods of identification of irradiated foods (Raffi *et al.*, 1994). The final reports of these programmes are a rich source for anybody looking for recent information about detection of irradiated food. The European Committee for Standardization (CEN) has at present adopted already five European Standards:

(1) EN 1784: detection of irradiated food containing fat by gas chromatography (GC) analysis of hydrocarbons
(2) EN 1785: detection of irradiated food containing fat by gas chromatography/mass spectrometry (GC/MS) analysis of 2-alkylcyclobutanones,
(3) EN 1786: detection of irradiated food containing bone by electron spin resonance (ESR) spectroscopy,
(4) EN 1787: detection of irradiated food containing cellulose by ESR spectroscopy; and
(5) EN 1788: detection of irradiated food from which silicate minerals can be isolated by thermoluminescence.

Regarding fish and seafood, validation of these CEN standards has been performed with fish bone (trout) in the case of ESR spectroscopy (Raffi *et al.*, 1992, Schreiber *et al.*, 1993) and with shrimps in the case of thermoluminescence (Schreiber *et al.*, 1996).

Recent studies of (ESR)-spectroscopy on fish bone demonstrate the promising potential of this method (Raffi *et al.*, 1989, Stewart *et al.*, 1991, Empis, 1995, Abdel-Rehim *et al.*, 1997). However, different kinds of crustaceae have been tested (Desrosiers, 1989, Desrosiers, 1996, Helle *et al.*, 1996, Morehouse, 1996, Stewart, 1996) but unfortunately the chemistry of the cuticle seems to be rather complex, leading to different ESR signals for various species and even to different spectra for identical species from various geographical regions (Stewart and Gray, 1996). An In-House blind trial on Norway lobster, however, yielded good results and even the radiation dose could be roughly estimated (Stewart and Stevenson, 1997). Good results were also obtained in a collaborative trial in Germany using ESR spectroscopy on brown shrimps and Norway lobster (Linke *et al.*, 1995), whereas in another inter-laboratory study in the United Kingdom satisfactory results were obtained for Norway lobster and crevettes, but not for pink shrimps (Stewart and Kilpatrick, 1997). Therefore, still more research is required in order to understand the variability of ESR signals from crustaceae. ESR studies on irradiated shellfish such as oysters and mussels, have shown promising results (Desrosiers, 1989, Raffi *et al.*, 1996a).

An interesting feature is the association of silicate minerals with various parts of shellfish including the intestines. By isolating these intestinally entrained minerals and analysing them by thermoluminescence, irradiation treatment can be detected. The analysis of dust from

herbs and spices, fruit and vegetables, bulbs and tubers meanwhile, is an established and very reliable methods to detect irradiation treatment even at rather low doses. Recent studies have extended the use of thermoluminescence of minerals isolated from shrimps to other shellfish such as various prawns and scallops (Schreiber *et al.*, 1994, Sanderson *et al.*, 1996). Also other seafood, e.g. squids (Pinnioja and Pajo, 1995) can be analyzed employing thermoluminescence. A further use of this technique is to apply it not to mineral isolates but to calcite shells or shell fragments (Raffi and Kent, 1996, Carmichael *et al.*, 1994).

An innovative method is photostimulated luminescence (PSL), which obviates the need of isolating minerals and just requires some shell fragments (Sanderson *et al.*, 1996). Interlaboratory trials using PSL are currently under way.

Regarding chemical methods, analysis of hydrocarbons relies on the radiolytical decomposition of fat. Also for fish and other seafood the pattern of hydrocarbons can be used to identify the radiation treatment (Nawar *et al.*, 1996, Morehouse, 1996, Helle *et al.*, 1996, Hwang *et al.*, 1997). Due to the relatively high content in unsaturated and poly-unsaturated lipids in fish and seafood, the pattern of hydrocarbons is mostly rather complex and further analysis by coupled chromatographic methods such as Liquid Chromatography-Gas Chromatography(LC-GC) has been proposed (Schulzki *et al.*, 1997).

In case where the fat content is low, e.g. in prawns, analysis of radiation-induced 2-alkylcyclobutanones has been successfully applied (McMurray *et al.*, 1996b). It has been proposed that the detection sensititvity of cyclobutanones in prawns can be further increased by immunoaffinity chromatography (McMurray *et al.*, 1996a).

Other chemical methods to detect irradiation treatment are the estimation of ortho-tyrosine formed from the protein part also of fish, shrimp and mussels (Meier *et al.*, 1996) or the analysis of the fragmentation of DNA by the comet assay, e.g. in trout and salmon (Cerda *et al.*, 1997).

Finally the application of microbiological methods should be mentioned. Changes in susceptibility to bacterial spoilage together with the relative rate of formation and the content of TVA and TVBN(Alur *et al.*, 1991, Alur *et al.*, 1994) , the relative changes in the microflora (van Spreekens and Toepoel, 1978, Jones *et al.*, 1995) as well as the measurement of bacterial turbidity developed in a clear nutrient medium susequent to inoculation with the test samples (Gautam *et al.,* 1998*)* have also been proposed as detection methods for the irradiation treatment for seafood.

Other detection methods not mentioned in this short chapter are referred to in various overviews (McMurray *et al.*, 1996c, Raffi *et al.*, 1994, Delincée, 1998).

It can be concluded that currently a range of analytical detection methods are available and that the application of treatment with ionizing radiation can now be identified for most seafood. Some of these methods are already internationally acknowledged and are routinely applied by national food control laboratories.

12. RE-IRRADIATION OF SEAFOODS

The General Standard of Codex Alimentarius for Irradiated Foods (Anon., 1984) allows re-irradiation only for products with low moisture content such as cereals, pulses, dehydrated food and other such commodities if the purpose is insect disinfestation. The treatment may be repeated as often as necessary until a cumulative 'overall average dose' of 10.0 kGy is reached. In any other application re-irradiation for the same purpose is considered substitute for good manufacturing practice and is unacceptable. Not considered as having been re-irradiated is a product prepared from raw materials which had been irradiated at lower doses for another technological purpose or a previously irradiated ingredient if it amounts to less than 5 % of the product. Again, a cumulative 'overall average dose' of 10.0 kGy should not be exceeded as a result of this treatment.

Applications of processing by ionizing radiation to seafood have the purpose of improving the hygienic quality and prolonging shelf-life (Kampelmacher, 1984). In both cases, the elimination or reduction of the number of microorganisms present is the goal of the process. If fresh seafood is stored under good handling practices the microflora will slowly grow and finally determine the end of shelf-life; this applies likewise to irradiated seafood. Consequently, re-irradiation of such irradiated seafood once the microflora has already re-multiplied and grown to a certain extent would be ineffective: the metabolic activity of the larger number of microorganisms would have contributed to the impairment of quality. These signs of chemical deterioration would remain even if the microflora was again eliminated. Hence, such practice does not serve a justified purpose and is unacceptable.

Theoretically, there is a single type of re-irradiation for microorganisms which would be justified. Microorganisms are most sensitive to radiation when in the cell-division state. A population found on a food always always contains all stages of the life-cycle of microorganisms. Any stress, including radiation, can be used to synchronize cultures. Thus, a first treatment with a low dose (below 1.0 kGy) would serve to synchronize the microorganisms which after a period of about 7 days are in the most sensitive state and could be treated with another dose of about 1.0 kGy. The synergistic effect of such treatment would be much larger than a single treatment with the cumulative dose. However, such approaches were only successful in the laboratory and could not be transferred to fish trade practices.

One of the greatest concerns of regulators and of consumer organisations surrounding food irradiation is the possibility that some processors who do not want to follow regulations, or want to cut corners or save money, may use irradiation in this manner. There exists a threat that low quality food could be irradiated, exhibit an extended shelf-life, and thus an inferior product has been manipulated to appear better than it really is. An often heard allegation is that food unsuitable for consumption might be cleaned up with the help of irradiation. Furthermore, a high quality product could be irradiated, exhibit an extended shelf-life, spoil at some point, and then be re-irradiated to enhance its appeal, and thus shelf-life. Re-irradiation is not considered a viable option, the signs of spoilage (changes in colour, odour, flavour, and general appearance) cannot be reverted or masked by radiation processing. Considering how rapidly most seafood spoils, there would be no chance for effective re-irradiation. Re-irradiation when used as an argument against processing food by ionizing radiation appears to be a chimera in the light of the facts and discussion given above.

13. COMBINATION PROCESSES

Generally, it should be understood that radiation processing is nearly always used as a combination process: If shelf-life is to be extended, a main spoilage factor is microbial activity; hence, eliminating microorganisms by radiation treatment indispensably requires measures to avoid re-contamination, i.e. combination with packaging. Packaging safeguards the beneficial effects of radiation processing. Even sprout inhibition of bulbs and tubers and ripening delay of fruit and vegetables can, in principle, be considered a combination treatment: it requires packaging in order to handle the produce and expose it to the radiation treatment. Consequently, packaging is a main combination treatment and covered earlier in Section 6.

The combination of irradiation with some other processing means can be used to further enhance shelf-life and quality (Campbell-Platt and Grandison, 1990, Hozowa and Sorman, 1991, Farkas, 1991, Farkas, 1990, Kilcast, 1991, Gould, 1996, Vas, 1981, Wierbicki, 1981, Wills, 1989, Thakur and Singh, 1995, Patterson, 1996). Many examples were listed in Section 10, however, some should be reiterated. The first consideration is irradiating products that are already in the frozen state, and maintaining that temperature throughout irradiation. Irradiation is also an excellent precursor to heating because irradiation sensitizes bacterial cells and spores present, making them easier to kill during cooking (Grecz *et al.*, 1981, Schubert and Stegeman, 1981, Rubio-Cabello *et al.*, 1980, Vincent *et al.*, 1990, Kim *et al.*, 1987). Pre-cooking may also be employed to inactivate some enzymes and at the same time stabilize the product. Heat inactivation of enzymes is indispensable if products are to be sterilized by radiation processing; radiation doses tolerable by food are not sufficient to inactivate enzymes. Many preservatives such as sodium benzoate, potassium sorbate and furylfuramide can be incorporated and will act synergistically with radiation to kill microbes, and to guarantee that any microbes that survive irradiation will still be inhibited (Maha *et al.*, 1981, Ninjoor *et al.*, 1981, Maha *et al.*, 1989, Licciardello *et al.*, 1986). Drip loss, especially of fillets, may be encountered by use of tripolyphosphate (Figueroa *et al.*, 1980). However, the main advantage of radiation processing of food is that it does not leave any residue; therefore, application of any chemical additive or preservative should, as far as possible, be avoided. Another avenue that can be combined with irradiation for enhancement of shelf-life and quality is dehydration (Kwon and Byun, 1995). By lowering the water activity extensively, many yeasts, moulds, and bacteria will not be able to grow and proliferate. Thus irradiating a dehydrated product can be very desirable (Ahmed *et al.*, 1982). Furthermore, the low water activity is an inhibitor of *Cl. botulinum* growth. As mentioned before, adequate packaging material can be used along with modified atmosphere (CO_2), vacuum, or oxygen scavengers to enhance the overall effect (Moral Rama, 1993).

14. WHOLESOMENESS OF IRRADIATED FISHERY PRODUCTS

The wholesomeness of any food, regardless of kind, composition, origin, handling etc., processed by ionizing radiation is established beyond doubt in the dose range of interest and suitable for practical exploitation of this technology. In 1980 the Joint FAO/IAEA/WHO/ Expert Committee on Food Irradiation (JECFI) concluded that the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard; hence, toxicological testing of foods so treated is no longer required. Further, The Committee considered that the irradiation of food up to an overall average dose of 10 kGy introduces no special nutritional or microbiological problems.

The committee was first convened in 1964 after a preparatory meeting in 1961; it had further meetings in 1969 when a few items were accepted temporarily for irradiation, in 1976

when several items received unconditional clearance and others received provisional acceptance; the final meeting was in 1980 with the conclusions given above (Anon., 1981). These were founded on more that 10 years of internationally co-ordinated research involving 24 countries as members of the International Food Irradiation Project (IFIP) which after reaching its goal was dissolved in 1983. The findings were reconfirmed including literature published meanwhile by another WHO Consultation on Food Irradiation (Anon., 1994). Many national committees have reviewed the situation and agreed to these general findings. On the basis of such internationally acknowledged findings about the wholesomeness of radiation processed food clearances and regulations have been issued in many countries. The basis for international trade is a General Standard on Food Irradiation of Codex Alimentarius (Anon., 1984). Recently, a Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation confirmed the wholesomeness also for any food irradiated for sterilization to elevated doses above 10 kGy: As long as the goal of elimination of all spoilage and pathogenic microorganisms is achieved and the products retain their sensory and physical properties, foods so treated can be considered safe and nutritionally adequate.(Anon., 1997c). Some of the research work on the wholesomeness of seafood are reported below as examples; full details are to be found elsewhere (Diehl, 1995).

The wholesomeness of irradiated foods is determined in terms of four criteria, namely, (1) absence of induced radioactivity (2) absence of pathogens and their toxins (3) avoidance of excessive loss in nutritive value and (4) absence of toxic, mutagenic or carcinogenic radiolytic products. It is now established that sources of gamma irradiation, namely, ^{60}Co and ^{137}Cs, used for shelf life extension or radicidation purposes, are unable to induce radioactivity in the treated food items. In addition, radioactivity cannot be induced using electron or X rays below 10 and 5 MeV respectively (Zaytsev *et al.*, 1975, Becker, 1983, Leboutet and Aucouturier, 1985, Miller and Jensen, 1987).

Microbiological aspects of irradiated foods including fish have been examined by several authors (see reviews, Hobbs, 1977; Teufel, 1981; ICMFH, 1983; Mossel, 1985; Farkas, 1989; Radomyski *et al.*, 1994; Monk et al., 1995). A dose of 4 kGy has been found to be most effective for more than adequate elimination of pathogens as judged by risk analysis. Radicidation also did not cause immediate flora changes (Mossel, 1985). Proper control of treatments and good manufacturing practices can ensure safety of irradiated foods. Guidelines for irradiation of fishery products to ensure product safety have been suggested by Giddings (1984).

The International Committee on Food Microbiology and Hygiene (ICFMH, 1983) concluded that modern food handling technology is adequate to control problems created by suppression of spoilage microorganisms and that food irradiation is an important addition to the methods of eliminating food borne pathogens. Microbiological safety of irradiated foods is comparable with that of foods preserved by other acceptable preservation methods (Farkas, 1989).

Irradiation of foods including fishery products can induce chemical changes affecting their nutritional quality. These changes depend on a variety of factors such as initial food composition, temperature during irradiation as well as treatment conditions such as the radiation source used, dose rate and absorbed dose. Much research has been performed on the nutrient value of irradiated foods (Underdal *et al.*, 1973; Kennedy and Ley, 1971; Adam *et al.*, 1982). Studies have shown that doses up to 50.0 kGy do not adversely affect the functions of fats, proteins, or carbohydrates when fed to animals (Diehl, 1995). The changes that occur are quite minor, and are rather difficult to detect in irradiated foods. It is important to consider that fats and carbohydrates are sources of energy in the human body, whereas proteins that are ingested

are sources of essential amino acids that the body uses in a variety of other ways, particularly as building blocks for other proteins. Thus, the damage caused to amino acids by irradiation in proteins in the food is of particular interest. The amino acids in haddock fillets were analyzed after irradiation at 53.0 kGy (Proctor and Bhatia, 1950), and very little if any difference was detected between the irradiated and non irradiated fillets (see Table III). More details of radiation chemistry of major food components can be found elsewhere (Elias and Cohen,1977;1983).

TABLE III. EFFECTS OF ELECTRON RADIATION (53 kGy) ON SELECT AMINO ACIDS IN HADDOCK FILLETS

	Amino acid content (parts of amino acid per 16 parts of nitrogen)	
Amino acid	Non-irradiated	Irradiated
Phenylaline	3.93	3.63
Tryptophan	1.16	1.08
Methionine	2.99	2.85
Cystine	1.04	1.04
Valine	6.29	6.69
Leucine	8.03	8.25
Histidine	1.85	2.00
Arginine	5.34	5.56
Lysine	9.70	9.29
Threonine	4.87	4.58

Source: Proctor and Bhatia, 1950

The nutritional quality of irradiated feed has been widely investigated. Irradiation of laboratory diet did not cause any adverse effects on protein digestibility, biological value, net protein utilization or amino acid composition (Ley *et al.*, 1969). Irradiation caused no adverse nutritional changes except a slight loss of thiamin in dried mackerel (Murray, 1981). Similarly, radiation induced losses of tryptophan (a limiting amino acid in shrimp) was negligible (Murray, 1981). Kennedy and Ley (1971) measured the effects of irradiation (6.0 kGy), cooking, and a combination of both treatments on the B-complex vitamins, nicotinic acid, riboflavin, and thiamin in cod fish fillets (Table IV). Irradiation did not affect nicotinic acid and a 4% loss caused by cooking was not increased by a combination of treatments, while, for thiamin the losses were 47% by radiation, 10% by cooking and 54% by combined treatment. It was concluded by the authors that irradiation followed by cooking produced a total loss which was the sum of the losses produced by each treatment.

TABLE IV. THE EFFECT OF RADIATION (6 kGy) AND COOKING (4 min AT 15lb/in^2) ON SOME B-COMPLEX VITAMINS IN COD (95% CONFIDENCE LIMITS IN PARENTHESES)

Vitamin	Unirradiated, raw (control)	Irradiated, raw		Unirradiated, cooked		Irradiated, cooked	
	Potency, mg/g*	Potency, mg/g*	% of control	Potency, mg/g*	% of control	Potency, mg/g*	% of control
Nicotinic acid	166 (164–168)	165 (162–168)	99 (97–101)	160 (158–162)	96 (94–98)	161 (159–163)	97 (95–98)
Riboflavin	1.98 (1.93–2.03)	1.87 (1.81–1.93)	94 (90–98)	1.80 (1.73–1.87)	91 (87–95)	1.67 (1.60–1.73)	84 (80–88)
Thiamin	2.67 (2.49–2.85)	1.41 (1.26–1.56)	53 (45–60)	2.40 (2.27–2.43)	90 (85–95)	1.22 (1.15–1.29)	46 (38–53)

Source: Kennedy and Ley, 1971

Results for cod show that particular radiation sensitivity of thiamin, as observed in halibut (Ziporin *et al*., 1957; Proctor and Goldblith, 1960) and swordfish (Lopez-Matas and Fellers, 1948); nicotinic acid and riboflavin are much more resistant than thiamin. These results were similar with cooking as reported by Read (1960) in a comparative study. It should be pointed out that the loss of any essential nutrient should be assessed with respect to its contribution to provide the normal dietary requirement of the nutrient. Fish are a good source of nicotinic acid, pyridoxine, biotin and vitamin B_{12}, in human nutrition, but are relatively poor in riboflavin, pantothenic acid and thiamin (Tarr, 1960). Diehl (1969) reported no loss in vitamin B_{12} in clam meat following heat processing after irradiation at a dose of 45.0 kGy.

The effects of irradiation on some vitamins in cod are shown in Table V. No significant decrease in the niacin concentration could be observed even at the highest doses used. Doses of up to 6.0 kGy to 10.0 kGy, however, resulted in a rapid degradation in thiamin. The concentration of alpha-tocopherol was only slightly lower in samples irradiated with 45.0 kGy as compared to the non-irradiated samples. Also, formation of any free radicals was not detected in the samples tested (Underdal *et al*., 1973).

TABLE V. THE THIAMIN, PYRIDOXINE, NIACIN AND A-TOCOPHEROL CONTENT OF NON-IRRADIATED COD AND COD IRRADIATED AT DIFFERENT DOSE LEVELS (mg /kg fish muscle)

Dose (kGy)	Thiamin	Pyridoxine	Niacin	a-tocopherol
0	0.51	1.75	16.8	7.0
1	0.57	1.46	16.4	6.0
3	0.87	1.64	17.9	7.0
6	0.07	1.54	16.5	6.5
10	0.07	0.95	18.2	5.5
25	0.02	0.81	15.4	5.0
45	0.02	0.46	13.5	4.5

Source: Underdal *et al.*, 1973)

Adam *et al.* (1982) demonstrated that gamma irradiation of vacuum-packed herring fillets at low temperatures (0°C) at 50.0 kGy did not affect the proportions of the polyunsaturated fatty acid components (see Table VI). Their results and other findings point to the importance of excluding oxygen during irradiation and subsequent storage of seafood rich in unsaturated lipids.

Wholesomeness testing procedures involving multigeneration animal feeding studies have become the most widely recognized techniques for establishment of toxicological safety and nutritional adequacy of irradiated foods. Parameters examined include body weights, food consumption, reproductive performance, longevity, pathology, liver function tests and tests for teratogenicity and mutagenicity (Adamiker, 1976). In a comprehensive survey, Barna (1979) reported 1223 studies on the wholesomeness of 278 irradiated foods, feeds and fishery products including sardine, salmon, Indian mackerel, shrimp, ocean perch, founder, white fish and fish meal. Apart from the parameters mentioned above, these studies also examined aspects related to protein utilization, food efficiency, water intake, growth, blood sugar level, serum protein level, urine characteristics, renal function, body temperature, behaviour etc. While some adverse effects have been found, the survey led to a general conclusion on the safety of irradiated foods. Neither stimulative nor adverse effects of consumption of irradiated foods were consistent, unambiguous or reproducible. Toxicological studies including chemical studies on radiolytic products found in radurized fishery products and tests for mutagenicity and teratogenicity have also revealed that irradiated seafoods are safe for consumption (Lewis, 1984; Thayer,1994).

Detailed wholesomeness studies of radurized Indian mackerel were carried out (Aravindakshan *et al.*, 1978; Chaubey *et al.*, 1978). A 90-day feeding study including single reproduction study in which Wistar rats were fed with diet containing irradiated mackerel at 35% level (protein content, 26%) did not show any adverse effects. There was no significant effect on body weight, protein efficiency ratio or fertility. Similarly no changes were noticed in haematological profiles, liver enzyme activities or in pathological data. Mutagenicity studies by Chaubey *et al.* (1978) showed that consumption of irradiated mackerel had no effects on induced dominant lethality in the male germ cells or in the micronuclei of the bone marrow.

TABLE VI. FATTY ACID COMPOSITION (g/100g OIL) OF UNIRRADIATED (RELATIVE ERROR = ± 8.5%) AND IRRADIATED (RELATIVE ERROR = ± 18.5%) HERRING OIL/WATER (40:60 W/V) EMULSION AND DEPENDENCE ON THE POST-IRRADIATION STORAGE TIME. RADIATION DOSE = 50 kGy

Fatty acid	Day 1		Day 28	
	Unirradiated	Irradiated	Unirradiated	Irradiated
14:0	3.8	3.6	4.0	4.0
16:0	19.8	19.0	18.5	18.1
16:1	6.0	4.7	4.6	4.3
18:0	2.4	2.4	1.7	1.9
18:1D9	29.1	27.5	27.9	27.4
18:1D11	3.4	3.1	3.6	3.7
18:2	1.2	1.1	1.3	1.2
18:3	0.5	0.4	0.6	0.5
20:1	2.3	1.9	2.9	2.7
20:5	5.9	3.9	6.0	4.2
22:1	5.1	4.9	5.3	5.3
22:6	6.3	4.2	5.3	3.9

Source: Adam *et al.*, 1982

Animal feeding experiments are costly, time consuming, cumbersome (Hickman, 1973). Therefore in recent years newer, less expensive, short term methods have been developed to assess the wholesomeness of irradiated foods (Takeguchi, 1983; Kilbey *et al.*, 1977; Chauhan, 1974). These tests have 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 deficient organisms, activation of viruses by DNA damage, chromosomal and nuclear abnormalities (Phillips and Elias, 1980). The detection of mutation per se is the aim of a number of tests such as Ames test, in which sensitivity to mutation in *S. typhimurium* is examined with respect to its growth requirements. Many studies of irradiated food have also employed in vivo mammalian short term tests. Prominent among these are dominant lethal, cytogenetic, micronucleus and host-mediated assays. Neither short nor multi-generation feeding studies have produced evidence of toxicological effects in animals due to their ingestion of irradiated foods (Thayer, 1994) including fish (Chaubey *et al.*, 1978).

15. SUMMARY

In summary, the preserving effect of ionizing radiation on fish and fish products has been well documented. For the irradiation of seafood products to be accepted, the treatment with irradiation must be shown not to lead to detrimental effects on the product and to be of advantage also to the consumer. Each particular seafood product must be examined thoroughly

before selecting irradiation as a course of action. The type of fish, harvest conditions, post-harvest handling, not to mention many other factors must be carefully considered and understood prior to preservation by irradiation. Some fish do not respond as well as other do to irradiation and thus radiation may not accomplish the goal. Furthermore, radiation processing must be integrated as one of several unit-operations into the existing lines of handling and production; this may require considerable changes of current practices. On the other hand, without such modification the full benefit of radiation processing of seafood will not be achieved. Overall, with very few exceptions, irradiation of fishery products is a not yet widely utilized technique. Not only do consumers and producers could benefit with extended shelf-life, but there is also a greater safety provided to the consumers health with the elimination of many potential harmful pathogens (microorganisms and parasites).

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Annex

TABLE A.I. CLEARANCES FOR SEAFOOD AND FROG LEGS BY COUNTRIES
(from ICGFI database as of July 1999)

Country	Code	Type of clearance	Date	Dose max (kGy)
FISH				
Bangladesh	3	UNCONDITIONAL	29.12.83	2.20
Brazil	5	UNCONDITIONAL	08.03.85	2.00
Chile	3,5	UNCONDITIONAL	29.12.82	2.20
Costa Rica	3	UNCONDITIONAL	07.07.94	2.20
Croatia	3	UNCONDITIONAL	21.06.94	5.00
Cuba	5	CONDITIONAL	01.01.91	3.00
Ghana	7	UNCONDITIONAL	15 01 98	2.00
	5	UNCONDITIONAL	15 01 98	3.00
	3	UNCONDITIONAL	15 01 98	5.00
India	3,5	UNCONDITIONAL	03.02.91	6.00
Mexico	3	UNCONDITIONAL	07.04.95	5.00
	5	UNCONDITIONAL	07.04.95	3.00
	7	UNCONDITIONAL	07.04.95	2.00
Pakistan	3	UNCONDITIONAL	07.03.96	5.00
	5	UNCONDITIONAL	07.03.96	3.00
South Africa	5	CONDITIONAL	09.03.87	2.00
Syria	3,5	UNCONDITIONAL	02.08.86	2.20
Thailand	5	UNCONDITIONAL	04.12.86	2.00
United Kingdom	5	UNCONDITIONAL	01.01.91	3.00
FISH (DRIED)				
Bangladesh	2	UNCONDITIONAL	29.12.83	1.00
Brazil	2,5	UNCONDITIONAL	08.03.85	2.00
Chile	2	UNCONDITIONAL	29.12.82	1.00
Costa Rica	2	UNCONDITIONAL	07.07.94	1.00
Croatia	3	UNCONDITIONAL	21.06.94	5.00
Cuba	2	UNCONDITIONAL	01.05.93	1.00
Ghana	3	UNCONDITIONAL	15.01.98	5.00
	5	UNCONDITIONAL	15.01.98	3.00
	7	UNCONDITIONAL	15.01.98	2.00
Indonesia	5	UNCONDITIONAL	10.02.95	5.00
Pakistan	2	UNCONDITIONAL	07.03.96	1.00
South Africa	5	CONDITIONAL	03.09.87	2.00
Syrian Arab Republic	2	UNCONDITIONAL	02.08.86	1.00
Thailand	2	UNCONDITIONAL	04.12.86	1.00
United Kingdom	3,5	UNCONDITIONAL	01.01.91	3.00
Viet Nam	2	CONDITIONAL	03.11.89	1.00
FISH (FROZEN)				
India	3	UNCONDITIONAL	02.03.91	6.00

TABLE A.I. (cont.)

FISH POWDER				
Korea, Rep. of	3	UNCONDITIONAL	14.12.91	7.00
FISH PRODUCTS				
Bangladesh	3	UNCONDITIONAL	29.12.83	2.20
Brazil	5	UNCONDITIONAL	08.03.85	2.00
Chile	3,5	UNCONDITIONAL	29.12.82	2.20
Costa Rica	2	UNCONDITIONAL	07.07.94	1.00
	3	UNCONDITIONAL	07.07.94	2.20
Pakistan	3	UNCONDITIONAL	07.03.96	5.00
	5	UNCONDITIONAL	07.03.96	3.00
Syrian Arab Republic	3,5	UNCONDITIONAL	02.08.86	2.20
Thailand	5	UNCONDITIONAL	04.12.86	2.00
FROG LEGS				
Bangladesh	3,5	CONDITIONAL	29.12.83	7.00
Croatia	3	UNCONDITIONAL	21.06.94	8.00
France	3	UNCONDITIONAL	08.05.88	8.00
Indonesia	3	UNCONDITIONAL	10.02.95	7.00
Mexico	3	UNCONDITIONAL	07.04.95	5.00
	5	UNCONDITIONAL	07.04.95	3.00
Netherlands	3	UNCONDITIONAL	01.08.92	7.50
FROG- LEGS (FROZEN)				
Indonesia	3	UNCONDITIONAL	10.02.95	7.00
INGREDIENTS FOR MARINADES (POWDER)				
South Africa	3	CONDITIONAL	31.07.91	10.00
SEAFOOD				
Croatia	3	UNCONDITIONAL	21.06.94	5.00
Cuba	5	CONDITIONAL	01.01.91	3.00
Ghana	7	UNCONDITIONAL	15 01 98	2.00
	5	UNCONDITIONAL	15 01 98	3.00
India	3	UNCONDITIONAL	02.03.91	6.00
Pakistan	3	UNCONDITIONAL	07.03.96	5.00
	5	UNCONDITIONAL	07.03.96	3.00
SEAFOOD (FROZEN)				
India	3	UNCONDITIONAL	02.03.91	6.00
SEAWEED (DRIED)				
South Africa	3	CONDITIONAL	25.05.88	10.00

TABLE A.I. (cont.)

SHELLFISH				
United Kingdom	3	UNCONDITIONAL	01.01.91	3.00
SHELLFISH POWDER				
Korea, Rep. of	3	UNCONDITIONAL	14.12.91	7.00
SHRIMP				
Bangladesh	3,5	CONDITIONAL	29.12.83	5.00
Belgium	3	CONDITIONAL	30.11.88	5.00
Croatia	3	UNCONDITIONAL	21.06.94	5.00
Cuba	5	CONDITIONAL	01.01.91	3.00
France	3	UNCONDITIONAL	10.10.90	5.00
Ghana	3	UNCONDITIONAL	15.01.98	5.00
	5	UNCONDITIONAL	15.01.98	3.00
	7	UNCONDITIONAL	15.01.98	2.00
India	3	UNCONDITIONAL	02.03.91	6.00
Indonesia	3	UNCONDITIONAL	10.02.95	7.00
Netherlands	3	UNCONDITIONAL	01.08.92	4.50
Pakistan	3	UNCONDITIONAL	07.03.96	5.00
	5	UNCONDITIONAL	07.03.96	3.00
Thailand	3	UNCONDITIONAL	04.12.86	5.00
United Kingdom	3,5	UNCONDITIONAL	01.01.91	3.00
SHRIMP (FROZEN)				
India	3	UNCONDITIONAL	02.03.91	6.00
Indonesia	3	UNCONDITIONAL	10.02.95	7.00
Thailand	3	UNCONDITIONAL	04.12.86	5.00

Explanations for 'Code' (purpose of treatment):

2 = disinfestation
3 = microbial control
5 = shelf-life extension
7 = parasite control

Note: Countries having generic clearances for seafoods are also listed under individual items such as fish and shrimp. If the approvals for a product include terms such as frozen, it indicates that this product cannot be irradiated under other conditions. In several instances countries specify different dose values for varying purposes; for details of the meaning of 'Dose Max (kGy)' the respective regulations should be consulted (e.g. the Netherlands regulate the maximum of an average dose, France regulates maximum dose)

Also refer to the ICGFI-Web Page (http://www.iaea.org/icgfi/) for more details on Clearance Database.

TABLE A.II. OPTIMUM RADIATION DOSES FOR MARINE FINFISH

NAME	SPECIES	PRODUCT FORM	OPTIMUM DOSE (KGY)	MAXIMUM DOSE (KGY)	SHELFLIFE (DAYS)	REFERENCE
Anglerfish	*Lophius americanus*	Tail	1.5	–	–	54
Blackback Flounder	*Pseudopleuronectes americanus*	Fillets or Dressed	4.5	9.0–9.3	22	48, 57
Bombay duck	*Harpodon nehereus*	Whole	1.0–2.5	5.0	18–22	45, 87,137,200, 201, 318
Butterfish	*Peprilus triacanthus*	Dressed	1.0–2.3	4.6–7.0	49	54, 57, 332
Carp	*Cyprinus carpio*	vacuum packed	1.5 &5.0	–	31–35	94, 95,171, 252
Channel catfish	*Ictalurus ictalurus*	Whole	1.0–2.0	4.0	20	108, 109, 110,288
Chubs	*Coregonus spp.*	Smoked	1.0	8.0	42	107, 341, 342
Cod	*Gadus morhua*	Fillets or Steaks	1.5–2.5	4.5–5.0	4–5 longer	13, 44, 56, 150, 211, 303, 306, 309, 338, 343, 345, 369
Pacific cod	*Gadus macrocephalus*	Whole or Fillets	0.5–1.0 (whole) 4.5 (fillets)	7.0	24	238, 362
Dogfish	*Squalus acanthias*	Fillets	2.0–	2.0	7	
Dover sole	*Microstomus pacificus*	Whole	0.5–1.0	–	–	362
Gray sole	*Glyptocephalus cynoglossus*	Fillets	1.0–2.0	–	29	243, 306
Lemon sole	*Microstomus kitt*	Frozen Fillets	2.5–5.0	5.0–10.0	–	67, 338
Petrale sole	*Eopsetta jordani*	Fillets	2.0–3.0	3.0	28–49	74, 240, 341, 342, 348
Haddock	*Melanogrammus aeglefinus*	Fillets	1.5–2.5	6.7	30–35	67, 259, 284, 306, 307, 308, 344, 345, 352
Halibut	*Hippoglossus hippoglossus*	Steaks	2.0–3.0	5.0	20	115, 303, 339
Halibut	*Paralichthys californicus*	Steaks	2.0	–	21–42	74, 242, 341, 352
Herring	*Clupea harengus*	Whole	1.0–2.0	5.0	10–14	303, 346
Lake herring	*Coregonus artedii*	Fillets	3.0	3.0	8	112
Herring smelt	*Argentina silus*	Smelt	0.5–1.0	–	6 longer	56
Kembung fish	*Rastrelliger negelectus*		1.0–2.0	2.0	12	347

TABLE A.II. (cont.)

NAME	SPECIES	PRODUCT FORM	OPTIMUM DOSE (KGY)	MAXIMUM DOSE (KGY)	SHELFLIFE (DAYS)	REFERENCE
Mackerel	*Scomber scombrus*	Whole	2.5	–	30–35	342, 343
Mackerel	*Rastrelliger kanagurta*	Whole	1.5	–	21–24	131, 152, 164, 165, 166, 200, 379, 380, 381
Nagli fish	*Sillago sihima*	Whole or dressed	2.0	3.0	19	7
Ocean perch	*Sebastes marinus*	Fillets	1.5–2.5	–	30	13,301,308,341,343,352
Ocean perch	*Sebastes alutus*	Whole or Fillets	0.5–1.0 (whole) 1.0–2.0 (fillets)	–	25–28	241, 243, 362
Yellow perch	*Perca flavescens*	Fillets	3.0	5.0	40–45	110, 113, 140, 34, 22, 10
Pollock	*Pollachius virens*	Fillets	1.5	2.3–2.5	28–30	13, 57, 67, 341, 343, 352
Pomfret	*Stomateus cinereus*	Whole	1.0	3.0	28 longer	4, 88, 132, 153, 200, 201
Rockfish	*Sebastodes spp.*	Fillets	1.5–2.5	–	20	115, 239
Sablefish	*Anopoploma fimbria*	Whole	3.0	–	7	351
Salmon		Fillets	1.0	3.0	20	71, 115, 236, 303, 339, 351
Sardines	*Sardinella melanura*	Whole	0.23	–	3 × longer	154
Threadfin	*Eleutheronesma tetradactylum*	–	1.0–2.5	–	3–4 × longer	4
Tuna	*Thunnus obesus*	Whole	2.0	–	–	11, 289
Trout	*Salmo gairdneri*	Fillets	1.0	–	28	94, 96, 151, 167
Lake trout	*Salvelinus namaycush*	–	3.0	7.0	26	140
Whitefish	*Coregonus clupeaformis*	–	1.5–3.0	–	15–29	141, 273
Whiting	*Merluccius spp.*	Whole	1.2	2.0–4.5	24–28	48, 57, 67, 76, 224, 227, 304,

TABLE A.III. OPTIMUM RADIATION DOSES FOR SHELLFISH

NAME	SPECIES	PRODUCT FORM	OPTIMUM DOSE (KGY)	MAXIMUM DOSE (KGY)	SHELF LIFE (DAYS)	REFERENCE
Baby clam	*Venerupis semidecus sata*	Meats	1.0–4.0	–	28	392
Soft-shell clams	*Mya arenaria*	Meats	3.5–4.5	–	30	257,308,334
Surf clam	*Spisula solidissima*	Meats	4.5	–	40–50	54
Dungeness crab	*Cancer magister*	Meats	2.0–2.5	–	28–42	240, 319
King crab	*Paralithodes camtschatica*	Meats	2.0	–	35	240, 241
Swimming crab	*Portunus pelagicus*	Meats	2.0	–	28	147, 216
American lobster	*Homarus americanus*	Meats	0.75	–	14 longer	72, 285
European lobster	*Homarus gammarus*	Meats	1.0–3.0	–	–	303
Norwegian lobster	*Nephrops norvegicus*	Tails	2.0–3.0	–	35–42	150
Mussels	*Mytilus smaragsinus*	Meats	1.5–2.5	–	42	216
Oysters	*Crassostrea virginica*	Meats	2.0	8.0	21–28	85, 126, 215, 223, 236, 243, 261, 341
Scallops	*Placopecten magellanicus*	Meats	0.75	1.5	28–43	125, 282, 283
Deep-sea shrimp	*Pandalus borealis*	Whole	2.0	–	34–41	150, 245
European brown shrimp	*Crangon vulgaris*	Whole	1.5	–	23	98, 99, 101
Pacific shrimp	*Pandalus jordani*	Whole	–	5.0	21	319
Tropical shrimp	*Paeneus spp.*	Whole	1.5–2.0	–	42–130	77, 123, 147, 181, 199, 203
White, pink, brown shrimp	*Paeneus setiferus, aztecus, duarum*	Whole	1.5–2.0	5.0	21–30	74, 188, 204, 262

TABLE A.IV. RADIATION RESISTANCE OF SEAFOOD BACTERIA OF PUBLIC HEALTH SIGNIFICANCE

BACTERIA	MEDIUM	D_{10} VALUE (KGY)	REFERENCE
Aeromonas hydrophila	Growth broth/Phosphate buffer/Ground bluefish	0.14–0.22	274
Bacillus cereus	Fish homogenate	0.10–0.15	182
Escherichia coli	Crabmeat	0.14	274
Escherichia coli	Grass prawns	0.39	156
Escherichia coli	Oysters	0.35	156
Escherichia coli	Soft-shell clam and mussels	0.40	208
Listeria monocytogenes 036	Fish homogenate	0.10–0.30	182
Proteus vulgaris	Oysters	0.20	208
Salmonella enteriditis	Grass prawns	0.48	156
Salmonella enteriditis	Phosphate buffer	0.25	253
Salmonella enteriditis	Oysters	0.50	253
Salmonella enteriditis	Shrimp homogenate	0.45	253
Salmonella typhimurium	Soft-shell clam and mussels	0.64	208
Salmonella typhimurium	Fish meal	1.74	208
Salmonella typhimurium	Phosphate buffer	0.225	253
Salmonella typhimurium	Fish homogenate	0.09–0.10	182
Salmonella typhimurium	Shrimp homogenate	0.30	253
Shigella dysenteriae	Shrimp	0.22	253
Shigella dysenteriae	Oysters	0.40	253
Shigella flexneri	Soft-shell clam and mussels	0.35	208
Shigella flexneri	Shrimp	0.41	208
Staphylococcus aureus	Grass prawns	0.29	156
Staphylococcus aureus	Soft-shell clam and mussels	1.0	208
Staphylococcus aureus	Crabmeat	0.8	208
Staphylococcus aureus	Oysters	1.5	208
Staphylococcus aureus	Shrimp	1.9	208
Streptococcus facaelis	Soft-shell clam and mussels	0.85	208

TABLE A.IV. (cont.)

Streptococcus facaelis	Shrimp	0.75	208
Streptococcus facaelis	Oysters	1.0	208
Vibrio cholera	Grass prawns	0.11	156
Vibrio parahaemolyticus	Fish	0.03–0.06	156
Vibrio parahaemolyticus	Seawater	0.05	156
Vibrio parahaemolyticus	Phosphate buffered saline	0.03–0.05	40
Vibrio parahaemolyticus	Shrimp homogenate	0.04–0.06	40
Vibrio vulnificus - O	Phosphate buffered saline	0.060	85
Vibrio vulnificus - T	Phosphate buffered saline	0.037	85
Yersinia enterocolitica F 5692	Fish homogenate	0.15–0.25	182

TABLE A.V. RADIATION RESISTANCE OF SEAFOOD VIRUSES OF PUBLIC HEALTH SIGNIFICANCE

VIRUSES	MEDIA	D_{10}
Hepatitis A — P.F.U	Clam/oyster	2.06
Hepatitis A — I.E.F.	Clams	1.6
Hepatitis A — I.E.F.	Oysters	1.48
Hepatitis A — In situ	Oysters	3.16
Hepatitis A	Overall	2.02
Poliovirus	Oysters	3.1
Rotavirus	Clams/oysters	2.4

Source: Mallet, et. al., 1991.

TABLE A.VI. RADIATION RESISTANCE OF SEAFOOD PARASITES OF PUBLIC HEALTH SIGNIFICANCE

PARASITE	MINIMUM EFFECTIVE DOSE (KGY)
Clonorchis spp. (in fish)	0.15
Clonorchis sinensis (isolated)	0.05
Opisthorchis viverrini	0.10
Gnathostoma spinigerum	7.0
Anisakis simplex	2.0–10.0
Anisakis cantonensis	2.0
Anisakis costaricensis	4.0
Paragonimus westermani	0.1

Source: Anon. 1992

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