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FOREWORD

The Microbiology Handbook series includes Dairy Products, Fish and Seafood, and Meat Products, published by Leatherhead Food International and RSC Publishing. The books in the series are designed as easy-to-use guides to the microorganisms found in foods. Each book provides a brief overview of the processing factors that determine the nature and extent of microbial growth and survival in the product, potential hazards associated with the consumption of a range of products, and growth characteristics for key pathogens associated with the product. All handbooks also contain a review of the related legislation in Europe and UK, guides to HACCP, and a detailed list of contacts for various food authorities. The books are intended as a source of information for microbiologists and food scientists working in the food industry and responsible for food safety, both in the UK and elsewhere.

Acknowledgements

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I thank the contributing authors for their collaboration, which has been the chief factor in ensuring the completion of this book. My gratitude is extended to Dr Peter Wareing, Training Manager, for his scientific input and Victoria Emerton, Technical Team Leader for her valuable editorial contribution. I also acknowledge and thank Jackie Apps, Alison Turner, and Ann Pernet for the work put into typesetting and indexing.
I dedicate this book to my husband - Goldwyn; thank you for all the support.

Rhea Fernandes
Leatherhead Food International
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INTRODUCTION

Fish and seafood are a main source of animal protein in the diet. Because of their health advantages over red meats, the consumption of fish and seafood has increased. Catches can be gathered from seas, rivers and lakes whose water can range from pristine to contaminated. Often contamination is from human and animal sources; thus, fish and seafood can be involved in the transmission of pathogenic microorganisms and toxins. Geographical region, season, and, for fish, whether they are pelagic (surface to mid-water) or demersal (bottom) feeders will influence the numbers and types of microorganisms present on freshly caught seafood.

The Microbiology Handbook- Fish and Seafood consists of the microbiology of seven different product categories: chilled and frozen raw fish, chilled and frozen prepared fish, molluscan shellfish, crustacean shellfish, cured, smoked and dried fish, fermented fish, and fish and shellfish toxins. The second edition of this handbook is a review of the entire book for currency of information. Key changes in this edition are the recent regulatory changes pertaining to food hygiene and microbiological criteria for foodstuffs.

Further Reading

General Fish/Seafood Microbiology


1. CHILLED AND FROZEN RAW FISH

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United States of America

1.1 Definitions

Fish are classified as any of the cold-blooded aquatic vertebrates of the super class Pisces typically showing gills, fins and a streamline body. In addition, ‘fish’ also refers to the flesh of such animals used as food. This super class of vertebrates includes all the bony and cartilaginous finfish, and excludes molluscs and Crustacea. However, some regulatory agencies such as the US Food and Drug Administration (FDA) will include molluscan shellfish, crustaceans, and other forms of aquatic animal as part of their ‘fish’ definition. In this chapter, “fish” will be used for fresh and seawater finfish.

Fish are an important part of a healthy diet since they contain high quality protein, but typically present a low fat percent when compared to other meats. In addition, most fish contain omega 3-fatty acids and other essential nutrients. Although fish is broadly similar in composition and structure to meat there are a number of distinctive features. Protein content in fish fillet varies typically from 16 - 21%. The lipid content, which can be up to 67%, typically fluctuates between 0.2 - 20%, and is mostly interspersed between the muscle fibres. Fish fillets are a poor source of carbohydrates, offering less than 0.5% (1). Fish fillet composition can vary significantly within the same species due to feed intake, migratory patterns, and spawning season. The lipid fraction is the component showing the greatest variation; it shows a typical season pattern especially in migratory species such as herring or mackerel. Fish can be divided into fatty and lean fish; lean fish are those fish that store most of their fat in the liver, while fatty fish have fat cells distributed along their bodies. Muscle composition and structure of fish also differ from those found in other meat. Fish flesh is dominated by the abundance of white muscle in relatively short segments, giving it its characteristically flaky structure. The connective tissue content of fish is also lower than that found in meat, typically 3 and 15% of total weight, respectively (1).
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*Chilled fish* is fish that has been cooled to, and maintained at or below 7 °C, but not below 3 °C during storage, transportation and sale.

*Controlled-atmosphere packaging (CAP)* refers to packaging in an atmosphere where the composition of the gases is continuously controlled during storage. This technique is primarily used for bulk storage.

*DMA* is dimethyl amine.

*Evisceration* is the removal of the viscera from a fish.

*Fresh fish* is raw fish that has not been processed, frozen or preserved.

*Frozen fish* is fish that has been cooled to, and maintained at or below -2 °C (normally below -12 °C) during storage, transportation and sale.

*Modified-atmosphere packaging (MAP)* refers to packaging systems in which the natural gaseous environment around the product is intentionally replaced by other gases, usually carbon dioxide (CO₂), nitrogen (N₂) and oxygen (O₂). The proportion of each component is fixed when the mixture is introduced, but no further control is exercised during storage.

*Organoleptic* refers to qualities such as appearance, colour, odour and texture.

*Quality* refers to palatability and organoleptic characteristics such as tenderness, juiciness, and flavour based on the maturity, marbling, colour, firmness, and texture of the fish.

*Raw fish* refers to fish that has not been cooked but excludes fish treated with curing salts and/or subjected to fermentation.

*Shelf life* is defined as the time of storage before microbial spoilage of a fish is evident.

*Spoilage* describes changes that render fish objectionable to consumers; hence, *spoilage microflora* describes an association of microorganisms that, through their development on fish, renders that fish objectionable to consumers.

*Spoilage potential* is a measure of the propensity of microorganisms to render fish objectionable to consumers through the production of offensive metabolic by-products.

*Superchilled fish* is fish that has been cooled to, and maintained at temperatures just below the freezing point, at -2 to -4 °C, during storage, transportation and sale.
CHILLED AND FROZEN RAW FISH

Vacuum packaging (VP) refers to packaging systems in which the air is evacuated and the package sealed.

1.2 Initial Microflora

The subsurface flesh of live, healthy fish is considered sterile and should not present any bacteria or other microorganisms. On the contrary, as with other vertebrates, microorganisms colonise the skin, gills and the gastrointestinal tract of fish. The number and diversity of microbes associated with fish depend on the geographical location, the season and the method of harvest. In general, the natural fish microflora tends to reflect the microbial communities of the surrounding waters. It is difficult to estimate how many microorganisms are typically associated with fish, since they heavily depend on the type of sample analysed and the protocol used for isolation. In fact, standard culture-dependent methods can only recover between 1 to 10% of total bacteria present in any given sample. More accurate, molecular-based methods have not yet been used to address this issue. Gastrointestinal tracts and gills typically yield high bacteria numbers, although these are influenced by water quality and feed. Fish harvested from clean and cold waters will present lower bacterial numbers than fish from eutrophic and/or warm waters. However, potential human pathogens may be present in both scenarios.

The autochthonous bacterial flora of fish is dominated by Gram-negative genera including: Acinetobacter, Flavobacterium, Moraxella, Shewanella and Pseudomonas. Members of the families Vibrionaceae (Vibrio and Photobacterium) and the Aeromonadaceae (Aeromonas spp.) are also common aquatic bacteria, and typical of the fish flora. Gram-positive organisms such as Bacillus, Micrococcus, Clostridium, Lactobacillus and coryneforms can also be found in varying proportions (1). It is crucial to mimic the environmental physico-chemical parameters when isolating bacteria from fish. For example, some species (most Vibrios) require sodium chloride for growth; whenever possible, several culture media containing sodium chloride and more than one incubation temperature should be used. It must be noted that mesophilic bacteria can rapidly overgrow psychrotrophic organisms.

Human pathogenic bacteria can be part of the initial microflora of fish, posing a concern for seafoodborne illnesses (2). These pathogens can be divided into two groups: organisms naturally present on fish (Table 1.I); and those that although not autochthonous to the aquatic environment, are present there as result of contamination (anthropomorphic origin or other) or are introduced to the fish during harvest, processing or storage (Table 1.II) (3).
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TABLE 1.I
Indigenous bacterial pathogens typically present in fish (3)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Temperature range</th>
<th>Estimated Minimum Infective Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium botulinum</em> - non-proteolytic type E</td>
<td>3 - 26 °C</td>
<td>0.01 - 1 µg toxin lethal dose</td>
</tr>
<tr>
<td>Pathogenic Vibrio spp.</td>
<td>10 - 37 °C</td>
<td>High for most species, 10^9 - 10^10 cells/g (exception: <em>V. vulnificus</em>)</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em> other vibrios</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aeromonas spp.</em></td>
<td>5 - 35 °C</td>
<td>Unknown</td>
</tr>
<tr>
<td><em>Plesiomonas shigelloides</em></td>
<td>8 - 37 °C</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

TABLE 1.II
Non-indigenous bacterial pathogens frequently present in fish (3)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Primary habitat</th>
<th>Minimum Infective Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Soil, birds, sewage, stream water, estuarine environments, and mud</td>
<td>Variable depending on the strain (&gt;10^2 cells/g)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Ubiquitous, human origin</td>
<td>10^5 - 10^6 cells/g. Toxin levels 0.14 - 0.19 µg/kg bodyweight</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Intestinal track of terrestrial vertebrates</td>
<td>from &lt; 10^5 - &gt;10^6</td>
</tr>
<tr>
<td><em>Shigella spp.</em></td>
<td>Human origin</td>
<td>10^9 - 10^10</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Fecal contamination</td>
<td>10^9 - 10^10</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Ubiquitous in environment</td>
<td>High (10^7 - 10^9 cells/g)</td>
</tr>
</tbody>
</table>

* Some sources considered L. monocytogenes as part of the natural aquatic flora

It is apparent from the above that there is potentially a very diverse range of organisms present on fish. However, numbers of pathogenic bacteria in raw fish tend to be low, and risk associated with the consumption of seafood is low (2, 4). In addition, during storage indigenous spoilage bacteria tend to outgrow potential pathogenic bacteria.
1.3 Processing and its Effects on the Microflora

1.3.1 Capture, handling and processing

Wild finfish are usually caught by net, hook and line, or traps, with very little control over the condition of the fish at the time of death or the duration of the killing process. This contrasts greatly with the meat industry, in which the health of each animal can be assessed prior to slaughter, and the killing process is designed to minimise stress. However, in recent decades, aquaculture practices have been expanding worldwide, offering better control of fish health prior to, and during harvest.

The length of time that set nets have been in the water or the time trawlers’ nets are towed, has an effect on the amount of stress and physical damage that the fish will suffer during capture. Physical damage such as loss of scales, bruising and bursting of the gut will increase the number of sites open for bacterial attack and spread. In addition, cortisone levels increase during prolonged stress and can alter the fillet quality.

After capture, the fish may be stored in the vessel for periods ranging from just a few hours to several weeks in melting ice, chilled brine or refrigerated seawater at -2 °C. Inadequate circulation of chilled brines may result in localised anaerobic growth of some microorganisms, and spoilage, with the production of off-odours. Used refrigerated brines can be contaminated with high numbers of psychrotrophic spoilage bacteria, and their re-use will increase the cross-contamination of other fish with such microorganisms. Increasingly, and especially when fish is stored on board for longer periods, freezing facilities (-18 °C) may be used to prevent the catch from deteriorating.

Fish may be eviscerated prior to storage at sea - a practice that may have both advantages and disadvantages. The action of intestinal enzymes and activity of the gut bacteria on the flesh around the belly cavity may produce discolouration, digestion and off-flavours in uneviscerated fish. In eviscerated fish, however, the cuttings provide areas of exposed flesh that are open to microbial attack. If evisceration is carried out at sea, care should be taken in removing all the gut contents and washing the carcass thoroughly prior to refrigerating, icing or freezing. The decision to eviscerate the catch at sea will depend greatly on the size of the fish and the duration of storage at sea, with fish such as tuna and cod being more commonly eviscerated than sardines, mackerel or herring.

During capture and storage, finfish will almost invariably come into contact with nets, decks, ropes, boxes and/or baskets, human hands and clothing. These contacts will not only increase the bacterial cross-contamination between fish batches but will introduce microorganisms from other sources such as humans, birds and soil. Of particular concern is the use of wooden or soiled plastic containers for storage and unloading at the quayside, in which the bacterial load...
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can be substantial. These containers are also used for displaying the fish during auction at the quayside, often in the absence of adequate refrigeration.

As with all foods, careful and sanitary handling during processing is required to reduce the risk of contamination with potential human pathogens, and to limit the loss of quality (5). Good Manufacturing Practice (GMP) and control of the sanitary conditions of the transport and processing environments are essential to limit additional risk of disease caused by fish consumption (6, 7). Monitoring of the seawater for algal growth in order to limit the risk of algal toxin ingestion, and of the quality of the water used for ice and to wash fish, cleaning of the work environment, use of effective detergents and disinfectants, and minimising handling will all reduce microbial cross-contamination.

1.3.2 Modified-atmosphere packaging

A natural atmosphere rich in oxygen (21%) is responsible for oxidative processes and for all aerobic respiratory life. Low oxygen levels have been shown to substantially prolong the freshness and quality life of refrigerated seafood products. MAP extends the shelf life of most fishery products by inhibiting bacterial growth and autoxidation.

In MAP, the natural atmosphere is replaced with a controlled gas mixture (carbon dioxide, nitrogen, oxygen etc.). Carbon dioxide is the most important gas in MAP of fish because of its bacteriostatic and fungistatic properties. In the absence of oxygen, partial fermentation of sugars occur leading to lower pH. Both carbon dioxide and low pH inhibit the growth of the typical spoilage bacteria such as Pseudomonas and Shewanella. Bacterial composition under MAP shifts from mostly Gram-negative to predominantly Gram-positive (lactic) bacteria. Brochothrix thermosphacta and psychrotrophic lactic acid bacteria (LAB) can produce spoilage characteristics; however, they are usually process contaminants, not part of the normal flora of the meat animals (8). Anaerobic atmospheres have less effect on fresh fish shelf life; fish have a higher post mortem pH, and specific spoilage organisms may use other terminal electron acceptors naturally present in the fish (trimethylamine-N-oxide (TMAO), ferric ion (Fe³⁺)). What is more, potential spoilage bacteria are among the psychrophilic and psychrotrophic flora present on temperate-water fish before death (9).

Packaging changes the intrinsic and extrinsic parameters affecting a product, from water activity (aₜ) through to physical damage. These changes can be deleterious, allowing more growth of spoilage organisms, for example, but if applied properly should extend the life of the product. Physical barriers not only protect from physical damage, but isolate the food in an environment different from the bulk atmosphere.

The atmospheric conditions surrounding a product may be passively or actively altered. By vacuum packing foods, a reduction in the oxygen tension is achieved which, in time, if there is some oxygen demand from the product, will result in fully anoxic conditions. However, by actively altering the composition of the
surrounding gas, a modified-atmosphere may contain any gas necessary for the desired effect.

Modified-atmosphere preservation of fish was first reported in the 1930s, but only in recent years has it seen a marked expansion in use and market share. This has been driven partly by increased consumer demand for fresh and chilled convenience foods containing fewer chemical preservatives. MAP has been applied to fresh meat and fish with a resulting commercially viable extension in shelf life (10). The microflora of meat is not the same as that of whole, gutted or filleted fish, and the MAP of fish has more challenges to overcome as a result of: a comparatively large initial load of bacteria present, which are able to grow rapidly at low temperature; the higher pH and reduction potential (Eh) of the fish muscle; the pathogens that may be able to grow before spoilage occurs; and the problems of muscle structure damage by the modified-atmosphere (11).

VP is one of the oldest forms of altering the interior gaseous environment of a pack, but residual oxygen and other electron acceptors may be sufficient to allow oxidative spoilage of fish (12).

The principal effect of raised carbon dioxide-MAP is an extension of the ‘lag’ phase of the growth of the bacteria on the fish, the inhibition of common ‘spoilage’ bacteria (Pseudomonas, Flavobacterium, Micrococcus and Moraxella), and the promotion of a predominantly Gram-positive, slower-growing flora (11).

Many additives have been tried in conjunction with changed atmosphere, including salt, phosphates, sorbates and chelating agents such as ethylenediaminetetraacetic acid (EDTA) (11). The products, after additive application, may not be considered ‘fresh’ fish.

Gases used in MAP of fish most commonly include carbon dioxide and nitrogen. High concentrations of carbon dioxide have the most pronounced microbial effect, but can dissolve into fish liquids and deform packages, discolor pigmented fish (11), and increase in-pack drip (12). Replacement of oxygen with nitrogen, an inert and odourless gas, does inhibit some aerobic bacteria and reduce the rate of oxidative rancidity. Sulphur dioxide, nitrous oxide and carbon monoxide have also been suggested as possible replacement gases in trace amounts for MAP/CAP, although less information on their effectiveness is available.

The single most important concern with respect to the use of MAP is the potential for outgrowth and toxin production by C. botulinum. Of particular concern are the psychrotrophic type E and non-proteolytic type B and F strains, as they are able to grow at temperatures as low as 3.3 °C and produce toxins, without overt signs of spoilage. Growth and toxin production have been detected in artificially contaminated packs of whole trout after 1 week’s incubation at 10 °C (13).

The (International) Codex Committee has published a Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003), which provides guidance relating to vacuum or modified-atmosphere packaging for specific fish products (14).

In the United States, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) has published a number of recommendations on
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the safety of MAP and VP refrigerated raw fishery products (15). It highlights temperature control as the primary preventive measure against the possible hazard of toxin production by *C. botulinum*, leading to the recommendation that the sale of MAP/VP raw fishery products be allowed only when certain conditions are met. These conditions include storage of the product at ≤ 3.3 °C at all points from packaging onwards, use of high-quality raw fish, adequate product labelling in respect of storage temperature, adequate shelf life and cooking requirements, and the use of a HACCP plan. It was also noted that organoleptic spoilage and rejection by the consumer should occur before the possibility of toxin production, at all times.

In the United Kingdom, the Sea Fish Industry Authority has published guidelines for the handling of fish packaged in a controlled-atmosphere (16).

The 1985 British Guidelines on MAP/CAP fish from the National Fisheries Institute state that:

1. Only the highest-quality fish, from both a microbiological and a chemical standpoint, should be used.
2. The only processing dip to be used is 5% potassium sorbate and 10% sodium tripolyphosphate.
3. The replacement gas should contain at least 40% carbon dioxide.
4. Subsequent storage must be at or below 3 °C.
5. Sensitive and accurate time-temperature indicators must be used to ensure that the product has not been temperature-abused.

Although all fish species need to be treated separately, grouping of fish types is possible to estimate the best carbon dioxide level to be used in MAP. For fatty fish, oxidative rancidity is a significant source of spoilage and hence the complete removal of oxygen and the use of oxygen-impermeable films are preferred. The addition of high levels of carbon dioxide to these fish, however, may result in unacceptable colour and textural change and hence little shelf life extension (11). For other less fatty marine fish, however, greater advantages are seen.

Storage studies have shown that the extension in shelf life of MAP cod fillets was proportional to carbon dioxide concentration up to around 50% (V/V), from where drip loss and gaping of the muscle meant that organoleptically classified rejection occurred sooner than with no carbon dioxide addition (12). The problem of water loss in MAP fish is common, owing to the dissolution of cellular structures; this problem occurs to a lesser extent with meat, but is recognised as a drawback of carbon dioxide application. Another problem facing the application of MAP to marine fish is the existence of carbon dioxide-resistant bacteria that can rapidly produce off-odours (e.g. trimethylamine and sulfides). The growth of these bacteria is reduced by high carbon dioxide concentrations but, as already stated, rejection due to alteration in fish texture with greater carbon dioxide makes this unsuitable (13).

Freshwater fish have different intrinsic bacterial flora and also do not contain such high concentrations of amines (e.g. TMAO) that can be reduced to produce
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off-odours, so lower carbon dioxide concentrations may have better preservative effects.

Gas mixtures of 40% CO₂/30% N₂/30% O₂ for white fish and 40 - 60% carbon dioxide with a balance of nitrogen for fatty fish have been recommended (16, 17, 18) and are probably the most widely used.

1.4 Spoilage

1.4.1 Fresh fish spoilage and methods of evaluation

Food spoilage can be considered as any change that renders the product unacceptable for human consumption (10). Spoilage of fish starts upon death due to autoxidation (oxidation of unsaturated lipids), reactions caused by activities of the fish’s own enzymes, and metabolic activities of microorganisms present in the fish. Over time, loss of the fresh characteristics may be simply measured by comparative visual and smell analysis.

Loss of freshness and spoilage cannot be separated as processes, but it is a commonly held view that loss of freshness is related to autolytic degradation and spoilage is more microbial in origin (1).

Degradation of whole fresh fish stored in ice generally follows a set pattern, and this pattern is the basis of freshness grading schemes. The eyes turn from convex and clear to concave and opaque, the gills from pink and shiny, with no smell, to brown and slimy with an intense off-odour; the skin turns from iridescent to dull and bleached with bacterial slime; and the flesh turns from bright and elastic to dull and soft (1).

Various methods for whole fish freshness evaluation by a trained panel have been proposed; these are summarised in Table 1.III.

<table>
<thead>
<tr>
<th>Name of scale</th>
<th>Scoring system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torry Scale</td>
<td>10 to 0 (≤ 4 reject)</td>
</tr>
<tr>
<td>Quality Index Method</td>
<td>0 to 24 (arbitrary reject)</td>
</tr>
<tr>
<td>European Grading Scheme</td>
<td>Extra, A, B, C (C=reject)</td>
</tr>
</tbody>
</table>

Significantly, rejection of whole fresh fish for human consumption may be made without chemical or microbiological evaluation, or for that matter evaluation of the taste of the fish, as traditional spoilage patterns of the external organs are typically very distinct.

When working with fillets, evaluation of taste is unavoidable as the visual pattern of degradation of the eyes and gills is unavailable for analysis. Taste panel evaluation of cooked fish may be used to score or grade colour, texture, smell and
taste of cooked fish. Generally, the cooked analysis of fish passes through four phases of spoilage:

i) Delicate sweet, sea-weedy taste, possibly slightly metallic.
ii) Neutral taste, little flavour.
iii) Traces of sour, fruity and/or bitter off-flavours; development of sickly sweet, cabbage-like, ammoniaal, sulphurous and/or rancid smells; texture becomes soft and watery or hard and dry.
iv) Enhancement of the spoilage characteristic of phase iii, spoiled and putrid.

The processes of degradation being analysed by the quality scoring methods above are a complex mix of physical, chemical, biochemical and microbiological actions. These processes are strongly influenced by the physical conditions of storage.

After death, rigor mortis is the first noticeable change in the fish. From being flaccid, the muscles harden as residual adenosine triphosphate (ATP) is reduced and the myosin and actin filaments bind to form actomyosin (18). After some time, rigor resolves, the muscles relax again and the fish returns to a flaccid state.

The pH of the muscle will drop after death depending upon the amount of residual glucose or glycogen that is reduced anaerobically to lactate with the co-production of ATP; this will generally correlate with the length and severity of rigor. Because fish tend to have relatively little residual glycogen compared with mammals, the pH drop of the muscle is correspondingly less; post-rigor values are typically in the range of pH 5.8 to 6.5 (18, 19).

After death, the Eh of fish muscle remains relatively high (20). To a varying extent, all marine fish use/have TMAO (21), which has been ascribed a number of possible functions as: a trimethylamine (TMA) detoxified waste product, an osmoregulator, an anti-freeze, or simply a waste product present due to bioaccumulation (22).

TMAO permits a high Eh to remain in the muscle tissue, as little endogenous reduction of TMAO occurs (18). However, bacterial reduction of TMAO to TMA, an intense odour compound, is significant and may even be responsible for the ultimate sensory rejection of fresh cod and other fish with high initial TMAO content (20, 23).

Changes in the resistance of the fish skin after death are used as the basis for tests that employ an electrode measurement of skin resistance (e.g. the Torrymeter, RT Freshmeter or Fishtester). As the fish degrades, the conductance generally increases; thus, measurements of the falling skin resistance may be made and compared with a calibration curve to estimate the time that the fresh fish has been stored in ice, or its remaining shelf life (1).

The process of ATP breakdown is used as an indicator of fish freshness (24). By measuring the concentrations of the six components, a ratio of concentration of the hypoxanthine (Hx) and inosine (HxR) to total concentration (ATP, ADP, AMP, IMP, HxR and Hx) gives a quantity (K-value, %), which increases from 0% towards 100% with time. During the initial storage, reduction in ATP and increase
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in hypoxanthine by endogenous enzymes allow this measurement to be used for determining freshness (19). However, hypoxanthine, which may also be formed by bacteria (25), is later significantly reduced by bacterial action, so the measurement is effective only during the initial loss of freshness. This measure is highly dependent upon the temperature of storage and may not reflect the rate of loss of quality equally at different temperatures.

Lipid oxidation and other oxidative changes lead to oxidative rancidity, colour changes, and are especially important in the spoilage of frozen fish, as microbial spoilage is limited by the low storage temperature (1, 7). Lipid oxidation can be a result of enzymic action or a cascade reaction initiated by free radicals (26, 27) produced by aerobic respiration and other forms of metal ion reduction (28). Oxidative rancidity is known to reduce the quality of fatty fish in particular (19, 26).

Chemical degradation continues after the initial post mortem phase; however, the importance of microbial action increases with time (5, 18). Quality indices based upon the products of microbial metabolism do not explain changes in quality until microbial growth produces measurable changes in the fish; therefore, these measurements are usually used to quantify the amount of spoilage, not to describe freshness.

Volatile bases are the best-characterised chemical indicators of fresh fish spoilage. Evaluation of Total Volatile Base-Nitrogen (TVB-N, also termed TVN), or a specific fraction of the volatile bases, for example the TMA fraction, using Conway diffusion chambers (29) allows determination of changes of mg-N/100 g fish. The Conway method (and variations of it) uses a strong inorganic base to volatilise the bases in the fish sample, and a segregated weak acid to absorb them; the residual acid is then titrated. The variation in post mortem fish pH may likewise influence the amount of bases being liberated to the air and consequently affect the odour characteristics of the fish. Comparing results for different fish species, however, does not show correlation between muscle pH and the amount of volatile bases contained within the fish at rejection. Neither does the change in pH during storage correlate well with the production of TVB-N.

During spoilage, the majority of volatile bases are produced from the soluble non-protein nitrogen of the fish (free amino acids and other low-molecular-weight nitrogenous compounds), as significant proteolysis is observed only during the latest stages of spoilage and after rejection (30, 31). For some fish species, a correlation can be made between the spoilage of the fresh fish and the production of TVB-N.

The major spoilage odours and flavours of fresh fish are undoubtedly principally microbial in origin, but rejection of whole fresh fish by sensory methods such as the EC grading scheme (32) is based upon non-specific odour detection and physical appearance.
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1.5 Factors Affecting Fresh Fish Spoilage

1.5.1 Temperature

By far the most effective method of reducing the rate of whole fresh fish spoilage is temperature control (1, 5). Fish spoil as a result of the chemical, biochemical and microbiological reactions taking place within and on the fish. All chemical reaction rate kinetics (and thereby microbial growth) are temperature-dependent; the lower the temperature of storage, the slower the spoilage processes proceed (within limits). Also, careful temperature control during storage is not only important in terms of quality loss, but also crucial for the assurance of consumer safety.

The application of ice storage increases the shelf life of fresh fish from a matter of hours at ambient temperatures to days or weeks. This increase has been reported to be moderately dependent on the temperature of the sea from which the fish are taken (33, 34). The reasons for the differences between tropical- and temperate-water fish spoilage rates may be many, including the ability of the endogenous bacteria to grow at low temperatures and lower endogenous enzyme activity.

1.5.2 Fish-spoilage bacteria

The parameters affecting the multiplication of microorganisms in foods have been categorised into two general groups: intrinsic (inherent qualities of the food) and extrinsic (qualities of the food environment) (35).

The factors are interactive and cannot be completely isolated. For fresh fish iced immediately after capture and continually stored in ice, the results of altering one parameter may have far-reaching consequences on others. For example, by altering the gas atmosphere (extrinsic), the pH of the fish muscle may change (intrinsic) and a different microbial population may develop due to the change in atmosphere and pH. The sum of these changes may result in a different spoilage profile.

1.5.3 Recognised specific spoilage organisms (SSOs)

The degree of spoilage leading to sensory rejection of fish is partly dependent on the perception of the consumer. Not all the bacteria growing on a food will lead to the production of objectionable characteristics; a minority are often associated with the majority of the spoilage. The concept of specific spoilage organisms (SSOs) is not new; yoghurt spoilage by yeasts and clostridial spoilage of cheese (5) are examples where it has been recognised for many years that a particular minority of the microbial flora present in the product is responsible for its spoilage. For fresh fish, realisation that the bulk of the microbial population on newly caught fish does not cause off-flavours and off-odours stems from work...
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started in the 1940s. During the 1970s, work with inoculated sterile fish blocks resulted in identification of a specific minority of microorganisms, which produced the characteristic spoilage compounds of the fish (30, 35, 36). These organisms are described as potential spoilers, but only if or when they reach numbers capable of producing sufficient spoilage compounds to effect rejection do they become the SSOs of the product (9, 35).

As previously noted, the spoilage of a product may be strongly influenced by the conditions under which the product is held; therefore, the characterisation of spoilage of each product must be made before the identification of the responsible agent(s) can proceed. Bacteria identified as being associated with the spoilage process of fresh fish are as follows:

1.5.3.1 Pseudomonas spp.

The Pseudomonadaceae family represent a large and poorly defined group of microorganisms. They are generally characterised as Gram-negative rods, motile with polar flagella, oxidase-positive, catalase-positive, obligate respiratory bacteria. The spoilage compounds associated with the growth of psychrotrophic Pseudomonas spp. on fish are diverse and in many cases species-specific. Pseudomonas spp. mediated spoilage is characterised by ‘fruity’, ‘oniony’ and ‘faecal’ odours from the production of ketones, aldehydes, esters and non-hydrogen sulphide sulphur-containing compounds such as methyl sulphide (36, 37). Members of the genus are able to produce pigments, and proteolytic and lipolytic enzymes that may affect the quality of fresh and, more especially, processed (e.g. frozen) fish products.

The spoilage of freshwater fish is generally ascribed to the growth of Pseudomonas spp. (9) and they are considered SSO of iced freshwater fish (35).

1.5.3.2 Shewanella putrefaciens

Sh. putrefaciens is considered an SSO of temperate-water marine fish species stored in ice; it is often isolated as about 1 - 10% of the total flora of fresh fish from temperate marine waters. It is also present in fresh water, and may play some role in the spoilage of freshwater fish. It is able to grow as fast as, or faster than the rest of the flora of ice-stored fresh marine fish (9).

The importance of Sh. putrefaciens to the spoilage of fresh marine fish has been recognised since the 1940s, although only since the late 1960s has it been realised that specific metabolites of Sh. putrefaciens growth may be used as indicators of spoilage (30). Sh. putrefaciens spoilage of fish is due to its biochemical action on muscle, i.e. its ability to reduce TMAO to TMA, produce hydrogen sulphide (H₂S) from cysteine, form methylmercaptan (CH₃SH) and dimethylsulphide ((CH₃)₂S) from methionine and produce hypoxanthine (Hx) from inosine monophosphate (IMP) or inosine, plus other characteristic compounds of the species responsible for spoilage. It is thus an important spoilage organism of gadoid fish such as cod,
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for which the most compelling evidence showing spoilage as a result of *Sh. putrefaciens* growth has been collected (12, 38). It is also able to produce hydrogen sulphide and a range of other off-odour compounds.

In terms of its taxonomy, *Sh. putrefaciens* strains are characterised as microaerophiles or anaerobes, they are heterogeneous, and recent taxonomic description using modern molecular methods bifurcated this species, with several new species being described. Initially, mesophilic strains of *Sh. putrefaciens* were identified as *Shewanella algae*, *Shewanella waksmanii*, *Shewanella affinis*, and *Shewanella aquimarina*. Later, additional psychrotrophic species such as *Shewanella baltica*, *Shewanella oneidensis*, *Shewanella gelidimarina*, *Shewanella frigidimarina*, *Shewanella livingstonensis*, *Shewanella olleyana*, *Shewanella dentritica*, and *Shewanella profunda* were described. In fact, *Sh. baltica* has been identified as the main H$_2$S producer in cod during cold storing (37).

*Sh. putrefaciens* may grow in the absence of oxygen using alternative terminal electron acceptors (ATECs), although, like all members of the Pseudomonadaceae, it is strictly respiratory. This wide-ranging respiratory capability is thought to be unique (39). The prevalence of the bacterium in so many environments, and its metal ion reduction ability has led to intensive investigation into its role in iron and sulphur cycles.

The ferric reductase activity of *Sh. putrefaciens* has been studied; as the ferric iron is insoluble, the bacteria have a significant problem to overcome - how to reduce a molecule that they are not able to take into their cells efficiently. The localisation of the reductase on the outer cellular membrane offers the bacteria a solution to this problem (40). Another distinct feature of the physiology of *Sh. putrefaciens* lies in its chemotaxis: the bacterium, unlike many other motile bacteria, does not show carbon-source chemotaxis, but does show strong chemotaxis up gradients of most of their alternative electron acceptors (39).

1.5.3.3 *Photobacterium phosphoreum*

Recognised for some time as being present on spoiling fish (30), *Ph. phosphoreum* increased in notoriety when it was proposed that reduction of TMAO to TMA limited the shelf life of MAP cod fillets (12). Because no other TMAO-reducing bacteria were present in sufficient numbers to produce the quantities of TMA that were related to rejection, it was proposed that this organism, owing to its cell size and activity, was capable of being in a significant numerical minority on the MAP fish but still able to yield the majority of the TMA thought to be responsible for the rejection (23, 41). Research has shown that approximately $10^7$ cfu/g of *Ph. phosphoreum* were required for 50% of taste panellists to reject a sample, whereas $>10^8$ cfu/g *Sh. putrefaciens* were required. In 50% N$_2$/50% CO$_2$ MAP cod, at the time of rejection, a population of *Ph. phosphoreum* sufficient to cause spoilage was found, but *Sh. putrefaciens* was not present in such numbers (23, 41).

*Ph. phosphoreum* has also been identified as responsible for histamine fish poisoning. This type of intoxication occurs when bacteria convert the histidine
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present in fish into histamine. Some Ph. phosphoreum strains have great capacity as histamine producers even under refrigeration conditions.

1.5.3.4 *Brochothrix thermosphacta* and lactic acid bacteria

*B. thermosphacta* is a well-characterised psychrophilic spoilage organism of meat. When inoculated into VP corned beef and sliced ham, *B. thermosphacta* did not produce off-flavours until 2 - 3 days after having reached 10^8 cfu/g (42).

Growing evidence suggests a role for *B. thermosphacta* in the spoilage of some MAP fish. Recent studies have investigated the dominance of *B. thermosphacta* on spoiling fish in a 40% CO₂/30% N₂/30% O₂ MAP (42). Acetate production has been reported as a good indicator of spoilage by this organism.

MAP studies have also demonstrated this organism’s sensitivity to oxygen and have shown that they are also inhibited by high CO₂ concentration (43).

1.6 Pathogens: Growth and Survival

Endogenous chemicals, algal toxins, human viruses, bacteria and higher parasites all present some risk associated with fish consumption (1, 5, 6). Of these, it is bacterial risks that increase after capture of the fish.

There are few human bacterial pathogens that can cause primary infections or disease and are capable of persisting in the aquatic environment. Fewer still are capable of growing on fresh fish. The remaining few bacteria present a major risk involved with the consumption of raw seafood such as sushi or oysters, but with proper cooking, these risks are substantially reduced (1, 5).

1.6.1 *Clostridium botulinum*

*C. botulinum*, a convenient but diverse species of bacterium with a number of different types, presents a potential risk. The heterogeneity of the types (A, B, C1, C2, D, E, F and G) is well documented (44). Types A, B, E, (and very rarely F and G) have been reported to cause human disease; types B, E and F include bacteria with minimum growth temperatures of approximately 3.3 °C (Group II) and these are non-proteolytic (i.e. they do not produce significant product spoilage). Adult disease is caused by the production of the botulinum toxin, a neurotoxin that causes flaccid paralysis and is associated with a variable (44) mortality depending upon dose, age, previous exposure and access to supportive treatment, including antiserum.

Review of outbreak data suggests fresh fish to be safe. Huss (1, 5) reports that fresh fish consumption has never been shown to cause human botulism; this is probably due to spoilage occurring before toxin elaboration. However, lightly preserved fish products are associated with botulism, especially type E botulinum intoxication. Of 404 intoxication outbreaks of type E *C. botulinum* recorded up to 1963, 75% (with a 35% mortality rate) occurred in Japan, where a particular
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fermented raw fish product that is consumed uncooked, I-sushi, accounted for the majority of cases (1, 5). Other lightly or semi-preserved (e.g. smoked, salted or pickled) fish have also been associated with botulism; again, the majority are associated with products not cooked immediately before consumption. The risk of toxin formation before apparent spoilage has been studied for some MAP fish (45), but as fresh fish are often cooked before consumption and the botulinum toxin is heat-labile, there remains only an ‘extremely small’ risk of intoxication if reasonable precautions are taken during handling, storage and preparation (11).

Despite the ubiquitous nature of type E _C. botulinum_ in the marine environment (isolated from around 90% of marine and environmental samples from northern European waters and its consequent presence in seafood (a surveyed incidence of as high as 65%) (2, 11), the risk posed to the consumer of fresh fish, whether stored aerobically or in modified-atmospheres, is small. Proper temperature control (<3.3 °C) will eliminate all risk, and proper cooking (boiling for 1 minute, or cooking at 80 °C for 5 minutes) (44) will substantially reduce risk.

1.6.2 _Vibrio parahaemolyticus_ and other vibrios

_V. parahaemolyticus_ is a marine organism, which can cause human gastroenteritis. It is generally undetectable in marine water below 19 °C but may grow in culture at temperatures as low as 5 °C and on food at 10 °C. Only about 1% of marine isolates produce a thermostable haemolysin, which is believed to be required for virulence. Generally, only shellfish are associated with the disease and no reported cases by the Center for Diseases Control, USA, were associated with finfish between 1978 and 1998 (46). The first report of the organism being a foodborne agent was with shirasu, a Japanese boiled and semi-dried sardine dish, which was probably contaminated from an uncooked food source or an excreting food-handler. In Japan, the majority of outbreaks are caused by consumption of raw fish products (e.g. sushi) (5), due to the cultural preference for raw fish dishes.

Since 1996, _V. parahaemolyticus_ cases have increased across the world. A unique clone of _V. parahaemolyticus_ O3:K6 is responsible for many of the recent _V. parahaemolyticus_ outbreaks, including epidemics in India, France, Russia, Southeast Asia, Japan, and North America. This strain has been responsible for 50 to 80% of all _V. parahaemolyticus_ infections since 1996 and is referred to as the pandemic strain (47). Currently, there is no specific guideline that describes a minimum level of _V. parahaemolyticus_ in sea water fish and shellfish that could potentially be hazardous to humans. Proper chilling, use of post harvest treatments and/or cooking of fresh seafood will reduce risks.

_V. vulnificus_ is associated with warm marine and estuarine waters. Human disease caused by the organism has only been observed in conjunction with marine bivalve (mostly oysters) and some crustacean consumption, and when in contact with contaminated water (no CDC reported cases from fish, 1978 - 2005). The bacterium causes primary septicaemia, especially in individuals with underlying diseases (patients suffer from immune and liver diseases or blood
disorders), which is often fatal (>50%). There is no risk associated with the consumption of properly chilled and cooked fresh fish.

*V. cholerae* O1, the causative agent of cholera, is historically associated with faecally contaminated water, but the bacterium is known to survive and grow in the shallow marine, and especially estuarine environment. It is particularly associated with disease following consumption of raw oysters from warm sewage-polluted waters. Again, there is no risk from the consumption of properly handled and cooked fresh fish.

### 1.6.3 *Aeromonas*

*Aeromonas* spp., and especially *Aeromonas hydrophila*, are associated with human diarrhoeal illnesses. They are aquatic organisms with an epidemiology that is yet to be fully understood. The major virulence factor appears to be the production of toxins with enterotoxic, cytotoxic, sodium channel blocking and haemolytic activity (48), although lack of data concerning infectious doses and the possibility that foodborne virulence is linked to the immune state of the host mean that full risk assessment is impossible. Some isolates are true psychrophiles, with minimum growth temperatures around 0 °C and optima of 15 - 20 °C; others are psychrotrophic mesophiles (49). Although the psychrophiles are unable to grow at body temperature, their potential to produce disease-causing exotoxin in food has not been fully examined (48).

It is known that *Aeromonas* spp. are present on fish and can grow to significant numbers during storage (1, 5, 17). Despite this, no more than circumstantial evidence exists linking the consumption of seafood to *Aeromonas* spp. infection, possibly due to under-reporting because of their likeness to *E. coli* on isolation media (50). Most cases have been sporadic rather than associated to large outbreaks. Chill storage may not eliminate growth, but proper cooking should substantially reduce risk of infection.

*P. shigelloides* is similar to *Aeromonas* spp. in its habitat, though it is a true mesophile with a minimum growth temperature of 8 °C and shows consequential seasonal variation in its environmental isolation. The bacterium has been documented as being responsible for a few fishborne outbreaks of gastroenteritis, and fish and shellfish are probably the major reservoirs for the organism (50). Similar problems as those encountered for *Aeromonas* spp. in assessing the risk of fishborne infection by *P. shigelloides* are encountered; however, proper chill storage will eliminate any risk associated with this organism, and proper cooking will substantially reduce risk.

### 1.6.4 *Listeria monocytogenes*

*L. monocytogenes* has been well documented as a foodborne human pathogen. Since 2000, listeriosis cases have been reportable to the CDC. In the US, *L. monocytogenes* incidence has been between 0.26 to 0.55 cases per 100,000
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persons. It is environmentally ubiquitous but its true frequency in the marine environment is not well studied. It is regularly isolated from seafoods (51). Being a psychrotroph, there is a possibility for growth of the organism on chill-stored fish, but cooking will significantly reduce the risk of infection. Owing to the dependence of the virulence of this organism on the immune state of the host (usually requiring lowered cellular immunity; at risk groups - pregnant women, foetuses, neonates, alcoholics, AIDS patients and patients undergoing immunosuppressive therapy) and a lack of data covering infectious doses, full risk analysis is not possible (52). The organism poses a serious risk in chilled products not cooked before consumption, but proper cooking will reduce any risk. D_{60} of 1.98 minutes in cod and D_{60} of 4.48 minutes in salmon have been reported (53).

1.6.5 Scombroid fish poisoning

The production of histamine and other biogenic amines with human immunological activity, within high histidine-containing fish (especially members of the Scombridae and Scomberesocidae families) is responsible for an intoxication termed scombroid poisoning (54). Like other toxins, it is not apparent to the consumer and it cannot be destroyed by cooking. Fortunately, scombroid poisoning is usually a mild intoxication and it is not a significant cause of death.

Of fish-transmitted human diseases scombroid poisoning is commonly reported in the US (118 outbreaks with 463 cases reported by CDC 1998 - 2002 (55)) and is also common worldwide. Decarboxylation of histidine by a wide range of bacteria including Enterobacteriaceae, some *Vibrio* spp., *Photobacterium* spp., *Clostridium* spp. and *Lactobacillus* spp., but more especially by *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei*, leads to the production of histamine, which has a maximum permissable level (for fish products from fish species associated with high amount of histidine) of 200 mg/kg fish in the EC. If fish are not properly refrigerated, there is a far greater risk of unacceptable histamine levels being attained before sensory rejection. Lightly preserved products, especially pickled fish, are exceedingly difficult to produce within the legal limits of histamine as the temperatures used during production can lead to rapid growth of histidine decarboxylating bacteria. Low-temperature storage of potentially toxigenic (<5 °C) fish at all times is the most effective way to control histamine production (6).

1.6.6 Parasites

Helminthic parasites can occur extensively in finfish but very few are capable of infecting humans. The most frequently reported parasites of human importance in fish are round worms of the genera *Anisakis* and *Pseudoterranova* and tapeworms of the genus *Diphyllobothrium*. Larvae of parasites such as *Anisakis simplex* are resistant to curing and marinating but can be easily destroyed by freezing at -17 to -20 °C for 2 hrs. Infections are mostly associated with the consumption of raw or
mildly processed fish such as sushi. A limited number of nematodes and trematodes found in finfish have also been identified as a cause of human disease (56).

In summary, risk of human disease caused by natural bacterial contamination (not through faecal pollution) of fresh fish is extremely low. The most effective control of all bacterial risks is continual chill storage in ice (0 °C) or freezing, which in many cases will eliminate the risk. Cooking properly immediately before consumption is also an effective way of reducing or eliminating risk of fresh fishborne disease.

1.7 Published Microbiological Criteria

Microbiological criteria for food defines the acceptability of a product or a food lot based on the absence or presence or number of microorganisms, including parasites and/or quantity of their toxins / metabolites per unit of mass, volume, area or lot (CAC, 1997; EC, 1997) Huss H.H., Ababouch L., Gram L. Assessment and management of seafood safety and quality FAO Fisheries Technical Paper. No. 444. Rome, FAO. 2003.

Criteria should set standards that are attainable by the currently accepted GMP and applied only when there is absolute need for it. Testing methods should be practical and the enforcement of such criteria should translate as a reduction of potential microbiological risks to consumers.

The most widely accepted microbiological criteria for chilled and frozen raw fish are those set for aerobic plate counts (APC) at 25 °C and *E. coli* proposed by the International Commission on Microbiological Specifications for Foods (ICMSF). An increase of APC to levels in excess of $10^6$ cfu/g is usually indicative of inadequate refrigeration, long storage under refrigeration or one of the former prior to freezing. Faecal coliform counts may be used instead of *E. coli* counts where this method is preferred. For fish from inshore or inland waters of doubtful microbiological quality, especially in warm-water areas and where fish are to be consumed raw, it may be desirable to test for *Salmonella* and *V. parahaemolyticus* (see Table 1.IV) (57).

<table>
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<th>Plan</th>
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Additional tests to be carried out when appropriate

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<th>Plan</th>
<th>n</th>
<th>c</th>
<th>$M$</th>
<th>$m$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>$10^3$</td>
<td>$10^7$</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>$10^3$</td>
<td>$10^7$</td>
</tr>
</tbody>
</table>
1.8 References


CHILLED AND FROZEN RAW FISH


FISH AND SEAFOOD


33. Sumner J.L., Gorczyca E., Cohen D., Brady P. Do fish from tropical waters spoil less rapidly in ice than fish from temperate waters? Food Technology in Australia, 1984, 36, 328-9, 334.


CHILLED AND FROZEN RAW FISH


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1.9 Further Reading

1.9.1 Processing


1.9.2 Spoilage


Gram L., Trolle G., Huss H.H. Detection of specific spoilage bacteria from fish stored at low (0 °C) and high (20 °C) temperatures. International Journal of Food Microbiology, 1989, 4, 65-72.


CHILLED AND FROZEN RAW FISH


Gram L., Trolle G., Huss H.H. Detection of specific spoilage bacteria from fish stored at low (0 °C) and high (20 °C) temperatures. International Journal of Food Microbiology, 1987, 4, 65-72.

1.9.3 Pathogens


1.9.4 Standards and criteria


2. CHILLED AND FROZEN PREPARED FISH PRODUCTS

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The Grimsby Institute
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2.1 Introduction

It was often thought that whilst the highest quality seafood products are processed and sold as whole fish and/or fresh fillets (e.g. Sushimi tuna), products of lesser quality, but still acceptable and meeting all safety criteria, are used in chilled and frozen prepared meals since many of the attributes on which quality is assessed are not present or noticeable (e.g. clarity of eyes, colour of gills, smell etc.), or could be disguised by sauces, colourings etc. This is no longer the case with many processed products now available in stores, emphasising the high quality of the seafood and the gourmet nature of the prepared products.

The same used to be said for the difference between fresh and frozen products, although recent developments in improved supply chains, and on-board freezing mean that products frozen at the point of capture are now perceived by the consumer as being of a better quality than a fresh product, due to the early cessation of spoilage.

Few seafood products are sold ‘ready-to-eat’, the exceptions being Sushimi (high quality raw fish), smoked (hot) mackerel, smoked (cold) salmon, and crustacea such as shrimp and crabs. The majority of seafood products produced require a cooking stage before consumption.

Even products where the seafood is only one ingredient within the recipe (composite food products) it should be noted that unlike other meat-based products, seafood (with the exception of some crustaceans) remains uncooked until the point of consumption. This has the result that any microbes present in the product have the capability to grow (with subsequent spoilage) unless strict temperature controls are applied. Even though heating prior to consumption will eliminate pathogenic microbes, it does not necessarily apply to the toxins that may have been produced.
FISH AND SEAFOOD

Recent trends in the market for food and seafood products, in particular, have shown the increasing importance of convenience foods. At the same time, the processing industry has developed a wide range of value added products, where the preparation has already been carried out, and the consumer only has to heat (cook) the product, often using the simplicity of a microwave oven.

2.2 Definitions

The traditional focus of the chilled and frozen fish market has shifted over the last few years, and this has produced a greater availability and diversity of products in the marketplace. The market spans fresh fish counter sales and frozen recipe dishes with fish as the predominant source of protein, and these are increasingly being presented with an ever-expanding range of accompaniments.

Seafood is the term used descriptively to include all categories of product, including freshwater fish, molluscs, prawns, etc. from both wild capture and aquaculture sources.

Marketing-style descriptions and process definitions are presented below to acquaint the reader with the variety and forms of seafood available.

Wet or chilled/frozen whole fish are whole fresh fish that may or may not have been filleted. This includes all categories of seafood including trout and other freshwater species, shellfish and molluscs. It is sold from wet fish counters on ice or pre-packed in modified atmosphere packaging (MAP). The expected shelf life attainable for this format is usually up to 8 days.

Warm-water/reef-caught fish were initially supplied to add colour and variety to wet fish displays, but these are now finding favour as products in their own right. They are typically flown into export markets, rather than frozen, as they tend to lose colour and sheen after defrosting; they are usually supplied filleted with head on. There is also a limited market for filleted material.

Doubts have been raised over the microbiological status and handling practices of reef-caught fish. Nevertheless, they are usually of a better standard than species such as tilapia, which can be farmed, and are well known for their diet consisting of waste materials. Microbiological hazards include Salmonella, Listeria spp., and pathogenic Vibrio spp., and it is worth noting the additional hazard to fish handlers from Vibrio vulnificus from injuries received during handling and filleting of spiny tropical fish.

Cold-water fish microbiological specifications are inappropriate for assessing freshness of warm-water fish. Shelf life on ice, or in MAP depends on catch conditions and treatment, but should be equivalent to cold-water products.

Prepared natural fillets are fillets of fish without any additional coating. They can be with or without skin and may contain pin-bones although the major skeletal bones are removed during filleting. Frozen fillets may have a surface layer of
frozen water that forms a protective glaze. Some products may be salted for flavour only (e.g. cod). Fresh fillets are sold from wet fish counters, or pre-packed in MAP or vacuum packaging (VP). The shelf life for MAP fillets is typically up to 5 days, which is approximately 30% (or 1 - 2 days) longer than that of non-pre-packed fish.

*Fish steaks* can be cut from a natural fillet or loin (e.g. tuna steaks) or band-sawn from a frozen block (e.g. swordfish steaks). Again, a protective glaze may be added to the frozen steak. They are sold from wet fish counters, or pre-packed frozen with added glaze.

*Cold-smoked natural fillets* are prepared from a range of cold water fish including herring, haddock, mackerel, and salmon. A typical cold smoking process may pre-treat the fillet by dipping in a 26% brine solution with a retention time of approximately 3 minutes. Added colours can be included with the brine solution to meet specific requirements for products e.g. annatto with smoked haddock. The result is fish with a typical residual salt content of 1.5 - 2.5%. Cold smoking is essentially a flavouring process, and typically uses natural smoke passing over the fillets suspended on racks in a kiln (oak chippings, sawdust with other woods adjusted for flavour profile and burn duration). A core temperature of around 27 - 30 °C for approximately 100 minutes is a typical processing condition. An alternative process using a liquid smoke in the brine is claimed to result in a similar flavour with reduced water loss in product and improved yields. The cold smoking process does not cook the fish or destroy microorganisms so the expected shelf life is not significantly increased from that expected for wet fish fillets, but may be increased (up to 1 month) by packaging methods like MAP or VP.

*Hot-smoked fish* are prepared from brined, natural fillets of fish such as herring, mackerel, salmon, etc., which may, in addition, be flavoured with herb dips or peppercorns. Hot-smoked fillets are prepared by brining fish fillets in a 26% salt solution with added natural colours to provide a range of product types, with a minimum residual salt content of 3.5% in the aqueous phase. They are then kiln-smoked to a minimum core temperature of 66 °C for 2 minutes. This process in effect cooks the product and reduces or eliminates microbial contaminants, resulting in a product that is ready-to-eat without any further cooking. These products are packaged in separate high-care facilities to minimise contamination with microbes either from the workforce or from raw products. The expected shelf life of these products is up to 8 days (or longer for some chilled, VP products) because of the high salt content acting as a barrier to spoilage.

*Pâtés* are prepared with raw fish, the ingredients premixed, and the product then shaped and heat-processed to cook the product before being chilled. The pâtés are sold from segregated ‘wet fish’ counters as a ready-to-eat product, in VP or through mail order.
FISH AND SEAFOOD

Seafood recipe meal centres (frozen or chilled) are places where these products are supplied without a source of carbohydrate, e.g. rice, pasta or potato. The protein source can be fish fillet or blocks, and may include molluscs or prawns that may or may not have undergone a minimal heat process during their primary processing.

Seafood ready meals (frozen or chilled) differ from seafood recipe meal centres in that they contain a source of carbohydrate such as a potato topping, rice or pasta. The majority are products made from various types of whitefish (cod, haddock, mixed whitefish.) Raw material is typically fish fillet, frozen blocks or canned fish and may include molluscs or prawns that may or may not have undergone a minimal heat process.

Breaded fillets/steaks and battered fillets are either natural or shaped fillets, or cut from a preformed frozen fish blocks into shapes; e.g. ‘fingers’ before being coated in batter and/ or breadcrumbs. There is an increasing market for high value fresh or thawed fish (e.g. tuna, salmon) coated and sold as chilled products, with an expected shelf life of around 7 days.

Fish cakes are products produced by mixing fish with potato and herbs, and formed into round cake shapes. The cakes are usually coated using a three- or a five-stage process, and heat-fixed. Band-saw dust and fish trimmings from fish-block preparation materials may be used. Other ingredients may be natural or reconstituted, and may also include additional ingredients such as fresh herbs and spices.

Prawns. Natural prawns from cold or warm waters can include those peeled, and in the shell. They are pasteurised products and treated as high risk raw materials.

Scampi are tails of Dublin Bay prawn or langoustine, coated in batter or breadcrumbs.

Surimi is the insoluble protein fraction of fish after washing and processing. It is often used as a protein supplement. With further processing, addition of ingredients such as colours, flavours, binders, etc., it can be used as a seafood analogue.

Other. This 'catch-all' category includes seafood salads, long-life seafood mixes using sorbate and benzoate as preservatives, jellied eels, taramasalata, and seafood mixes, which may include molluscs, cephalopods, fish nuggets/bites with dips and seafood-based snack products.
2.3 Initial Microflora

2.3.1 Seafood

Other chapters have discussed in some detail the indigenous spoilage, and pathogenic flora associated with seafood. In summary, the microbial flora of seafood is associated with the gills, skin, and intestines of the fish. Only after death, will the saprophytic flora start to invade the surface flesh (1, 2). Dalgaard et al. and his co-workers have described a large celled microorganism found in spoiling fish and identified it as *Photobacterium phosphoreum* (3). A primary role in fish spoilage has been assigned to this organism.

After capture and during any subsequent processing, handling or preparation stage, other potentially pathogenic and spoilage bacterial contaminants may be introduced from a wide range of sources including the environment, product handlers, and ingredients added to the final product. Contamination will, on the whole, remain on the surface of the fish fillet except where “gaping” or separation of the muscle blocks occurs, which may become a focus of contamination deep within the muscle. The edible tissue of some species of fish are more susceptible to gaping than others, whilst gaping is also dependent on a range of other factors that effect the muscle composition, including seasonal variation, storage, and processing conditions such as dehydration or poor filleting practices.

The concern is that fish may be visibly acceptable but any subsequent washing process will have little effect on removing contaminants, as a result there is an increase in spoilage rate thereby reducing the product shelf life, and an increased risk from a potentially unsafe product (especially those that receive a minimal heat process, i.e. searing or bar marking).

Tuna that does not meet the highest of grades based on colour, fat content, sensory assessment of freshness etc. is not used for sushi or other high risk (and high value) products; it may be used for other products or markets depending on the product specification. Tuna caught and discarded as unsuitable for sushi or other high-risk uses is often diverted for canning. Grading is based on size of fish (>25 kg), species (Bluefin>Bigeye>Yellowfin>Albacore), or visible damage from gaff marks (4). Recent public opinion has favoured the use of dolphin- and turtle-friendly long lining to the less discriminatory netting, and have emphasised the origin and sustainability of the fish as key marketing points.

Frequent rebaiting and prompt removal of caught fish is essential to limit rapid spoilage, and the potentially toxic by-products of histamine-producing bacteria (5, 6). The Food and Drug Administration (FDA) has produced guidelines for handling and processing of tuna, specifying internal temperature limits for fish and for limiting temperature abuse during handling and grading (1).

For a summary of background information on the hazards associated with fisheries products and ingredients, see Table 2.I.
2.3.2 Non-seafood ingredients

The range of ingredients used in the preparation of chilled and frozen prepared seafood products is very diverse, and so are the potential microbial contaminants associated with them. Table 2.1 provides an overview of common raw materials, and associated spoilage and pathogenic microorganisms; however, it is essential that during new product development a full hazard identification and risk analysis be conducted to ensure that the microbial risk for specific ingredients are identified.

**TABLE 2.1**
Spoilage and pathogenic microflora associated with common raw materials and ingredients used in prepared frozen and chilled products

<table>
<thead>
<tr>
<th>Raw material</th>
<th>Associated microflora</th>
<th>Pathogenic microflora/toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables (Untreated)</td>
<td>Lactobacilli, coryneforms, spore-formers, coliforms,</td>
<td>Salmonella, <em>Escherichia coli</em>, <em>Clostridium perfringens</em>,</td>
</tr>
<tr>
<td></td>
<td>Pseudomonads, moulds, yeasts</td>
<td><em>Clostridium botulinum</em>, <em>Listeria</em>, <em>Shigella</em>, <em>Bacillus cereus</em>, <em>Aspergillus</em>, <em>Penicillium</em>, <em>Fusarium</em>, protozoa and viruses</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato flake and powder</td>
<td><em>Bacillus</em>, moulds</td>
<td><em>B. cereus</em></td>
</tr>
<tr>
<td>Rice</td>
<td><em>Bacillus</em></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td><em>Bacillus</em>, moulds</td>
<td><em>Staphylococcus aureus</em>, <em>staphylococcal toxin</em>, <em>B. cereus</em></td>
</tr>
<tr>
<td>Flour</td>
<td><em>Bacillus</em>, moulds</td>
<td><em>Bacillus licheniformis</em>, <em>Bacillus subtilis</em></td>
</tr>
<tr>
<td>Dried herbs and spices</td>
<td><em>Bacillus</em>, <em>Clostridium</em></td>
<td><em>B. cereus</em></td>
</tr>
<tr>
<td>Dairy products</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Lactobacilli</em>, <em>Staphylococcus</em>, anaerobic spore-formers,</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em>, yeasts</td>
<td></td>
</tr>
<tr>
<td>Water/Ice</td>
<td><em>Enterobacteriaceae</em></td>
<td><em>Cryptosporidium</em>, <em>Vibrio cholerae</em>, <em>Aeromonas</em>, enteric viruses</td>
</tr>
</tbody>
</table>

2.4 Processing and its Effects on the Microflora

2.4.1 Selection of raw materials/ingredients

It is important that the potential microbial contaminants of all ingredients, raw materials, intermediate processing, product assembly, and, for chilled products, shelf life determination, be considered at an early stage during new product development, by the HACCP team or by a hazard and risk assessment programme. In addition, the effects of processing and combination or use of ingredients on identified microorganisms should also be considered with respect to both the safety and quality/shelf life of the product.
Any preparation stage or process should be suited to the raw material, but will also need to ensure that the optimum shelf life can be safely obtained. Intermediate processing and use of chill for storage of work-in-progress will help to optimise available life of perishable components. For some components, this may be an incidental effect, as a heat process is often required for functional or organoleptic reasons. Rapid controlled cooling of heat-processed components will help to minimise spoilage, and potential opportunities for growth of food-poisoning bacteria.

Figures 2.1 - 2.5 illustrate basic principles in the preparation of chilled and frozen recipe dishes. Generic examples have been used to illustrate only the major points, as the full complexity of the processes used for specific products cannot be covered in a general text.

It is recommended that the principles of HACCP are applied to novel processes, line extensions and process reviews.

2.4.2 Raw material tempering

Whenever frozen seafood is used as the raw material it is normally tempered before processing; there are a number of distinct methods used. All have the same intention, to evenly raise the temperature of the entire frozen product from -20 °C or lower, up to -5 °C so as to improve the cutting and handling properties during processing. Examples of tempering techniques include the use of heated air, defined holding time at a chill temperature (0 – 3 °C), or the use of microwave technology.

It is critical that the time and product temperature during tempering be controlled to avoid elevated temperatures (>5 °C) on the product surface allowing microbial growth or the onset of spoilage in raw material. The use of water for rapid thawing of product is not recommended since it not only rapidly thaws the product surface, but it also poses a risk of cross-contaminating other products undergoing tempering.

Effects on the microflora are minimal during tempering (7), but it is assumed that any band sawing or dice process will introduce heat from blade friction, and contamination from equipment to clean fish muscle. Band-sawn materials or fish dice are normally promptly further processed. Block-sawn dust that is required for use in fish cakes is swiftly transferred to a chiller to avoid water loss and spoilage from process contaminants.

2.4.3 Preparation of fish component of chilled and frozen recipe dishes

2.4.3.1 Coated products

Seafood products are coated with a cereal-based batter or crumb with up to 30 - 50 % of the final product weight accounted for by the batter or crumb. Where the raw materials are frozen, the core temperature, depending on the size of the fillet
processed, may not rise above 0 °C during processing. The processing of three examples types are described in Figure 2.1 (2.1A - Scampi, 2.1B - Shaped Fillets, 2.1C - Fish Cakes).

2.4.3.1.1 Whole-fillet / scampi

Scampi (Nephrops norvegicus), after landing, are placed into plastic boxes and covered in ice to preserve their condition. This material is transferred to a processing facility with the ice replaced as necessary to ensure that the product is maintained at the temperature of melting ice.

On arrival at the processing plant, the scampi is washed in potable water to remove foreign bodies. It is then size-graded, frozen in a nitrogen blast freezer and weighed into sacks for later processing.

The scampi meat (tails) is removed intact from the shell using potable water whilst still frozen.

The “blown” meat is graded, drained and then refrozen for storage until required for further processing. Any broken pieces or fragments of frozen tail meat are kept for use in the production of a reformed scampi product, by mincing and mixing with a binding agent (as described in section 2.4.3.1.2).

Frozen cores are usually batter-enrobed, with the coating heat-fixed, quick-frozen, chilled or sold chilled on defrost with an expected shelf life of 6 - 7 days.

Individual Quick Frozen (IQF) fish fillets or steaks may also be coated using a similar process.

**Fig.2.1. Raw material preparation stages for chilled or frozen coated fish products**
2.4.3.1.2 Shaped products

This type of product falls into two categories; those that use frozen blocks of whole fillets, or those where the trimmings, off-cuts and by-products from other processes (maybe minced) are reformatted give a final product that is still 100% fish.

Where frozen blocks of fillets are used they will be “tempered” (temperature raised to around -7 °C) to facilitate efficient cutting with a band-saw to a specified thickness. Shapes may then be cut from the individual slabs using either hydraulic shape cutters or continued cutting with band-saw type blades (e.g. fish fingers). Waste material produced from this type of processing is often reused in the third category (fish cakes).

2.4.3.1.3 Fish cakes

Fish cake preparation is used as an example of the production of fish products with fresh or dried ingredients added during the process.

Fish raw material can be obtained from a number of sources

i) By-product of fish shaping or cutting/dicing process - block-sawn dust.
ii) Fish-filleting waste - off-cuts or belly flaps.
iii) Fresh/fillet/minced fish block.

Waste fish generated as a by-product of another process will always be susceptible to spoilage and water loss. This is especially the case with block-sawn dust, as the physical structure of the material has been destroyed. Therefore, material will need to be transferred swiftly to a chiller, or promptly processed to avoid further quality loss. This type of material will be susceptible to contamination by Enterobacteriacea and possibly Staphylococcus spp.. It is for this reason that all by-products from primary processing, that are to be reused for other foods, are handled and stored as any other food product. Spoilage of fillet pieces or trimmings will not be appreciably different from that of fillet, unless it is poorly handled.

The remaining dried ingredients are mixed with the fish, and water is added to rehydrate the components. The mix is shaped and formed ready for packing or for enrobing.

The fish component of the cake will, because of its nature, spoil more rapidly than the dry ingredients; but, once the dry components have been rehydrated, additional spoilage organisms may be introduced, such as aerobic spore formers and Staphylococcus spp. from potato flake.

Herbs or seasonings used in the recipe may contribute additional spoilage organisms or, more seriously, pathogens such as Salmonella and Listeria.

Fish cake cores may be batter-enrobbed, with the coating heat-fixed, quick-frozen, chilled, or sold as a chilled product after thawing, with an expected shelf life of 6 - 7 days.
In all of the above examples, rapid automated processing with the minimum of delays will minimise product spoilage by pseudomonads, vibrios and other fish-associated spoilage organisms, as well as ensuring that there is minimal contamination from human processors. Scampi tails are particularly susceptible to contamination with Enterobacteriaceae, and possibly *Staphylococcus* spp. because of the intensive handling required during processing.

Control of temperature limits the potential for growth of spoilage organisms and pathogenic bacteria, such as *Listeria* and *Vibrio* spp., which can occasionally be isolated from protein-handling processes. Spore-forming anaerobes such as *C. perfringens* could, potentially, rapidly multiply if strict temperature control was not maintained.

### 2.4.4 Battering/enrobing process

Figure 2.2 illustrates the typical stages of enrobing or breading.

A large variety of product formats and types can be batter-enrobed, e.g.

- a. Tempered band-sawn materials
- b. IQF/pre-formed fillets
- c. Natural fillets
- d. Fish products, i.e. fish cakes

Portions or fillets may be batter-enrobed, with the coating heat-fixed, quick-frozen, chilled, or sold as a chilled product after thawing, with an expected shelf life of 6 - 7 days.

Breaded or battered fish fillets are fully heat-processed before consumption.

Products are preformed and presented to the process (either three- or five-stage) frozen or chilled. The first stage, which is product-dependent and not always required, is the addition of a pre-dust, which typically consists of flour, rusk or crumb. The function of this is to improve the adhesion of subsequent coatings, absorb any excess moisture on the surface of the fish portion, and to carry flavourings where required. The product then passes along a wire mesh belt through a ‘waterfall’ enrober that gives an even coating of batter to the entire product; any excess is removed with an air knife.

If a crumb coating is required, the crumb is poured onto the product followed by repeat enrobing and crumbing to complete the five-stage process.

Batter contains water, flour, starch, and may include milk powder, egg, spices, and if required, raising agents. The batter in the enrober is continuously recirculated and fresh ingredients (normally a dry pre-mixed recipe) are constantly added to a pre-mix vessel with potable water.

Initially, a characteristic flora will develop from the batter components, e.g. lactobacilli from the milk powder and flour, *Bacillus* spp. from flour, milk powder and spices, and Enterobacteriaceae from flour. Over time, some of this initial flora
associated with the ingredients will be diluted out, replaced in part by process contaminants and bacterial flora from seafood.

![Flow diagram outline of a typical three- and five-stage enrobing/breading process](image)

The length of production run and temperature of the batter are key factors in deciding the balance of the dominant flora within the enrober. With inadequate temperature control of batter systems, contaminants such as Enterobacteriaceae can proliferate and develop during the process, but because batter is heat-set, this is not of great significance to the microbiological standard of the finished product. *Staph. aureus* and *B. cereus* can proliferate under certain conditions, and both of these can produce heat-stable toxins that can survive a batter heat-fix process. The origin of contamination for the two organisms is often unclear, but it is believed that poor hygiene regarding product and food handlers, and, in the case of *B. cereus*, dry batter mixes are all potential sources.

It is advisable that some control mechanism is employed to prevent toxin-forming organisms from multiplying to dangerous levels. This can be achieved by frequent clean-downs and adequate hygiene, time-limitation or production run temperature control. The FDA has issued clear guidelines on acceptable
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temperature regimes and run times permitted for batter enrobers. A temperature no greater than 10 ºC for 12 hours cumulatively or 21.1 ºC for no more than 3 hours is recommended (1).

Well-managed breading and pre-mix systems that are segregated from batter enrobers present little hazard (7).

Vegetable quality is an important characteristic often overlooked in the selection of vegetables for frozen and chilled products. The vegetables must withstand processing, and must not show evidence of disease, as this could introduce off-flavours and reduce shelf life. Figure 2.3 illustrates vegetable processing and treatment for inclusion in chilled and frozen fish recipe dishes.

2.4.5 Recipe products

These products involve the combining of seafood with other food ingredients as part of a recipe, to produce a specific product. There are two main categories.

2.4.5.1 Seafood recipe meal centres (frozen or chilled)

These products can be defined as the central component of a meal, but they are supplied without a source of carbohydrate e.g. rice, pasta or potato. The protein source can be fish fillet or block, and may include molluscs or prawns that may or may not have undergone a minimal heat process during their primary processing. For example, canned fish may be used in their preparation owing to it having wider availability and low wastage.

Fig.2.3. Flow diagram outline for recipe dish ingredients preparation
The products will often be presented with vegetable toppings, pastry, or breadcrumbs, and may include sauce accompaniments.

The presentation of vegetables has a significant effect on the product quality. For example, larger diced pieces allow for easier handling and a reduction in spoilage but are less attractive to the consumer.

For premium products, all-year-round fresh vegetables are preferred, not just as accompaniments, but also as components in recipe meals. Any assumptions regarding the application of good agricultural practice to produce should be made with caution. For example, the use of night soil or incorrectly applied manure or sewage sludge for ground preparation may contaminate produce with a wide range of pathogens. A number of examples of direct faecal contamination of vegetables exist in the literature (8), with cases of foodborne disease traced to *Listeria monocytogenes*, *Salmonella typhi*, and *E. coli* O157, for example.

Products must be washed thoroughly to remove pests and soil, and reduce spoilage organisms and potential pathogens. However, any processing stage, i.e. chopping and mixing of material, will disperse the saprophytic flora of vegetables - lactobacilli, coryneforms, spore-formers, coliforms and pseudomonads - throughout the mix. Obvious signs of vegetable soft rot due to mechanical damage or infection by *Erwinia* spp. (7, 8) will, with the accompanying release of additional nutrients by processing, encourage more rapid decay and loss of structural integrity. The opportunity for growth of *L. monocytogenes* should be taken into account when setting shelf life or holding times of vegetable components, even those with pH-modified dressings. This is a concern for chilled products that may have a shelf presence for up to 6 days including day of manufacture.

Washing with a biocide will at best achieve a 2 - 3 log reduction in bacterial numbers on the surface of vegetables. In an attempt to improve washing and reduce chlorine taint, acidification of hypochlorite and chlorine dioxide have been proposed and recommended as an improved alternative. Although the processes are claimed to be superior to using unmodified hypochlorite for washing, control is more onerous.

Blanching of vegetables can be an effective means of reducing or removing surface contamination. However, if the process is not appropriately controlled, loss of quality, colour and water will occur. Once vegetables have been blanched, they should be stored under temperature-controlled conditions, treated as a high-risk ingredient, and protected from any post-processing contamination.

2.4.5.2 Seafood ready meals (frozen or chilled)

These differ from seafood recipe meal centres in that they contain a source of carbohydrate such as a potato topping, rice or pasta. The majority are products made from various types of whitefish (cod, haddock, and mixed whitefish). Raw material is typically fish fillet, frozen block or canned fish, and may include molluscs or prawns that may or may not have undergone a minimal heat process.
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Food-poisoning incidents traced to rice and caused by *B. cereus* are well known and are still a common issue in the UK (9). Manufacturers of chilled products with an extended shelf life must recognise the potential for growth and spoilage of psychrotrophic strains of *B. cereus*, especially in those products that have a vegetable component or rice accompaniments.

Chilled pasta is becoming more popular than dried pasta as a component in many product lines. An incident involving *Staph. aureus* toxin is well documented (10, 11), but temperature-abused pasta can also spoil rapidly by mould growth.

Potato toppings made from flake or powder will normally have little associated spoilage flora. On hydration, spore-formers can proliferate if adequate control of temperature is not maintained. Spices and herbs added to form toppings along with breadcrumbs or other coating materials will similarly contribute a spore loading (primarily *Bacillus* spp.) to the product (12, 13).

Products or dried ingredients contaminated with either spores of aerobic bacteria (e.g. *B. cereus*), if subjected to temperatures between 20 and 40°C, can germinate and rapidly multiply. This is a particular issue especially where products are heat treated to eliminate vegetative cells, and then cooled slowly allowing germination and re-growth. For this reason all products, where composite meals are heat-treated, should be chilled to <5 °C as quickly as possible using either blast chillers or other equipment specifically designed for this purpose.

Placing such products in a standard chill store to cool down could result in condensation, and an increased risk of contamination from *L. monocytogenes* and other bacteria that may be present on chilled surfaces such as ceilings.

2.4.6 Preparation of non-fish components of chilled and frozen recipe dishes

2.4.6.1 Sauce manufacture

The variety of ingredients and components used in sauce manufacture is extremely wide, with the range always expanding as new tastes and cuisines are developed. Sauces or marinades are added to fish to broaden appeal or to accompany components. Sauces can be multi-component preparations or more simple preparations, such as butter garnish with black pepper. Typically, the preparation process must ensure the eradication of all vegetative pathogens from the ingredients, rely on the use of microbiologically sound ingredients, or use barrier or hurdle principles to preclude the growth of pathogenic microorganisms.

If the sauce contains modified starches for freeze/chill stability, a heat process in excess of 73 °C will be required to rehydrate this component fully. Alternatively, a process equivalent to at least a 5D *Listeria* kill will be necessary. Appropriate handling facilities or equipment will be required to protect the product, such as high-risk/low-risk barriers and dispensing and/or holding at above 63 °C.
Fig. 2.4. Flow diagram outline for recipe dish ingredients preparation

Time limitation may minimise the potential for spoilage by process contaminants or at the dispensing unit. Without barriers, the sauce component will spoil rapidly and the possibility of vegetative pathogens from post-process contamination or raw components growing to significant numbers cannot be ruled out.

Acidified or low-pH sauce formats using acetic acid or vinegar normally require a lesser heat process for the equivalent effect, and handling of processed sauce can be less rigorous than, for instance, that of a dairy-based sauce.

Marinade-type products are becoming more popular. The format uses flavour compounds - herbs, spices, flour products, etc. suspended in an acidified sauce with an oil component. It is expected that this product format would have a restricted shelf life to limit potential for growth of psychrotrophic bacilli or any survival of enteric pathogens (12, 13).

Materials prepared with regard to good hygienic practice need only be assembled in a tray or pouch before chilling or freezing in readiness for distribution.

Figures 2.4 and 2.5 illustrate the recipe dish ingredients preparation and assembly and packaging, respectively.
2.4.7 Effects of packaging on chilled products

Products with minimal additional ingredients may use packing techniques designed to minimise microbial growth. These include:

2.4.7.1 Vacuum packing

A range of fresh chilled products including salmon (smoked and fresh portions), and tuna loins are packed by placing the product in a plastic pouch and removing all the air. The objective of this method is to deny the aerobic bacteria (both spoilage and pathogens) the oxygen that they need to grow. The oxygen-free conditions will however allow other anaerobic bacteria such as *C. botulinum* to grow, unless the temperature is strictly controlled to below 5 °C throughout the supply chain.

2.4.7.2 Modified-atmosphere packaging

Originally developed for the red meat industry, the method replaces air in the sealed packaging with a defined mixture of gases, generally carbon dioxide and nitrogen, so replacing the oxygen necessary for aerobic growth of spoilage microorganisms. Increased levels of carbon dioxide will also result in a slight decrease in the pH of the surface tissues, as it dissolves in the surface moisture to form a weak acid, which may restrict either microbial growth or enzyme activity.
2.5 Spoilage

During processing, temperature control is important in preserving chilled integrity of ingredients, and in influencing the final eating quality of the product. This is also true of chill storage and the distribution chain.

The literature has good information on spoilage of individual components of recipe meals, but little on in-pack spoilage.

The fish component of recipe dishes, except for some crustacea, is typically uncooked or at best lightly processed to retain eating characteristics. This is considered to be the one major difference between fish and meat products, where the protein component is nearly always heat-processed. Spoilage of this material is not expected to differ greatly from the processes described elsewhere. Spoilage will be most readily detected in proteinaceous components of ready meals; initially, by a loss of texture, followed by moisture loss, and finally flavour loss. Beyond this, advanced spoilage gives further changes in texture and the production of characteristic ‘off-odours’.

It is not clear whether the additional contaminants from components or processing will accelerate this typical seafood-type spoilage.

2.5.1 Chilled storage

For chilled products or chilled components of frozen products, the microflora of interest will be mainly psychrophilic or cold-adapted (14) spoilage organisms such as Pseudomonas spp., Shewanella putrefaciens and Ph. phosphoreum. However, in numerical terms, a large number of nutritionally non-fastidious spoilage organisms are commonly isolated from seafood. Not only will these organisms have a numerical advantage over other proposed spoilage organisms, but they will also be readily adapted to the nutrient source and conditions, and therefore will have a greater opportunity to dominate other spoilage flora numerically over the course of product shelf life (15).

At refrigeration temperatures (5 - 10 ºC) Enterobacteriaceae are often found to proliferate and produce a characteristic type of spoilage that includes, but is not limited to, the slow development of ammonical and sulphuric ‘off’ odours and flavours(16); at slightly higher temperatures (10 ºC), Vibrionaceae will succeed (17).

Aeromonas spp. and Yersinia spp. have both been suggested as possible spoilage or pathogenic contaminants of chilled recipe meals (18). The case for growth and contamination of products with Aeromonas is stronger, as this organism is a common contaminant of freshwater fish species (19). Nonetheless, both organisms are capable of growth at chill temperatures, but a conclusive case for pathogenicity has yet to be presented for Aeromonas spp. (20).
2.5.2 Frozen storage

Maintenance of product temperature at \(-18 \, ^\circ C\) will not permit bacterial growth or enzymic spoilage. The conditions and freezing process most favourable for good-quality products will, however, also be those that have the least effect on the viability of microorganisms (21).

As a note of interest, freezing also has a protective role in improving the safety of raw materials by, for example, eliminating nematodes in products that do not have a heat treatment step.

By retaining the intrinsic spoilage flora of seafood, the shelf life of products is limited by the natural spoilage process described earlier. This also ensures that any post-process bacterial contamination or spore-forming bacteria in the product will not grow, producing characteristic off-flavours, which would warn the consumer of the potentially unsafe nature of the product.

Products that are developed with an extended shelf life beyond the 6 - 7 days typical of fish products/recipe meals must consider the potential for growth of non-proteolytic \textit{C. botulinum}; this should also take into account shelf life of product components used in the assembly of the final product. The ACMSF (22) issued clear advice on this topic and has recommended that the shelf life of products without barriers (23) is restricted to less than 10 days.

2.5.3 Enzymic spoilage

Post-harvest changes in the quality of fish due to enzymic activity and \textit{rigor mortis} are well known and understood (10). Still, enzymic processes are often ignored or overlooked as insignificant or unimportant because of the rapidity of fish spoilage. However, where the variety of components and ingredients used in manufacture is varied and complex, spoilage should not be considered a purely microbial phenomenon as taints and off-flavours can develop by non-microbial means (e.g. auto-oxidation of fats/oils).

Spoilage can be either a function of bacterial growth or the microbial release of free enzymes into solution. The type and nature of the enzymes and their activity will depend on the bacterial species. For example, a recent report examining fish gut bacteria from cold waters characterised a proteolytic enzyme, released by a pseudomonad with demonstrable activity to \(0 \, ^\circ C\) and a peak in production at \(10 \, ^\circ C\) (15). It is not unreasonable to expect that spoilage bacteria release many extracellular enzymes. Other examples of bacterial proteolytic and lipolytic enzymes produced by \textit{Alicigenes} spp., \textit{Pseudomonas} spp., \textit{Bacillus} spp., \textit{Serratia} spp. and \textit{Aerobacter} spp. within dairy products are well known. These have been proved to impart off-flavours.

Characterisation of the enzymes has demonstrated residual activity even after a heat process that would destroy vegetative cells (24). This reinforces the need for prompt processing and temperature control of ingredients and finished products.
2.6 Pathogens: Growth and Survival

There are only two species of pathogenic bacteria that can truly be said to occur naturally in fish. These are *C. botulinum* type E and *Vibrio parahaemolyticus* (25). In addition, agricultural run-off or sewage can contaminate in-shore species, especially molluscan filter feeders, with pathogenic microorganisms or viruses. Cases quoting contamination of seafood with organisms such as *S. typhi*, *Vibrio* spp. and *Campylobacter* are recorded in filter feeding molluscs such as mussels.

Additional hazards from marine bio-toxins, histamine intoxication, etc., are dealt with elsewhere in this book.

Hazards attributable to the non-seafood components are listed below and this information, along with previous discussion, may be of benefit within any risk assessment for chilled and frozen prepared fish products.

2.6.1 Bacillus cereus

This is a common contaminant of dust, soil, air, water, and many raw or processed foods (26). A number of other *Bacillus* spp. have been linked with food poisoning and should also be considered.

Germination of spores requires favourable conditions, or a heat shock followed by holding at a temperature suitable for growth. Inappropriate holding times and temperatures will, in some cases, permit rapid growth of *B. cereus*. Numerous quoted figures exist in the literature regarding cell counts required before toxin production occurs, and some effects related to product type and time to toxin production have been noted. Counts of between $10^3 - 10^4/\text{g}$ should be treated with some concern (26).

Incidents in such diverse materials as cream, potato, spices and herbs and vegetable shoots have been reported (12, 13, 27).

2.6.2 Clostridium botulinum

There are reports of rapid spoilage of vegetables by *C. botulinum* when held in favourable conditions for growth (28) i.e. low oxygen or anaerobic conditions. This may occur where storage under gas such as carbon dioxide is used to reduce ripening and so prolong storage.

2.6.3 Clostridium perfringens

A study examining *C. perfringens* in seafood noted a transient population of spores in the guts of fish feeding adjacent to rivers and sewage outfalls (29). Therefore, any risk of *C. perfringens* contaminating seafood will depend on the source of raw material, and this issue may be of greater consequence for warm-water, farmed or free-living crustacea (30).
Vegetable raw materials are potentially contaminated through soil conditioning with animal manure.

2.6.4 *Listeria monocytogenes*

Interest in this organism has been characterised by a number of very serious incidents associated with a variety of product types (27). An acceptance has now developed in the UK of the presence at low levels of *L. monocytogenes* as a contaminant in foods (31). Sources of contamination are many because of the widespread distribution of this organism in the environment. However, the possibility of a sanitiser-resistant biofilm colonising processing environments should be viewed with some concern (32). *L. monocytogenes* tends to grow poorly at refrigeration temperatures in seafood (33); but, when introduced or inoculated into sterile components of chilled prepared recipe dishes, the restrictions on growth rate seen with seafood will not apply. Rates of contamination with smoked seafood are much higher than those with unsmoked products, and this is believed to be due to a tolerance to brine used in the smoking process (32).

*L. monocytogenes* has been frequently isolated from fish-processing plants and is thought to be able to colonise this type of environment (32, 33) due to its ability to grow at low temperatures and form “biofilms” on surfaces. It is unclear, at the present time, if any other potentially pathogenic organisms, indigenous or other, will colonise factory environments in a similar fashion except for possibly *Vibrio* spp.

2.6.5 *Shigella spp.*

*Shigella* spp. are considered primarily to be waterborne organisms but epidemiology indicates that contamination of vegetables and possibly in-shore caught seafood could act as vehicles for infection by *Shigella* (34). Chilling may help *Shigella* to survive, but under the appropriate conditions, growth may occur in foods (26).

2.6.6 *Staphylococcus aureus*

There are a number of underlying reasons for the development of *Staph. aureus* enterotoxins in foods. These include inadequate refrigeration and preparing foods far in advance of planned service, infected food handlers, and inadequate cooking or holding within the temperature growth range (27). These conditions will allow the production of the toxin, which is not affected by any subsequent cooking process, and will therefore cause “food poisoning” even in a product that is cooked before consumption.

All the above points should be considered within any risk assessment of the potential for growth and toxin production by *Staph. aureus* (35).
Illnesses caused by *Staph. aureus* enterotoxins and *B. cereus* emetic toxin are often confused owing to similar symptoms, and their association with similar materials, such as batter mixes, pasta, pastry, etc. (36).

### 2.6.7 Viruses

Concerns over contamination of seafoods with viruses (due to species-specific nature of viruses) are generally limited to molluscan shellfish from waters or crops contaminated with sewage.

### 2.7 Published Microbiological Criteria

Due to the complexity of many processed food products, with respect to the number and types of ingredients used, it is not surprising that Microbiological Standards (defined as mandatory compliance required (37)) are limited to those raw materials in which specific hazards occur, and not to final products. In the EU these are defined under European Commission (EC) Regulation 2073/2005 (38), although the only seafood products specifically identified are raw “shelled and shucked products of cooked crustaceans and molluscan shellfish”.

Microbial guidelines, as published by for example, the Institute of Food Science and Technology (IFST) (39) or the British Retail Consortium (BRC) (40), are not mandatory, but provide the industry with an indication of the microbial level at which action is required to ensure product safety.

Microbiological specifications do not necessarily relate to product safety, but are levels that are determined as satisfactory by the customer (within the supply chain) as part of the specification of raw materials /final product.

Microbial criteria and the monitoring of microbial levels within a product are important for monitoring trends within production systems, and are a recognised means of providing data on manufacturing standards, although due to long analysis times required, the fresh product is often consumed before the results are obtained, and as such the monitoring is for verification purposes rather than control.

The criteria themselves will have a range of levels that are dependant on the point in the processing or supply chain at which they apply. For instance,

- A dairy-based sauce that includes a non-pasteurised cheese will contribute significant aerobic total counts, but these may be eliminated by a subsequent heat-treatment stage.

Microbial levels at the end of manufacturing (m) will be set much lower than those at the end of shelf life (M) which in turn should still be safe to consume, unless the criteria is for a pathogenic organisms where only non detection is acceptable.

The following tables (Table 2.II and 2.III based on IFST Guidelines (39)) provide example guidelines for seafood products. It should be remembered that...
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the microbial criteria for other products (rice, pasta, batter etc) should also be consulted when establishing the specifications for raw materials and final product.

TABLE 2.II
IFST Guidelines for part-cooked foods – e.g. coated fish, fish fingers, fish cakes

<table>
<thead>
<tr>
<th>Pathogens and toxins</th>
<th>Organism</th>
<th>GMP*</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens Criteria for absence not generally applicable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indicators and spoilage organisms</td>
<td>Organism</td>
<td>GMP*</td>
<td>Maximum</td>
</tr>
<tr>
<td>E. coli</td>
<td>&lt;10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2.III
IFST Guidelines for processed foods - fish products, ready meals

<table>
<thead>
<tr>
<th>Pathogens and toxins</th>
<th>Organism</th>
<th>GMP*</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp. Not detected in 25 g</td>
<td>Not detected in 25 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes Not detected in 25 g</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. cereus &lt;10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staph. aureus &lt;20</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. parahaemolyticas (warm-water fish) Not detected in 25 g</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine (&lt;5 mg/100 g)</td>
<td>&lt;50 ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(scrombroid fish) (&lt;5 mg/100 g)</td>
<td>(&lt;5 mg/100 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indicators and spoilage organisms</td>
<td>Organism</td>
<td>GMP*</td>
<td>Maximum</td>
</tr>
<tr>
<td>APC (heat treated)</td>
<td>&lt;10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Product-dependent</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>&lt;10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>&lt;10</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

*GMP = Values found immediately after production under good manufacturing conditions.
Maximum= Levels at end of stated shelf life
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An authoritative survey of specifications can be found in Shapton & Shapton (41).

2.8 References


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17. Gram L., Trolle G., Huss H.H. Detection of specific spoilage bacteria from fish stored at low (0 ºC) and high (20 ºC) temperatures. International Journal of Food Microbiology, 1987, 4, 65.


CHILLED AND FROZEN PREPARED FISH PRODUCTS


3. MOLLUSCAN SHELLFISH

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3.1 Definitions

3.1.1 General features of the mollusc

The phylum Mollusca, which includes oysters, clams, mussels, cockles, scallops, squid, octopi, chitons, snails, slugs, whelks, abalones, and tooth shells are a group of soft-bodied animals that usually secrete external protective shells. The phylum consists of five classes: Amphineura, Gastropoda, Pelecypoda, Scaphopoda, and Cephalopoda. The class having species of economic and public health importance is the Pelecypoda. The class Pelecypoda includes all of the bivalves such as clams, mussels, oysters, cockles, and scallops. In these forms, the foot is compressed to form a muscular spade for digging and the head is greatly reduced, lying well within the mantle cavity. Pelecypods have little need for locomotion. In most species, the shell, composed of two valves hinged together dorsally, completely encloses the body. Strong muscles, the adductors, can hold the shells tightly shut against enemies (1).

Bivalves feed by filtering large volumes of water across their gills to obtain oxygen and food. For example, oysters and clams can process about 3.6 litres, and 2.7 litres of water per hour, respectively. Plankton and algae form the bulk of their diet from seawater. In the process of filtering water, they also trap bacteria, viruses, chemical contaminants and other impurities on the mucus of the gills. Special tracts of cilia move this mucus toward the mouth, where it is eventually swallowed and digested. The digestion process requires less than 2 hours in actively feeding adult bivalves (2). Many microorganisms ingested by shellfish survive the digestive process (3, 4).

The term shellfish in this context is limited to oysters, clams, mussels, cockles and scallops. Other species of shellfish such as crabs, lobster, and shrimp, while
commercially valuable, are not as vulnerable to contamination through pollution of their beds as the bivalves. Whatever contaminants are in the water will eventually get into the shellfish, and usually at higher concentrations than found in their habitat. The habitat of oysters and mussels are sessile, dwelling on the bottom or attached to structures in the water column. Clams are benthic and live burrowed in the mud, but maintain contact with the water by means of a siphon tube (5).

### 3.1.2 International commercial products

Globally, many species of bivalves are edible (Table 3.1). The following are descriptions of the various bivalves and their forms of preparation for human consumption (6):

**TABLE 3.I**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Country</th>
<th>Scientific nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft (shell) clam</td>
<td>Pacific/Atlantic North America</td>
<td>Mya arenaria</td>
</tr>
<tr>
<td>Hard clam</td>
<td>Pacific/Atlantic North America</td>
<td></td>
</tr>
<tr>
<td>(also used for Quahaug/quahog)</td>
<td>Pacific - North America</td>
<td>Saxidomus nuttali Venus mortoni</td>
</tr>
<tr>
<td></td>
<td>Pacific - South America</td>
<td>Protothaca thaca</td>
</tr>
<tr>
<td></td>
<td>Pacific – Japan</td>
<td>Meretrix spp.</td>
</tr>
<tr>
<td>Quahaug (also known as hard clam and hard shell clam)</td>
<td>Atlantic – North American/Europe</td>
<td>Mercenaria mercenaria Venus mercenaria</td>
</tr>
<tr>
<td>Coquina clam</td>
<td>Atlantic – Europe/North America</td>
<td>Donax spp.</td>
</tr>
<tr>
<td>Hen clam</td>
<td>Japan</td>
<td>Mactra sachalinensis</td>
</tr>
<tr>
<td>Butter clam (also called the Washington clam)</td>
<td>Atlantic/Pacific-North America</td>
<td>Saxidomus giganteus</td>
</tr>
<tr>
<td>Mogal clam</td>
<td>Japan</td>
<td>Saxidomus nuttali</td>
</tr>
<tr>
<td>Little neck clam (also called rock Cockle Pacific littleneck)</td>
<td>Pacific – North American</td>
<td>Protothaca staminea or Paphia staminea</td>
</tr>
</tbody>
</table>
### MOLLUSCAN SHELLFISH

<table>
<thead>
<tr>
<th>Shellfish Type</th>
<th>Region</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulf clam</td>
<td>Gulf of Mexico</td>
<td><em>Titaria cordata</em></td>
</tr>
<tr>
<td>Pismo clam</td>
<td>Pacific – North America</td>
<td><em>Trivela stuttorum</em></td>
</tr>
<tr>
<td>Surf clam (also called bar clam)</td>
<td>Atlantic – North America</td>
<td><em>Spisula solidissima</em></td>
</tr>
<tr>
<td>Freshwater clam</td>
<td>Japan</td>
<td><em>Corbicula spp.</em></td>
</tr>
<tr>
<td><strong>Oysters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common oyster</td>
<td>Europe</td>
<td><em>Ostrea edulis</em></td>
</tr>
<tr>
<td>Portuguese oyster</td>
<td>Europe</td>
<td><em>Crassostrea angulata</em></td>
</tr>
<tr>
<td>Blue point oyster (also called American oyster)</td>
<td>Atlantic – USA</td>
<td><em>Crassostrea virginica</em></td>
</tr>
<tr>
<td>Pacific oyster</td>
<td>Pacific – North America/Australia/New Zealand</td>
<td><em>Crassostrea gigas</em></td>
</tr>
<tr>
<td>Western oyster (also called Olympia oyster)</td>
<td>Pacific – North America</td>
<td><em>Ostrea lurida</em></td>
</tr>
<tr>
<td>Cocke</td>
<td>South America</td>
<td><em>Ostrea chilensis</em></td>
</tr>
<tr>
<td>Cockle</td>
<td>Japan</td>
<td><em>Ostrea laperousei</em></td>
</tr>
<tr>
<td>Sydney rock oyster</td>
<td>Australia</td>
<td><em>Crassostrea commercialis</em></td>
</tr>
<tr>
<td>Rock oyster</td>
<td>New Zealand</td>
<td><em>Crassostrea glomerata</em></td>
</tr>
<tr>
<td>Dredged oyster</td>
<td>New Zealand</td>
<td><em>Ostrea lutaria</em></td>
</tr>
<tr>
<td><strong>Cockles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common cockle</td>
<td>North Atlantic – Europe/North Africa</td>
<td><em>Cardium edule</em></td>
</tr>
<tr>
<td>Spiny cockle</td>
<td>Atlantic/Mediterranean</td>
<td><em>Cardium corbis</em></td>
</tr>
<tr>
<td>Knotted cockle</td>
<td>Atlantic/Mediterranean</td>
<td><em>Cardium aculeatum</em></td>
</tr>
<tr>
<td><strong>Mussels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue mussel</td>
<td>North Atlantic – Europe/Pacific – New Zealand</td>
<td><em>Mytilus edulis</em></td>
</tr>
<tr>
<td>Common mussel</td>
<td>Pacific – North America</td>
<td><em>Mytilus californicae</em></td>
</tr>
<tr>
<td>Horse mussel</td>
<td>Europe</td>
<td><em>Modiolus modiolus</em></td>
</tr>
<tr>
<td>Bearded horse mussel</td>
<td>Atlantic/Mediterranean</td>
<td><em>Modiolus barbatis</em></td>
</tr>
</tbody>
</table>
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Mussel
- Mediterranean – South East Europe: Mytilus galloprovincialis
- New Zealand: Mytilus canaliculus
- Australia: Mytilus planulatus

Scallops
- Scallop: Atlantic: Pecten varius
- Coquille St. Jacques: North East Atlantic: Pecten maximus
- Iceland scallop: North Atlantic: Chlamys islandica
- Bay scallop: West Atlantic: Argopecten irradians
- Weathervane scallop (also called Alaska scallop): Pacific – North America: Pecten caurinus
  Pacific – North America: Pecten aequisulcatus
  Pacific – Japan: Pecten laquatus
- Common scallop: Japan: Pecten yessoensis
- Sea scallop (also called giant smooth): Atlantic – North America: Pecten magellanicus or Placopecten magellanicus
- Commercial scallop: Australia: Pecten meridionalis
  New Zealand: Pecten novaeezealandiae
- Great scallop: Atlantic/Mediterranean: Pecten jacobaeus
- Calico scallop: Atlantic – North American: Aequipecten gibbus
- Queen scallop: Atlantic: Chlamys opercularis
- Variegated scallop: Atlantic/Mediterranean: Chlamys varius
- Scallops saucer: Australia: Amusium balloti

3.1.2.1 Clam

Clam is the common name of a large group of often edible, mostly marine bivalve molluscs. Different species are variously called quahog, geoduck, hard-shell or soft-shell clam, littleneck, cherrystone, and other names. A mantle tissue encloses the body of the clam, laterally compressed, and two symmetrical shells (valves) held closed by two large muscles and joined by a dorsal hinge joint. The muscular foot is used to burrow in mud or sand. Buried clams leave tubes, or siphons, extended above the surface to maintain water currents needed for respiration and feeding. Most clams are only a few centimetres in their maximum dimensions, but
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the giant clam of the Indian and Pacific oceans may be nearly 130 cm long and weigh 227 kg.

*Live:* in shells.

*Fresh:* in shell, or shelled meats.

*Frozen:* in shell, or shelled meats.

*Smoked:* meats; after precooking and brining, the meats are smoked and packed in oil in cans or jars.

*Dried:* Hoshigai (Japan) shelled meat, skewered with bamboo sticks, sun-dried.

*Canned:* meats are steamed open and the meats removed from the shells; washed, trimmed and packed, either whole or minced, in cans with either brine or clam liquor, then heat-processed; smoked meats are also canned. Canned soups include clam chowder, clam madrilène and clam liquor.

3.1.2.2 *Oyster*

Oysters are marine bivalve molluscs, having rough and irregularly shaped shells that are closed by a single adductor muscle. Oysters lack a foot or have a reduced foot, and no siphon. They live free on the bottom or adhere to stones or other objects in shallow water along the seacoasts, tidal waters or in brackish water in the mouths of rivers. These molluscs feed on minute plants and animals carried to them by the current.

*Live:* in shell

*Fresh:* in shell or shelled meats, uncooked (shucked).

*Frozen:* shelled meats, uncooked.

*Dried:* shelled meats, boiled and then sun dried for 3 to 10 days before packing in boxes (Hong-Kong)

*Smoked:* meats usually canned in edible oil.

*Semi-preserved:* meats cooked and packed in spiced vinegar.

*Canned:* meats removed from the shell by steaming, packed in weak brine; uncooked meats packed unprocessed, hermetically sealed.

*Soups:* oyster stew, oyster soup, oyster bisque, all in cans.
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3.1.2.3 Mussel

Mussels are marine bivalve molluscs that live either partially buried in the sea bottom or attached to rocky surfaces by means of byssus threads. They have filibranch gills, in which the individual branches, or filaments, are united, and held apart, by interlocking tufts of hair-like cilia. The familiar marine mussel is asymmetrical, with the posterior ends of the shells (valves) broad and rounded, and the anterior end smaller and pointed. The posterior halves of the valves contain a large adductor muscle that holds the valves closed. In some species, the shell is often characterised with a lustrous nacreous or pearly lining inside their shells.

Live: whole with shells.

Fresh: cooked meats.

Smoked: meats may be canned in edible oil.

Canned: cooked meats, in mussel liquor, brine, vinegar solution; sauces, also with other ingredients, mussel in butter sauce, mussel paste.

Semi-preserves: cooked meats with vinegar-acidified brine and spices; or packed with jelly.

Salted: cooked meats bottled in brine; cooked meats packed in dry salt for transport.

Bait: used live extensively for baiting fish hooks for line fishing.

3.1.2.4 Scallop

Scallops are marine bivalve molluscs living in shallow waters of protected bays with sandy or muddy bottoms. The shells are fluted and round with a broad, flattened ear or wing at the hinge area. They have a radially ribbed shell with the edge undulated and swim by opening and closing the valves. The common scallop is about 5 cm long. Internally, scallops possess a single large adductor muscle, composed of striated muscle fibres (used for fast swimming) and unstriated muscle fibres (used to hold together the two halves of the valves). The adductor muscle is the only edible part of the animal. Unlike other sessile bivalve molluscs, scallops can swim or leap by a series of rapid closings and openings of the valves.

Fresh: shelled meats (often only the adductor muscle and the roe are eaten.)

Dried: peeled, gutted, boiled, smouldered and afterwards dried (Japan).
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Frozen: shelled meats.

Canned: in own juice, in sauce, and butter.

3.1.2.5 Cockle

A marine bivalve mollusc having a shell with convex radially ribbed valves. Primarily inhabits shallow waters near tidal zones where they bury themselves in the sandy or muddy bottoms. Cockles range from about 1 to 15 cm in length and have two valves of equal size.

Live: in shell.

Fresh: meats removed from the shell by boiling.

Salted: meats either lightly or heavily dry salted, depending on length of journey; also bottled in brine.

Vinegar cured: bottled in malt vinegar after brining.

Canned: in brine.

3.2 Initial Microflora

The microflora of molluscan shellfish directly reflects the environment from which the shellfish is harvested. The initial microflora of molluscan shellfish is comprised of the natural commensal microorganisms and the microorganisms accumulated from the water during feeding. Because molluscan shellfish are filter feeders, they accumulate pathogenic microorganisms from polluted waters. Whatever contaminants are in the water will eventually get into the shellfish (7). The microflora are dependent upon a number of factors such as season, and the environment, which affects the temperature, salinity, pH, nutrient concentration and pollutants of the water column. For example sessile and benthic shellfish dwelling on the bottom or burrowed in marine sediment are especially vulnerable to pollutants (sewage and/or wastewater) introduced into the shellfish water beds. Raw and partially cooked shellfish, mostly in oysters, clams and mussels, are vectors of disease transmission (8). The following pathogenic microorganisms causing illness in humans are characteristically associated with molluscan shellfish:

\[
\begin{align*}
Vibrio parahaemolyticus & \\
Vibrio cholerae O1 & Vibrio vulnificus \\
Vibrio fluvialis & Vibrio cholerae non-O1 \\
Vibrio hollisae & Vibrio mimicus \\
Vibrio furnissii & 
\end{align*}
\]
Aeromonas hydrophila
Staphylococcus aureus
Shigella spp.
Campylobacter coli
Bacillus cereus
Hepatitis non-A, non-B
Snow Mountain virus

Plesiomonas shigelloides
Salmonella spp.
Campylobacter jejuni
Escherichia coli
Hepatitis A
Norwalk virus
Other viral agents

A variety of commensal microflora are found in molluscan shellfish. The microflora of molluscan shellfish at harvest consists predominantly of Gram-negative rods while the Gram-positives constitute a minor portion of the flora. The largest groups found are the Pseudomonads, Vibrios, and Aeromonads. Other microorganisms found in lesser numbers are the Achromobacter spp., Flavobacterium spp., Pseudomonas spp., Acinetobacter spp., Micrococcus spp., Enterococci spp., Bacillus spp., Alcaligenes spp., Moraxella spp., and yeast cells of Rhodotorula rubra and Trichosporon spp. (9, 10, 11, 12, 13).

Water temperatures affect the concentrations of microflora in the water. At harvest, molluscan shellfish have an Aerobic Plate Count (APC) of approximately 1000 to 100,000 /g.

There are microorganisms, observed microscopically, that have never been cultured on laboratory media. They are termed viable non-culturable microorganisms. For example, spirochetes of the genus Cristispira and Saprospira, have been observed microscopically in the digestive tract of Eastern and Pacific oysters, but have never been cultured (14, 15).

In summary, the initial flora composition and concentration of bivalves are highly variable, responding to the environment in which they are harvested. Temperature, salinity, nutrients, pollution point sources and hydrological events will greatly influence the initial microflora in bivalves.

3.3 Processing and its Effects on the Microflora

3.3.1 Oysters, clams, mussels, and cockles

The processing of bivalves takes two forms, shellstock (live) which is shellfish in the shell, and shucked shellfish that is shellfish, whole or in part, from which one or both shells have been removed. Flow diagrams of the major processing steps of molluscs are presented in Figures 3.1 and 3.2.

3.3.1.1 Shellstock

3.3.1.1.1 Processing description

The method of processing shellstock begins with the harvester collecting shellstock by the utilisation of tongs, rakes, dredges, mechanical harvesters, or by
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hand (Figure 3.1). Shellstock are given a wash to reasonably free them of bottom sediments and detritus as soon as practicable, after harvesting. Water used for shellstock washing is from approved growing areas, or from other sources approved by the State Shellfish Control Agency (SSCA). The shellstock is transported dry, usually in open-mesh containers and/or burlap sacks, by truck or boat. If by boat, usually the boat will be docked by the processing facility with shellstock piled on the deck of the boat or dock. Shellstock are placed in dry-cooler storage containers in the facility, or immediately placed in refrigerated trucks, for transportation to the point of sale.

Shellstock may be wet-stored at the processing facility in tanks of clean seawater, or in bay-approved shellfish-growing waters prior to sale or processing. There are three primary reasons for wet storage: first, shellfish can be harvested from remote areas at convenient times and held live for brief periods at locations close to the point of sale, in containers from which they can be retrieved easily. Secondly, wet storage also may be used to de-sand those shellfish species that tend to accumulate sand in their mantles and gills thus making them more palatable. Thirdly, wet storage may be used to increase palatability by increasing salt content of shellfish harvested from low salinity waters.

Fig. 3.1. Generic flow diagram of live shellfish from harvester to point of sale
3.3.1.2 Effects of microflora during processing

The initial microflora found on or in shellstock is influenced by sources of shellstock, handling, and temperature storage of shellstock to point of sale. There is a positive relationship between sewage-polluted shellfish and enteric disease transmitted by shellfish (8, 16, 17). Shellfish may contain higher levels of pathogens than are found in the water in which they grow. The safety of shellfish is predicated by the cleanliness of the growing area waters from which they are harvested, and the sanitary practices applied during harvesting and shipping. The most important factor in controlling bacterial growth in shellstock is temperature. Shellstock should be refrigerated at temperatures at or below 10 °C (50 °F). At this temperature the multiplication of faecal coliforms (E. coli, Klebsiella, Enterobacter, and Citrobacter species) and Vibrios, including V. vulnificus, are prevented, with the exception of A. hydrophila (18). Generally, Food and Drug Administration (FDA) recommends that potentially hazardous food such as shellfish be held at 7.2 °C (45 °F) or below (19). Oysters will remain alive in the shell for approximately two weeks if kept at the recommended temperatures; however, with prolonged storage the microbiological quality of the bivalve decreases. Generally, shellstock are not refrigerated on the harvest boat; when it reaches the processing facility, shellstock are placed in refrigerated trucks for transport or placed in wet storage. If long delays occur before reaching the processing facility and/or refrigeration, the bacterial counts in the meats will increase. Furthermore, there is evidence that in the absence of adequate refrigeration, for a considerable length of time, some pathogenic organisms will survive and multiply in shellfish (20, 21, 22).

Harvest conditions are another factor that influences the growth of bacteria in shellstock. At harvest, bivalves tightly close their shells thus trapping water and microflora. Over a period, without adequate refrigeration, metabolic waste accumulates in the shell serving as nutrients for bacterial growth. Several studies have established the increase of bacterial counts in shellstock during post harvest period before the shucking process (18, 23, 24, 25).

Water used for shellstock washing should be of good sanitary quality, to avoid possible contamination of the shellstock. The washing of sediment and detritus soon after harvesting will prevent further contamination. Several studies have established that pathogenic Vibrios and other bacteria may be present in marine sediments throughout the year (21, 26). Washing shellstock helps prevent quantities of sediment and bacteria being mixed with, and thus contaminating the shucked meat at the point of sale.

Wet storage operations are highly variable and may range from temporary storage near-shore in approved areas to on-shore tanks using re-circulating, synthetic seawater for the purpose of de-sanding and salt uptake. Shellfish in wet storage tanks are similarly subjected to pollution if the tank water is obtained from a polluted source. An outbreak of infectious hepatitis in Sweden in 1956, involving 691 cases was attributed to oysters contaminated in a wet storage area (27). Excessive sediment on the shells and dead shellfish may increase bacterial
loads in the tanks and lead to increased microbial levels in the shellfish during storage. Thus, washing and culling shellfish prior to storage is essential.

Another potential risk with the use of wet storage is the commingling of bivalve molluscs with other marine species such as crab, lobsters, and fish. It presents a risk of cross-contamination from the non-molluscan animals to the bivalves.

3.3.1.2 Shucked shellfish

3.3.1.2.1 Processing description

The processing of shucked shellfish is limited to washing of shellstock, removal of shellfish (shucking), washing, and packing shellfish into containers (Figure 3.2). Sealed containers are placed in boxes and covered with crushed ice for chilling or refrigeration and transportation. The process of shucking involves forcing a knife between the valves and cutting the adductor muscle of one valve freeing the shellfish from one valve. To remove the shellfish attached to the remaining valve, the knife is used to cut the remaining adductor thus freeing the shellfish (meats). Meats are collected in either perforated or non-perforated buckets for delivery to the washing station. Washing of meats with potable water is accomplished by placing the meats on a skimmer table (the stand-supported, perforated tray in which shucked shellfish are spray washed and/or drained) or in a blower-washer (a tank-like device for immersion washing of shucked shellfish). In the blower-washer, the meats are in a tank of water and agitated by the introduction of air from the bottom of the tank. After washing of meats, the tank is opened through a drain gate and chute, through which the washed meats are discharged for rinsing and then packed in containers. Containers are placed in packing boxes for shipping at refrigerated temperatures.

3.3.1.2.2 Effects of microflora during processing

The same considerations as previously discussed, for the effects of microflora during the processing of shellstock, are applied to the shucking operation of shellfish. Shucking and washing of meats under sanitary conditions prevents the proliferation of bacterial populations. If shellfish are not reasonably clean at the time of processing, a considerable quantity of the adhering material will be mixed into the shellfish during the shucking process, thus contributing to high bacterial counts in the final product (28). In addition, shellstock is subjected to contamination when stored on the floor in standing water, or subjected to splash from foot traffic. Due to the nature of the shucking operation, clothing becomes very soiled. Under these conditions if the shuckers enter the packing room, the finished product may become contaminated. Shuckers routinely use either cotton or rubber gloves during the shucking operation. Studies have shown the use of rubber gloves reduces the contamination level, whereas cotton become wet and
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retains bacteria during the shucking thus increasing the bacterial load in shellfish (29). Separate rooms or lockers for storage of clothing, aprons, and gloves help prevent the contamination of shellfish. There is a tendency to store such articles on the shucking benches or in packing rooms, which may lead to contamination of product.

![Fig 3.2. Generic flow diagram of shucked shellfish from harvester to point of sale](image)

Shellfish are subject to contamination during the shucking and packing process when the following are not adhered to (19):

1. Shucking buckets and storage containers are kept at adequate height above the floor to prevent contamination from floor splash.
2. Shucked meats are thoroughly drained, cleaned and delivered to the packing room or prechilled and placed in temporary refrigeration (≤ 2 hours) at 7.2 °C or less, within one hour.
3. Shucking buckets are completely emptied at the packing room and no overage is returned to the shucker.
4. Shucking containers are rinsed clean with running water, and sanitised before each filling.
Unacceptable practices in the packing step can affect the microflora of shellfish. Controls that should be applied include using clean packing equipment, clean gloves, and packing the shellfish in a timely manner. All equipment coming in contact with the shellfish such as colanders, shucking pails, skimmers, blowers and other utensils that have cracked, rough, or inaccessible surfaces are apt to harbour accumulations of organic material in which bacteria may grow.

Refrigeration and shipping of shucked shellfish in wet ice is highly recommended. Shucked shellfish are held and transported at temperatures of 7.2 ºC or less. Shucked shellfish are an excellent medium for the growth of bacteria. Studies have shown that bacterial growth is significantly reduced at storage temperatures of less than 7.2 ºC, and that storage in wet ice is the most effective method for refrigeration of shucked meats (30, 31, 32).

### 3.4 Spoilage

There is a lack of information in the literature regarding spoilage microorganisms in shellfish. Much of the work has been done with oysters and clams. Earlier studies, at the turn of the century, showed that organisms such as *Achromobacter*, *Pseudomonas*, *Flavobacterium*, *Micrococcus*, *Proteus*, *Alcaligenes*, and *Pseudomonas fluorescens* are the responsible agents of spoilage in oysters held at various temperatures (10, 33, 34, 35, 36).

Shellfish, particularly shucked meats, are an excellent medium for growth of bacteria. As described previously, keeping shellfish refrigerated is the most effective method of retarding deterioration and spoilage. The initial flora found in shellfish is the major factor of spoilage in bivalves. The environment has an effect on the bacterial population found in shellfish. These organisms may play a significant role in the spoilage processes. The spoilage process in shellfish may be divided into three stages: increase of acidity, abundant gas production, and proteolysis (10). The high incidence of proteolytic bacteria and other types capable of fermenting glucose in the natural flora may be responsible in post-mortem spoilage of shellfish. For example, in oysters, glycogen levels range from 0.47 to 6.8% (37). Glycogen, a carbohydrate, is stored in the bivalve as a source of energy. Glycogen is hydrolysed by the initial flora found in shellfish, thus producing acid and gas (38). This process changes the bacterial profile to microorganisms favouring low pH; these include lactobacilli, streptococci, and yeasts. Lactobacilli seem to be the predominant microflora in shellfish, particularly oysters stored at 7 ºC, whereas bacterium such as *Pseudomonas*, *Achromobacter* and *Flavobacterium* levels decrease during storage.

In summary, anywhere during the process of shellfish from harvesting to the point of sale, where there is a breakdown of temperature control or prolonged storage of shellfish, spoilage will occur. Temperature abuse will result in high bacterial counts.
3.5 Pathogens: Growth and Survival

This section deals with bacterial pathogens frequently present in shellfish. Descriptions of outbreaks or reports of growth of the organism in shellfish are presented. These organisms can be present in the shellfish from the point of harvesting, during processing, and to the point of sale. They can be introduced to the shellfish from the environment or added during processing. The control of growth and survival of these organisms can be reduced by the use of good manufacturing practices including immediate and proper refrigeration, prevention of cross-contamination, and harvesting of shellfish in the cooler months.

3.5.1 Vibrios

Vibrios are Gram-negative facultative rods naturally occurring in the marine water (estuaries, coastal areas), primarily in brackish or saltwater. Diseases caused by Vibrios are enteric in nature, ranging from epidemic cholera to sporadic cases of diarrhoea.

3.5.1.1 Vibrio parahaemolyticus

V. parahaemolyticus causes gastroenteritis lasting 24 - 48 hours with abdominal pain, diarrhoea, nausea, headache and fever. It is a common marine bacterium, widespread in both polluted and unpolluted waters. Major outbreaks are associated with the warmer months. Sporadic cases are frequent along the coast of the United States, and very common in Japan, where outbreaks occur regularly. This bacterium is present in all the major processing steps of a shellfish plant, from harvesting to finished product (i.e. shellstock and packing of shellfish in jars) (29). V. parahaemolyticus multiplies rapidly at temperatures above 20 ºC. Large numbers of bacteria must be present to reach potentially infective levels of >10^7 colony forming units per g (39); however, there is a recent report of low levels of V. parahaemolyticus possibly producing illnesses (40). Between May and September 1997, there were over 250 cases of illness on the West Coast of North America extending from California to Canada. The majority of the illnesses were associated with the consumption of raw or partially cooked oysters and clams. One of 250 cases reported involved shucked products with low levels, approximately 23 cfu/g, of V. parahaemolyticus. Products involved were from the same processor, lot and retail outlet. V. parahaemolyticus illness is usually a result of eating raw or partially cooked shellfish (8, 41). The ability of the bacterium to cause gastroenteritis is highly correlated with the production of a heat-stable haemolysin (42), although non-hemolytic strains have been associated with illness (43, 44). Fortunately, only a small percentage of strains in the marine environment are potentially pathogenic (45). The incidence of V. parahaemolyticus from shellfish is low, and estimates based from a hospital study show less than 0.5 cases/100,000 population/year (46).
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Control of *V. parahaemolyticus* growth in shellfish meats is temperature dependent. Inadequate refrigeration will accelerate the growth of *V. parahaemolyticus*, which have been implicated in shellfish-related disease outbreaks (31). They are sensitive to cold, and rapidly become inactive at low storage temperatures.

3.5.1.2 *Vibrio vulnificus*

The symptoms of *V. vulnificus* are fever, chills, and nausea within 24 - 48 hours of onset. Death has been reported to occur within 36 hours from onset. It is one of the most severe foodborne infectious diseases, with a fatality rate of 50% for individuals with septicaemia. Healthy individuals are susceptible to gastroenteritis. High-risk individuals, those who have liver disease, diabetes, cirrhosis, leukaemia, or immunosuppression, are particularly susceptible to primary septicaemia; these individuals are advised not to eat raw shellfish. Oysters are the most hazardous food associated with *V. vulnificus*. The bacterium is widespread in estuarine waters particularly in warm waters of >20 °C. Primarily, the organism has been found in the Gulf of Mexico; however, the organism has also been found in cooler temperatures from estuaries of Washington and Oregon (47). The bacterium is an estuarine species that causes wound infections, gastroenteritis, or primary septicaemia. All isolates are considered pathogenic. The occurrence of *V. vulnificus* causing septicaemia and death has been associated with the consumption of oysters (8, 48). Again, like *V. parahaemolyticus* the estimated annual incidence rate is very low, in the range of 0.4 - 0.8 case/100,000 population/year (8, 46, 49). Control of *V. vulnificus* is by rapid refrigeration during warm weather months. Hazards from this bacterium can be controlled by thorough cooking of shellfish, and preventing cross contamination once the product is cooked.

3.5.1.3 *Vibrio cholerae non-01*

Illness caused by *V. cholerae* non-01 is generally a less severe gastroenteritis than that caused by *V. cholerae*. It causes diarrhoea, abdominal cramps, fever, nausea, and vomiting. Onset of symptoms occurs in 48 hours and can be severe, lasting 6 - 7 days. The organism attaches to the intestine and release of a toxin is suspected. *V. cholerae* non-01 has been associated with septicaemia, primarily in immunocompromised individuals with a mortality rate exceeding 50% (50). *V. cholerae* non-01 is ubiquitous in the estuarine environment. In one study, 23 of 24 major USA-West Coast estuaries were positive for *V. cholerae* non-01 (51). The widespread distribution of this bacterium is comparable to other studies in the Gulf and Atlantic Coasts (52, 53, 54, 55, 56).

*V. cholerae* non-01 is a common bacterial cause of molluscan shellfish-associated illness. Illnesses are usually associated with the consumption of raw oysters as described in Table 3.II (8, 46, 57, 58). Control of oyster-associated
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*V. cholerae* non-01 illnesses is by cooking seafood thoroughly, preventing cross-contamination of cooked seafood, and harvesting of oysters in colder months when *Vibrio* counts in water are the lowest (59).

### 3.5.1.4 *Vibrio cholerae* 01 and 0139

Symptoms of epidemic cholera vary from mild watery diarrhoea to acute diarrhoea with characteristic rice water stools. Illness can include abdominal cramps, nausea, vomiting, dehydration, and shock. Death may occur after severe fluid and electrolyte loss. Onset of disease is 6 hours to 5 days. Serotype Ogawa biotype El Tor and 0139 are responsible for epidemics in South American, and southern Asia including India and Bangladesh, respectively. Poor sanitation and contaminated water supplies will spread the disease, whereas excellent sanitation facilities in developed countries are responsible for the near eradication of epidemic cholera. In developing countries, *V. cholerae* 01 is transmitted by faecal contamination of food or water. There is evidence of strains of *V. cholerae* 01 establishing in the U.S. Gulf coast environment, and the transmission of the disease by consumption of raw, undercooked, or cross-contaminated shellfish (57, 60). The presence of these strains appears to be in low numbers and non-virulent (59). The control of *V. cholerae* 01 in molluscan shellfish, particularly in oysters, is the same as previously discussed for the non-01 strains. In addition, processors should know the product source, e.g. imported product from a country experiencing an epidemic.

### 3.5.1.5 Other *Vibrio* spp.

There are several other *Vibrio* spp. associated with shellfish-borne illness outbreaks, including *V. fluvialis*, *V. mimicus*, and *V. hollisae*. (8, 61, 62, 63). The frequency of infection of these organisms is no different from other *Vibrios*; however, the pathogenic severity is lower than *V. cholerae* 01, *V. parahaemolyticus* and *V. vulnificus*. In general, these organisms cause gastroenteritis lasting 24 - 48 hours with abdominal pain, diarrhoea, nausea, headache and fever. They are common marine bacteria, widespread in both polluted and unpolluted waters. Illnesses are associated with the warmer months. Control of infection from these organisms is no different from the other *Vibrios*.

### 3.5.2 *Campylobacter jejuni*

*C. jejuni* requires reduced levels of oxygen (3 - 5%) and 2 - 10% carbon dioxide for optimal growth conditions. It is considered a fragile and sensitive organism to environmental stresses such as oxygen, drying, low pH, salinity, acidity and heat. It causes diarrhoea, abdominal pain, fever, and nausea. Onset of illness is from 3 to 5 days after ingestion of contaminated food, and illness lasts 7 - 10 days.
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TABLE 3.II
Molluscan shellfish associated outbreaks caused by *Vibrio* spp.\(^a\) (8)

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Incidents</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard clams</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Oysters</td>
<td>279</td>
<td>362</td>
</tr>
<tr>
<td>Soft Clams</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mussels</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Scallops</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Includes *V. parahaemolyticus*, *V. cholerae* 01 and non-01, *V. vulnificus*, *V. fluvialis*, *V. mimicus*, and *V. hollisae*

Infecitive dose varies with host susceptibility and virulence of the strain. Human feeding studies suggest that about 400 - 500 cells/gram of *Campylobacter* may cause illness (64, 65, 66). *Campylobacter* spp. have been isolated from shellfish beds throughout the world (67, 68, 69, 70, 71), and have been implicated as the causative agent in illnesses from the consumption of raw oysters and clams (68, 72, 73). In one case, infection occurred among participants of a firemen’s banquet. Sixteen of 28 participants became ill with campylobacteriosis after ingesting raw clams. Although, the incidence of *Campylobacter* infection is low from the consumption of molluscan shellfish, *Campylobacter* appear to survive well in oysters during storage. *C. jejuni* survived for over 20 days in market-shucked oysters stored at 4 °C, whereas at 10 °C *Campylobacter* survive only for 5 days (71). This finding is significant in that *C. jejuni* does not multiply in shellstock at refrigerated temperatures; however, they survive well at refrigerated temperatures. Recently, *C. jejuni* were recovered, ranging from 0.4 to 114 cells, from Pacific shellstock oysters purchased from a retail grocery store (74). Campylobacters do not survive well in the environment due to the organism’s lack of resistance to environmental factors; however, the organism is being continually inoculated into the environment by waterfowl and farm run-offs and thus eventually contaminating shellfish growing waters. To reduce the risk of shellfish-borne campylobacteriosis, control of *C. jejuni* begins in the harvest of shellstock from approved waters, and their immediate proper refrigeration. Proper care should be taken if the finished product is to be packaged in modified-atmosphere packaging (MAP).

3.5.3 Other organisms of concern

3.5.3.1 *Aeromonas hydrophila* group and *Plesiomonas shigelloides*

*A. hydrophila* group (75, 76, 77, 78) and *P. shigelloides* (79, 80) are ubiquitous in the marine environment. They have been implicated in limited shellfish-associated gastroenteritis cases (8, 81, 82, 83, 84, 85), although their role as a pathogen is highly controversial and circumstantial. The seasonality of
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*Aeromonas* and *Plesiomonas* is similar to the *Vibrios*, being isolated more often during the warmer month. They survive well in shellstock or shucked oyster meats held at refrigeration temperatures. In one study, after 7 days storage of shucked oysters at a shellfish processors’ cooler kept a 5 ºC *Aeromonas* counts ranged from 500,000 to 3,600,000/gram (29). Similar results were seen by other investigators (86, 87) of the outgrowth of *A. hydrophila* group stored at refrigeration temperatures. Control of these organisms in shellfish products is limited. They are easily destroyed with mild cooking temperatures. Caution should be exercised by individuals with haematological malignancies (88), suggesting that these individuals are at higher risk when consuming raw or partially cooked shellfish containing these bacteria.

### 3.6 Published Microbiological Criteria

The published microbiological criteria below are included as a guide, and where indicated are intended to be used for official control purposes.

<table>
<thead>
<tr>
<th>Test</th>
<th>Acceptable Levels in Molluscan Shellfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Plate Counts</td>
<td>&lt;500,000 cfu/g¹</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>&lt;230/100 g²</td>
</tr>
<tr>
<td>Enterotoxigenic <em>E. coli</em></td>
<td>&lt;1000 per gram, negative for heat-labile toxin (LT) or heat-stable enterotoxin (ST)¹</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Negative for the presence¹</td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>Negative for the presence of toxin producing 01 or non-01 organisms ¹</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>&lt;10,000 MPN per gram²</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>negative for staphylococcal enterotoxin or when the viable MPN count is &lt;10,000²</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>negative for the presence³</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>Under review⁴</td>
</tr>
</tbody>
</table>

¹ Source (89), FDA established action levels or levels of concern for certain microbial pathogens in molluscan shellfish. The Agency will consider enforcement action against the shipment of molluscan shellfish if the levels exceed as stated. Enforcement action is considered on a case-by-case basis taking into account all the factors associated with the specific situation.

² Source (90), FDA/Administrative Guidelines

³ Source (91), FDA/ Administrative Guidelines, Pathogen monitoring of Selected High Risk Foods Domestic including: milk, dried milk/whey drink powders; non-dairy frozen desserts; soy products; prepared sandwiches. Note: Although molluscan shellfish are not included in the selected high risk food, action is considered on a case-by-case basis taking into account all the factors associated with the specific situation.

⁴ FDA and ISSC recommending establishment of an educational program to alert at risk population of presence of *V. vulnificus* in molluscan shellfish.
3.7 References


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MOLLUSCAN SHELLFISH


MOLLUSCAN SHELLFISH


FISH AND SEAFOOD


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MOLLUSCAN SHELLFISH


3.8 Further Reading


4. CRUSTACEAN SHELLFISH

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4.1 Definitions

*Crustaceans* are invertebrate animals and represent a class of the phylum Arthropoda. They possess a segmented body, jointed limbs and a chitinous exoskeleton, and are usually aquatic, e.g. crabs, lobsters, crayfish and shrimp (1). Crayfish, lobsters and crabs have ten legs; the front pair ends in claws.

*Shrimps, prawns and scampi* are temperate species commonly eaten by man and include members of the families *Nephrops norvegicus* (scampi), *Pandalus borealis* (deep-water shrimp), *Pandalus montagui* and *Crangon crangon* (inshore shrimp). Species eaten from tropical waters include *Penaeus* spp., *Parapeneopsis* spp., and *Trachypenaeus* spp. (deep-water shrimp). Shrimp can be purchased in the raw or cooked state in a variety of forms:

*Entire shrimp, with shells and heads on or off* are usually packed, with additional water to form a glaze, and frozen in 1 and 2.5 kilo pack (boxes or bags).

*Peeled ready-to-cook shrimp* are raw, peeled product, packed and frozen in 0.5 and 1.0 kg polythene bags.

*Peeled and cooked shrimp* are ready-to-eat after controlled thawing, and available in 0.5 and 1.0 kg polythene bags.

*Breaded shrimp and scampi* are battered and coated in breadcrumbs and sold frozen to the consumer for cooking prior to consumption. Such products are usually only partially cooked before freezing.
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Crabs, lobsters and crayfish are traditionally kept alive after capture and then cooked to kill immediately before consumption.

Cooked and chilled, or frozen crab, lobster and crayfish are available for the consumer owing to the growing demand for ready-to-eat products, e.g. lobster tails and cooked crabmeat presented decoratively in their shells.

Pasteurised crabmeat is edible meat removed from the exoskeleton by hand, packed into polythene bags, stored and transported as frozen product.

4.2 Initial Microflora

As with finfish, the level and type of the initial microflora of crustacean shellfish will reflect a combination of factors, which include the environment from which they have been captured or harvested; their feeding and living habits; the geography of the area they are captured from or are grown in; the season; and the temperature and quality of the waters in which they exist. Following capture or harvest, the flora will change depending upon the methods of handling and/or the environmental conditions to which they are exposed. Microbiological risks associated with crustacean shellfish increase in proportion to the degree of handling that the product undergoes, particularly after it has been cooked. For example, during the peeling of cooked shrimp, the potential exists for cross-contamination from both the processor and from the environment. Control measures to prevent these potential hazards occurring include training and supervision of operatives as well as Good Hygienic Practices (GHP). Poor hygienic procedures will increase the probability of contamination with bacteria of public health significance. The structure of the circulatory system of crabs is not closed, which means that the haemolymph can be a reservoir of bacteria, particularly for members of the genus *Vibrio* (2).

Spoilage begins following death once the immuno-response system of the crustacean shellfish fails to function; hence, for crabs and lobsters, spoilage bacteria are of little importance as, traditionally, only live carcasses are processed, e.g. boiled to kill the crustaceans and cook their meat prior to consumption. However, with the increasing demand for ready-to-eat/cook crustacean products, there is a growing market for fresh lobster tails or cooked crabmeat in crab shells. If these products are subjected to temperature abuse, storage above the recommended storage temperature, this presents more favourable conditions for growth by both spoilage and pathogenic bacteria.

In comparison, shrimp die immediately after capture, so that spoilage may occur during landing and transportation to the processing plant. As with finfish, it is important that shrimp are handled and iced correctly in order to keep them in as fresh and uncontaminated a state as possible.

The microbial flora of crustaceans from temperate waters differs from that of crustaceans from warmer, tropical waters. Spoilage bacteria associated with temperate crustaceans include *Pseudomonas* spp., *Shewanella putrefaciens* and
members of the *Acinetobacter-Moraxella* group. In contrast, the genera associated with tropical species include Enterobacteriaceae, *Vibrio* spp., *Pseudomonas* spp., *Alcaligenes*, corynebacteria, *Micrococcus* spp., and *Achromobacter* spp. Hence, from the time of capture and processing, bacterial contamination and spoilage are inevitable. The rate of spoilage will be a function of time and temperature, whether GHP are in place, and will be dependent upon whether the crustaceans were harvested from cool or warmer waters.

As with finfish, there is evidence that the shelf life of tropical crustaceans is longer than that of temperate-water species stored correctly in ice - for example, up to 16 days for tropical species compared with 8 - 10 days for temperate water species (3). The psychrotrophic bacteria responsible for causing spoilage are not present on freshly captured crustaceans from tropical waters in such high levels as are found in crustaceans from cooler waters and, thus, the rate of deterioration of the crustacean shellfish by these organisms is reduced, especially if they are stored in ice or iced water.

### 4.3 Processing and its Effects on the Microflora

Crabs, lobsters and crayfish are harvested from cages or pots containing bait. They are then taken to market alive in either holding tanks, chilled sea water, or transported directly to processing plants. These animals can survive out of water if the surrounding environment is kept cool and damp. The claws are usually bound together to prevent the animals fighting and causing damage to each other. As the muscles of crustacean shellfish decompose rapidly after death dead animals are rejected.

In comparison shrimps die quickly after capture. A large proportion of shrimps are produced by aquaculture, or are found in brackish water around the shores, so they are either sold on the local market or are processed in local processing plants. Shrimps can be processed whole, with “head on” or “head off” whereby the edible tail is removed from the head, gills and thorax. The latter process can expose the shrimp muscle to incidents of cross-contamination if GHP are not followed; however, by removing the head and thorax a source of enzymes is eliminated that will prevent the occurrence of black spots. When the shrimp arrive at the processing plant they are washed and then sorted into size. Shrimp might be further processed with the shell on, or with the shell and central vein removed. Scrupulous hygiene is important at this stage to prevent the cross-contamination of the shrimp tails. Shrimp will then be glazed and frozen in a raw state, or cooked, glazed and frozen (see Figure 4.1).

#### 4.3.1 Prawns and shrimps

An example of the process used for preparing frozen, uncooked prawns and shrimp is presented in Figure 4.1. After capture and transport to the processing plant, they are washed, graded according to size, peeled and cooked, chilled
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frozen, and packed to meet the customer’s specification. The cooking stage will eliminate the intrinsic microbial flora of the shrimp as well as any contaminants that have been added during handling and transportation to the processing plant. After cooking, Good Manufacturing Practice (GMP), including GHP, should be followed to ensure that the product is not re-contaminated with microorganisms present in the processing environment or from the workers.

Breaded products introduce an added risk of spore forming bacteria, particularly the pathogens *Bacillus cereus* and *Clostridium perfringens* originating in the flour, spices and cooking batter.

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**Fig. 4.1. Flow diagram - frozen uncooked prawns**
CRUSTACEAN SHELLFISH

4.3.2 Cooked crabs and lobsters

As indicated earlier, since crabs and lobsters are traditionally consumed immediately after cooking, there is no associated spoilage problem. However, with the growing demand for ready-to-eat products from crab and lobster meat, there is a new hazard of contamination of the cooked meat by workers during its physical removal from the exoskeleton. If hygienic procedures are not followed, contaminating microflora may include enteric bacteria, bacteria of public health significance, viruses or parasites.

Once removed and cleaned, the meat is either repacked and sold in the shells, or packaged and processed. Processed crab and lobster meat can also be bought as packages of meat that have been subjected to a pasteurisation step, or in hermatically sealed cans that have undergone an appropriate heat treatment.

4.4 Spoilage

The main genera of Gram-negative bacteria responsible for the spoilage of crustacean shellfish are the same as those that cause spoilage in finfish; they include *Sh. putrefaciens* and *Pseudomonas* spp.

4.4.1 Prawns and shrimps

Freshness indicators for prawns and shrimps are for the shells to be crisp and dry and feel sharp and cool to the touch, and have a sweetish, slightly iodine smell. In comparison, spoiling prawns and shrimps have shells that are soft, wet and ‘soapy’ or ‘jammy’ to the touch. Heat is produced if the shrimps decompose in a confined space, and a strong ammoniacal odour is produced, indicative of protein degradation and the growth of the principal spoilage bacterium *Sh. putrefaciens*.

4.4.2 Crabs and lobsters

Crabs and lobsters should be alive when boiled or sold. If boiled, the tightness of limbs will indicate whether they were killed by immersion in boiling water or, for crabs, immediately prior to boiling. The shells have a crisp, bright and dry appearance, whilst boiled crustaceans have a pleasant slightly sweet smell. With spoiled crabs and lobsters, there is no indication of life. If the tail drops, the joint between the tail and carapace is open and the limbs hang loosely. The shell will be soft, dull and sticky, with visible decomposition or green discoloration seen through the membrane exposed by the dropping tail. An offensive odour is also produced.
4.5 Pathogens: Growth and Survival

4.5.1 *Salmonella* spp.

The genus *Salmonella* contains a wide variety of ‘species’ pathogenic for man or animals, and usually for both. They are mesophilic microorganisms usually residing in the intestinal tracts of human beings and warm-blooded animals. Hence, *Salmonella* species are more commonly found in crustaceans reared by aquaculture or growing in faecally contaminated brackish water compared with species of marine origin (4, 5, 6, 7). Moreover, animal manure, a common pond fertiliser used in aquaculture, often contains *Salmonella* and other enteric pathogens (8) and has been demonstrated to contaminate crustaceans growing in these environments. Aquatic birds and the hands of workers have also been identified as sources of the pathogen. *Salmonella* cannot be completely removed from raw shrimp during processing, even if GMP is followed, although efficient cooking will quickly eliminate it (6).

4.5.2 *Listeria* spp.

One of the earliest accounts of the presence of *Listeria monocytogenes* in crustaceans was by Russian workers in 1959 (9). Following this, nearly 30 years elapsed before the potential hazard of *L. monocytogenes* in contaminated crustacea was recognised in the United States of America (USA) when the pathogen was isolated from Mexican frozen cooked crabmeat (10). Although foodborne illness had not been associated with the product, in compliance with the Food and Drug Administration’s (FDA) ‘zero tolerance policy’ for *L. monocytogenes* in cooked ready-to-eat foods, the first in a series of Class I recalls was issued in May 1987 to retrieve nearly 4 tonnes of tainted crabmeat (11). An import alert was put in place in June 1987, which required automatic detention of all Mexican frozen crabmeat that tested positive for *Listeria* and *Escherichia coli* (12).

The pathogen was later identified in frozen raw shrimp (13) and lobster tails (14) - cooked prior to consumption. Further surveys identified *Listeria* spp. in 24.5% of frozen raw shrimps from different countries (13). The above results prompted the FDA to develop a compliance programme for the pathogen, as well as for *Salmonella*, in domestic/imported shrimps (15), and to increase testing of many other domestically produced seafoods under the General Pathogen Surveillance Programme (14, 16).

Results of a survey carried out in England and Wales between 1987 and 1989 demonstrated that *L. monocytogenes* was absent from 40 samples of cooked prawns, shrimps and cockles sold (17). However, later surveys in 1990 recovered the pathogen from retail raw, cooked and smoked fish, fish fingers, shrimp and shellfish (11).

The presence of *L. monocytogenes* in cooked ready-to-eat crustaceans is recognised as a potential risk to selected groups of individuals: the very young and
old, pregnant women and the immuno-compromised. To date, no incidence of foodborne illness caused by *L. monocytogenes* has been attributed to crustaceans. However, the pathogen can grow at temperatures as low as 3 °C.

### 4.5.3 *Staphylococcus aureus*

*Staph. aureus* is not a member of the intrinsic flora associated with crustacean shellfish. The main source of contamination is the hands of processing personnel; about 50 - 70% of healthy individuals carry toxin-producing strains of *Staph. aureus* on their hands, as part of the natural bacterial flora, whilst boils and cuts are also common sources of the pathogen. Crustaceans have been linked with food intoxications attributed to *Staph. aureus* because of the ubiquity of this organism in the processing environment, combined with the degree of handling of these types of product, particularly crabmeat (18, 19). Control should be achieved by promoting GHP and staff training programmes.

### 4.5.4 *Clostridium botulinum*

*C. botulinum* type E is commonly associated with fish and fishery products, although contamination by types A and B may occur from the environment. The pathogen has resistant spores, so is able to survive heat treatments that eliminate vegetative cells, e.g. pasteurisation and cooking. Once the competing microflora has been removed by a heat treatment, GMP should be followed in the processing plant to ensure that the final product is held at 3 °C, especially in products that have been vacuum packaged.

Researchers have demonstrated that inoculated shrimp supported toxin production by *C. botulinum* type E at 10 °C but not at 4 °C (20).

### 4.5.5 *Vibrio spp.*

*Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are species that have been reported to be associated with raw crustaceans harvested from estuarine waters (21, 22) as well as from undercooked crustacea when harvested from contaminated waters (23, 24, 25). Crabs are covered in a chitin exoskeleton, which can become colonised by *V. cholerae*; therefore, it is important that the cooking stage is controlled to eliminate the pathogen from the product and that procedures are in place to prevent any cross-contamination of the cooked product.

*V. cholerae* is divided into two groups, *V. cholerae* O1 and *V. cholerae* non-O1. The former, the causative agent of cholera, is widely distributed in aquatic environments, although more recently the non-O1 type has also been demonstrated to cause foodborne illness in crustaceans. An epidemic that began in South America in 1991, which was eventually attributed to a ship discharging its bilge into the coastal waters of Peru, was associated with seafood consumption,
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and caused major economic losses to South American countries that export shrimp to the United States.

*V. parahaemolyticus*, a marine halophile, occurs in crustaceans from warm environments. It is more commonly implicated in countries where crustacean shellfish are eaten raw, e.g. Japan, South America.

*V. vulnificus* is an invasive and lethal pathogen associated with wound infections, but has the potential to cause fatal foodborne illness (26). The organism is widespread in estuarine waters and is a common isolate from harbour water in warm climates. Contamination by this organism can be controlled by rapid chilling of crustaceans after harvesting and avoidance of time/temperature abuse during distribution and processing chains.

### 4.5.6 Campylobacter jejuni and Campylobacter coli

*C. jejuni* has not, to date, been implicated in a case of foodborne infection associated with crustaceans. However, the potential hazard exists as *C. jejuni* was isolated from 36 (15%) of 240 samples of freshly hand-picked blue crab (*Callinectes sapidus*) meat, whilst 5.8% of samples contained *C. coli*. Quantitative levels were below limits of detection in all cases (<0.30 MPN/g) (27).

### 4.5.7 Aeromonas hydrophila and Aeromonas sobria

*A. hydrophila* and *A. sobria* are known to be fish pathogens and are commonly isolated from ponds used for aquaculture. However, they have not been confirmed to be causal agents of foodborne illness associated with crustaceans (28), although *A. hydrophila* has been isolated from prawns (29). *A. hydrophila* is able to grow well at refrigeration temperatures (0 - 2 °C) so that GHP coupled with controlled low temperature storage is essential to prevent the pathogen from reaching levels that may present a hazard.

### 4.5.8 Parasites

The use of animal excreta in aquaculture has also been associated with foodborne parasitic infections of crustaceans, particularly trematodes (6). Parasitic infections are controlled by either cooking infected material prior to consumption, or freezing at a temperature of -23 °C for 7 days (30, 31, 32).

### 4.5.9 Viruses

Crustaceans grown in waters contaminated with sewage may become carriers of viruses such as Hepatitis A, Norovirus, Caliciviruses, Astroviruses and non-A, non-B Hepatitis viruses (31). Of these, Hepatitis A virus presents the most serious hazard; however, in a survey carried out in the United States between 1977 and
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1981, only 0.47% of outbreaks attributed to Hepatitis A virus were attributed to a foodborne origin (33). Both Hepatitis A virus and Norovirus are stable pathogens and are relatively resistant to inactivation (34). They can survive low pH levels, consistent with that in the stomach, or chlorine concentrations similar to those found in drinking water. Norwalk viruses can also withstand heating to 60 °C.

4.5.10 Allergens

Allergens have not been reported to be a significant hazard with crustacean shellfish, although it is one of the European Union (EU) legislated allergens. The risk of such hazards is far higher with fin fish and molluscan shellfish.

4.5.11 Chemical pollutants and biotoxins

Researchers have demonstrated that crabs feeding in waters contaminated with algae do have potentially high levels of algal toxin domoic acid (DA) in their viscera, which could be transferred to the meat during unskilled removal from the shell (35). The toxin causes amnesic shellfish poisoning (ASP) when consumed, which results in a persistent and apparently permanent loss of short-term memory (36). No foodborne incidents have been attributed to this source to date.

4.6 Published Microbiological Criteria

The global legislative requirement for controlling the safety (and quality) of crustacean shellfish is to apply a preventive, risk-based system based upon the seven principles of Hazard Analysis and Critical Control Point (HACCP) supported by pre-requisite or good practices programmes at all stages of the food chain, from farm to fork (37, 38, 39). Therefore any microbiological criteria need to be part of this HACCP-based system to verify that the system is under control.

A summary of microbiological criteria for crustacean shellfish is listed below. Provisions laid down concerning the use of additives would also apply to these types of products.

4.6.1 EU Legislation

Microbiological standards for cooked crustaceans and shellfish, produced or imported into the European Union (EU), are included in the Commission Regulation (EC) No. 2073/2005. Selected parts from this regulation is presented below.
4.6.1.1 Microbiological criteria applicable to the production of cooked crustaceans (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs)

4.6.1.1.1 Chapter 1 - Food safety criteria

L. monocytogenes

Prepared crustacean products fall into the category described in the regulation as “Ready-to-eat foods available to support the growth of L. monocytogenes other than those intended for infants and for special medical purposes”. During capture and preparation there is a zero tolerance for the presence of L. monocytogenes on the product. However, to take into account the issues of natural habitats being contaminated with low levels of this pathogen there is a slight loosening of the regulation at the point of sale and for the duration of the products shelf life (refer to Table 9.I, Food Category 1.2).

If the level of L. monocytogenes should be above this level then the consignment is considered to be unsatisfactory.

Salmonella

All cooked crustaceans and molluscan shellfish should be free from Salmonella at the point of sale and for the duration of their shelf life (refer to Table 9.I, Food Category 1.16). Should any sample test positive for Salmonella then the product is declared to be unsatisfactory.

4.6.1.1.2 Chapter 2 - Process hygiene criteria

Process hygiene criteria for E. coli and coagulase-positive staphylococci in shellfish and shucked products of cooked crustaceans and molluscan shellfish are laid down (refer to Table 9.II, Food Category 2.4.1).

4.6.2 International requirements

International requirements are based upon having a risk-based, preventive management system in place at all stages of the supply chain. The requirements for this preventive approach is described as follows (37):

- Fish and fishery products should be prepared in plants certified by the local competent authority. All certified plants should comply with the GHP (39);
- The fisheries industry should take responsibility, implement and maintain safety management systems based upon HACCP;
- The national competent authority is responsible for the certification of fish processing and manufacturing plants, verification of effective systems, and
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issuing of certificates of compliance for export products. This includes auditing and inspection programmes;

- National surveillance and monitoring programmes should be in place to demonstrate that all identified hazards are under control, e.g. biotoxins, and to identify potentially emerging hazards;

Within this requirement is the flexibility for countries and trading blocks to set specific standards required to deliver an equivalent requirement with those at national level. Such standards need to be science-based, especially if they are more stringent than those recommended by the Codex Alimentarius Commission.

Verification that GHP have been followed during the handling and preparation of crustacean shellfish for consumption is usually done by assessing the level of E. coli and/or coagulase-positive staphylococci in the products. Legal requirements generally require that these groups of indicator organisms are present in levels <100 cells/gram of product. Pathogenic bacteria such as Salmonella spp. should be absence is 25 grams multiplied by the number of subsamples tested.

4.6.2.1 United States

The FDA brought into force a Federal Mandate Seafood Rule in 1995 which forms the foundation for good practices required for the handling and processing of imported fish and fishery products into the U.S. The best practices include GHP and HACCP (37). All fish and fishery products produced in and imported into the U.S. after 17 December 1997 are required to demonstrate that HACCP principles were applied to their handling and processing. The USFDA has a zero tolerance for Salmonella in fish and fishery products; this includes farmed shrimp. The US FDA considers that the presence of pathogenic microorganisms in seafood is an indicator of adulteration (32).

4.7 References

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4.8 Further Reading

4.8.1 Spoilage and pathogens


4.8.2 Pathogens - general


5. CURED, SMOKED AND DRIED FISH

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5.1 Definitions

Curing is the preservation of fish by removing or displacing available water in the fish flesh to a level that discourages microbial growth, and before significant spoilage takes place. It includes the salting, drying and smoking processes, their combinations and/or variations.

Fish curing is practised worldwide, both to preserve and to create a variety of new products. The processes take anywhere from a few hours to a few days or even months, dependent on the country or the type of product.

Salting is both a method of preserving fish, where the salt replaces moisture in the fish flesh, and a preliminary operation to some smoking, drying, and marinating processes. Fish can be salted in three different ways or their combination:

*Dry salting:* The fish is headed, split and opened out flat, and salting is done by stacking the fish in layers, alternating fish layers with a layer of salt, and the extracted moisture is allowed to drain away. This can be called ‘kench’ curing and is mainly used for non-fatty white fish.

*Wet salting or brining:* The fish is immersed in a brine solution in which different salt concentrations can be used according to the process. Usually, fish is immersed for a few hours in a relatively low concentration brine, to assure a salt concentration in the fish flesh that is appropriate for flavouring purposes prior to the use of other preservation techniques, rather than for preservation itself. The immersion in concentrated brines for long periods is usually used for fatty species and for longer-term preservation, and is sometimes known as ‘pickling’.
Dry / wet salting. This is a mixture of a dry and a wet salting process. The fish is mixed with dry salt but in a watertight container. As the water is drawn out of the flesh by the salt a brine is formed, and the fish can be immersed in such brines for long periods. When the process is running in a closed system, this ‘brine’ can be collected and placed again over the fish several times, depending on the type of product. Usually, as concentrated brines are formed, it can also be named ‘pickling’ or ‘blood pickling’.

Marinating or pickling. Marinades are acidified brines, where an organic acid, usually vinegar, is used as a preservative instead of salt. Thus, the preservative effect is due to the low pH used in the process. Salt, as well as other flavourings (sugar, spices), are added to the marinade. There are some marinating processes where salt is used for the first cure and a firmer product is obtained. The products are usually consumed raw. Included in this group are some lightly preserved products where no acid is added to the marinade, such as ‘gravad’ fish.

Salt-boiling. Salted-boiled fish are the products obtained on either boiling fish in a brine, or boiling it with dry salt and water, under normal pressure, without other preservation methods applied after boiling. The principle of preservation of the first method is the inactivation of enzymes and killing the non-spore-forming organisms, thus delaying spoilage for a few days. Longer shelf life is achieved in the products when salt is added, prior to or during the boiling process, thus reducing the water activity of the product.

Drying is the preservation of fish by removing the moisture content of the fish flesh by evaporation, thus making the product unsuitable for microbial growth. Food products can be dried in three different ways:

Air or contact drying. Heat is transferred to fish from heated air or a heated surface, and the air movement removes the vapour. This is the common method of drying fish. Traditionally, this was done naturally by exposure of the fish to the air.

Vacuum drying. The product is dried by contact with heated surfaces or by radiation; evaporated water is removed by vacuum pump. It is not commonly used for fish products.

Freeze drying. Fish in contact with refrigerated plates freeze, the ice sublimes and the vapour is removed from the fish by vacuum pumps. After the freeze-drying process, the product rehydrates quickly but does not retain reabsorbed moisture on cooking. It has found limited application with fish.

Smoking is an ancient process for preserving and flavouring food. The original principle of preservation was due to a combination of lowered water activity of the product and the uptake by the product of bactericidal and antioxidant components.
of smoke. The secondary objective is to impart sensory characteristics such as smoke colour and smoke flavour to the product. With the exception of some Asian countries, smoking is now used mainly to give the fish a characteristic flavour, and the preservative effect is only slight.

*Smoked fish* is prepared by subjecting the fish to the direct action of smoke from wood or wood sawdust burning. A dry or wet salting step is always used in the process before exposure to the wood or sawdust smoke.

*Smoked-flavoured fish.* After the salting step, fish can be smoked by means other than the direct action of smoke, such as injection or dipping in a solution of liquid smoke, which impart the flavour of smoke to fish.

*Liquid smoke* is a natural aqueous condensate of wood smoke that has been aged, and filtered to remove tars and particulate matter. It is commonly used in place of traditional smoke. It can be injected into the product, or added to the brine with other flavourings.

*Cold-smoking.* In this process, the fish is subjected to heat for a period that does not coagulate protein. Smoking can be either traditional, by exposure to the wood or sawdust smoke, or by means of smoke-flavouring processes, or liquid smoke. Usually, temperatures below 30 °C are used.

*Hot-smoking.* In this process, the fish is subjected to heat for a sufficient period of time to coagulate protein throughout the fish. It can be smoked either traditionally, by exposure to the wood or sawdust smoke, or by means of smoke-flavouring processes with liquid smoke. Usually, process temperatures are over 60 °C.

*Clear smoke.* Is an illegal method (in the European Union (EU)) employed to treat tuna to ensure a bright red colour is maintained during storage and transport. The process was designed so that the smoke is filtered to remove all smoke particles, leaving only elevated levels of carbon monoxide, which irreversibly combine with the haemoglobin in the tissue to maintain the red colour.

5.2 **Initial Microflora**

The initial microflora are those of the raw fish from which the products are made. However, the salt used in the processes may contain halophilic bacteria, spore-forming bacteria or osmophilic moulds. Also, the sugar used in some products may contain yeasts and moulds as well as spore-forming bacteria. The different spices used in the immersion brines are also a potential source of microorganisms, including pathogens. The smoke itself may contain spores of moulds.
5.3 Processing and its Effects on the Microflora

Owing to the wide variety of products, processes and producers’ private recipes, it is impossible to establish standardised processing stages in the curing of fish. Therefore, only the most common processes can be described here.

When working with salting, smoking or marinating processes, all using salting steps, it is important to understand the concept of salt concentration, or salt in the water phase, and salt content.

Salt concentration means the percentage of salt in the water contained in the fish. This should be distinguished from the salt content, which is the percentage of salt in the whole weight of fish that is water plus protein plus fat, etc. Salt in the water phase is the amount of salt compared with the total amount of water and salt in the fish. Salt in the water phase links the two conditions of moisture and saltiness.

\[
\text{% Salt in the water phase = } \frac{\text{% salt content}}{\text{% salt content + % moisture}} \times 100
\]

Another important point to remember when wet salting is used is the concentration or strength of a brine. Different brine strengths are required according to the curing processes and/or the type of product. The strength of the solution is described as degrees of saturation (brineometer degrees). A 100º brine solution means that the solution is saturated and no more salt can be dissolved; a 0º brine saturation means pure water. The dissolution of salt in water is dependent on the temperature. Thus, it is important to state the temperature. At 18 ºC a 100º brine is produced when the solution contains 26.4% salt (Table 5.1). When a 100º brine is used, sometimes the salt crystallises onto the surface of the fish; a 90º or above brine (237.6 g of salt or more per litre of brine) may cause salt burn in this way. When a milder brine, about 50º (132.0 g of salt per litre of brine), is employed, it causes swelling because the fish absorbs water from the salt solution (1). For smoking salmon, the fish is usually salted with a 70 - 80º brine (184.8 - 211.2 g of salt per litre of brine).

**TABLE 5.1**

Brine strength at 18 ºC (after Bannerman (2))

<table>
<thead>
<tr>
<th>Brineometer degrees</th>
<th>Weight of salt (g/litre of brine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>26.4</td>
</tr>
<tr>
<td>20</td>
<td>52.8</td>
</tr>
<tr>
<td>30</td>
<td>79.2</td>
</tr>
<tr>
<td>40</td>
<td>105.6</td>
</tr>
<tr>
<td>50</td>
<td>132.0</td>
</tr>
<tr>
<td>60</td>
<td>158.4</td>
</tr>
<tr>
<td>70</td>
<td>184.8</td>
</tr>
<tr>
<td>80</td>
<td>211.2</td>
</tr>
<tr>
<td>90</td>
<td>237.6</td>
</tr>
<tr>
<td>100</td>
<td>264.0</td>
</tr>
</tbody>
</table>
5.3.1 Salting

Salting of fish for preservation purposes has been used since pre-history. Salting can be done in a number of different ways. Usually it is followed by other preservation techniques such as drying and/or smoking.

The salt used should be of food-grade quality, low in calcium and magnesium, and essentially free from iron and copper (3). However, if very pure sodium chloride is used for curing purposes, it tends to lead to a slight yellowing and softening of the product. When pure salt is used, addition of levels of 0.15 - 0.35% and 0.15% calcium and magnesium, respectively, are recommended to help make the product whiter, but the concentration of copper and iron must be kept under 0.1 ppm and 10 ppm, respectively (1). Also, calcium and magnesium ions of impure salts can form a barrier to the passage of sodium ions from the surface to the bulk of the fish, and such delays, mainly in tropical conditions, can lead to the decomposition of the centre of thick muscles before it reaches the level of salt concentration sufficient to prevent spoilage. This condition is known as ‘putty fish’ (4).

‘Salt burn’ might occur when the surface of the fish dries out too quickly as a result of the small grain size of the salt, which consequently inhibits the diffusion of the water from the bulk of the product to the surface, and a ‘putty’ fish product is also obtained (1).

5.3.1.1 Dry salting / Kench curing

Usually, lean white fish is used for this purpose. This is because fatty fish turn rancid quickly, and salt can speed this oxidation process. The most common fish used in the northern hemisphere is cod (Gadus morhua), caught in North Atlantic waters. For a light salting, the fish is mixed with one part of salt per eight parts fish, and for a heavy salting a ratio of 1:3 salt:fish is used (1). The fish is then stacked in piles of about 2 metres and the water is allowed to drain away; the fish is restacked with fresh salt after about a week. It takes about 3 weeks until proper salt penetration and moisture loss are achieved. A ‘green cured’ product is obtained when the water content has been reduced to about two-thirds of the initial amount, and the salt has penetrated throughout the fish (3). See Figure 5.1.

‘Green cured’ split cod with about 60% of the backbone removed, having a short section at the end of the tail, can be dried further to produce ‘klipfish’ or ‘bacalão’, for which there is a high demand in Mediterranean countries.

It is common for the dried salted cod in the Mediterranean countries to be made using solar salts, which include calcium and magnesium. The calcium and magnesium impurities, however, are likely to cause hydroscopicity of the product and, when the air humidity is high, outgrowth of halophilic bacteria may occur, producing characteristic pink patches. On the other hand, when rock salt is used the ‘dun’ patches caused by moulds are prevalent (1).
Dry salting can also be used for small species without the need for opening the fish flat. Only small fish can be salted whole. For large fish it is advisable to split it before salting.

The greatest barriers to salt penetration are the skin and scales, and the uptake of salt is dependent on the type of fish (fatty or lean) as well as its presentation (whole, eviscerated and/or split). Where fish are salted in their whole form, it appears that water activities much less than 0.90 are rarely achieved, so that the risk of spoilage is high. Food-poisoning organisms, such as *Staphylococcus aureus* could survive and grow at such water activities in the product, although toxin production would be unlikely (4).

![Diagram of processing of dried salted cod](image)

Fig. 5.1. Processing of dried salted cod

### 5.3.1.2 Wet salting or brining

As referred to above, when a light brine is used, the process is mainly for flavouring purposes prior to the use of other preservation processes, such as
CURED, SMOKED AND DRIED FISH

smoking. The brining step for smoking purposes also helps the product to develop a desirable glossy appearance.

For preservation purposes, a saturated brine is used. The main objective of the brining method of salting fish is to limit air penetration during processing; thus, limiting the amount of rancidity that can develop. This is why it is the preferred method for fatty fish. Fresh sardines, headed and gutted, can be cured in a saturated brine solution for 6 days and, after being drained and pressed at 465 kg/m² for 15 hours; they can be stored at 28 °C, with a shelf life of about 4 weeks (water activity \( a_w \) about 0.78) (1).

5.3.1.3 Dry / wet salting

In this process, the fish is dry-salted, but as it is placed in containers, the liquid that comes out of the fish forms a brine, and can be collected and replaced again over the fish. There are several variations of this process. For example, if split fish is dry-salted, the water that comes out of the fish forms a brine in which the fish stays immersed for 2 - 3 days. It is then taken out and dried in the sun or in kilns until about 35% moisture in the product is obtained; the product obtained is called gaspé-cured (5). See Table 5.11.

The fish are kept immersed in their own brine near saturation point for several days, with pressure being applied to the top of the container to help the brine to drain away, the brine is then replaced over the fish, and a red burgundy colour will develop in the product. Here, the fish enzymes play an important role by maturing or ripening the product. Anchovies are the main fish used for these purposes, but mackerel, sardines, herrings and other fatty fish can be used. Histamine formation is of importance when using scombroid and some clupeidean species. Histamine-forming bacteria do not generally grow at water activities below 0.90 (1).

<table>
<thead>
<tr>
<th>Moisture (%)</th>
<th>1.5</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
<th>3.5</th>
<th>4.0</th>
<th>4.5</th>
</tr>
</thead>
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<tr>
<td>40</td>
<td>3.61</td>
<td>4.76</td>
<td>5.88</td>
<td>6.98</td>
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<td>5.26</td>
<td>6.25</td>
<td>7.22</td>
<td>8.16</td>
<td>9.09</td>
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<tr>
<td>50</td>
<td>2.91</td>
<td>3.85</td>
<td>4.76</td>
<td>5.66</td>
<td>6.54</td>
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<td>5.98</td>
<td>6.78</td>
<td>7.56</td>
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<tr>
<td>60</td>
<td>2.44</td>
<td>3.23</td>
<td>4.00</td>
<td>4.76</td>
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<td>3.23</td>
<td>3.74</td>
<td>4.26</td>
<td>4.76</td>
</tr>
</tbody>
</table>
5.3.2 Salt-boiling

Fish can be boiled after being dry-salted in a watertight container and heated for at least 1 hour, or can be boiled by being placed in a basket, which is dipped into a boiling brine for 10 - 30 minutes. The first process can give a longer shelf life, up to 1 - 2 months, because of the salt content of the product, whereas the latter method preserves the fish for only a few days. As referred to above, only the non-spore-forming organisms are eliminated in the process and, even if the product is hermetically sealed, outgrowth of bacterial spores after processing may occur (4). Other preservation techniques can be used for extending the shelf life of these products.

Traditional jellied eels are a product where pieces of fish are cooked in boiling water, containing 1 kg of salt for every 25 kg of fish, until the flesh is soft. The cooked portions are poured into bowls containing gelatin dissolved in water (10% solution) and sometimes a small amount of vinegar. Once cooled, they are packed for consumption within 2 weeks at chill temperature (6).

The Indonesian ‘pindang’ is made by boiling the fish in a saturated salt solution and it is sold as is, and lasts for 3 - 6 days without refrigeration (1). However, the occasional implication of pindang in cases of foodborne illness, and even death, underlines the need for careful control (4). Another salt-boiled Indonesian product, ‘ikan kaju’, has a shelf life of several years, but it has further smoking, drying and salt-boiling steps until a hard translucent block is obtained (4).

5.3.3 Marinating / pickling

Fish can be brined in solutions based on organic acid instead of salt. The product is usually consumed raw with the high strength acid solution softening the bones enough for them to be eaten. In a second step, flavouring (sugar, salt, and spices) is added to the brine. A much firmer product is obtained when the fish is salted prior to acidification. Usually, herring or other fatty species are used. The fish can be soaked in a high concentration of vinegar (about 4 - 7%) for as long as 3 weeks. Afterwards, it can be repacked in milder brine (1 - 2% acetic acid and 2 - 4% of salt) for distribution and sale (1). Another variation uses sour cream instead of the second brine step.

Herring can also be cured for several days in an 80 to 90º brine with 2.5% 120-grain distilled vinegar, then packed in barrels with a 70º brine. After this, they can be cut and cured for 3 days in a solution with 3% white distilled vinegar, and 6% salt, and packed (3).

Ceviche, a Peruvian product, is made of different varieties of fish, placed in a marinade of lime juice, usually flavoured with onions and hot chillies. The lime juice plays the role of the vinegar, giving a cooked appearance to the product.

All these products are ‘semi-preserves’. They will have a salt content >6% (w/w) in the water phase, or a pH <5 and they are stabilised for a limited period, usually requiring refrigeration below 10 °C for longer-term storage. There is epidemiological evidence that these products have been the cause of some
foodborne diseases related to the presence of biotoxins, bacterial toxins and parasites (7).

At pH levels below 4.5, marinated products will keep for several months at a temperature of 4 °C. Some bacteria and enzymes are still active in the presence of acid and salt, and the flesh will break down. This bacterial and enzymic action can produce a typical flavour desirable in some semi-preserves in which salt or a mixture of salt and sugar is used to preserve the fish (8). There are several products named marinades (matjes, gravad fish, etc.) where no acid is added to the process. These can be considered as 'lightly preserved' products with a salt content <6% sodium chloride (w/w) in the water phase and low acid content (pH >5.0) (7). They are not cooked prior to consumption and they have a limited shelf life even at refrigeration temperatures.

Foodborne pathogens, such as *Listeria monocytogenes* and *Clostridium botulinum*, are likely to survive in these mild-processed products.

Matjes are made of fat young herring, gutted, mixed with salt, then mild-cured with special seasonings and packed in barrels with 80 º brine (5). Gravad fish is a Scandinavian product made of salmon fillets, which are placed in a mixture of salt, sugar and spices. Fresh dill is placed between the layers of fish, and the stacks are placed in the refrigerator, under a light pressure. It takes about 2 days to marinate completely, depending on the thickness, the temperature and the amount of pressure applied (5). It is important to assure the quality of the raw material, it being preferable to use frozen salmon or to freeze the product at the end of the process to avoid parasite survival.

### 5.3.4 Air drying

This is probably the oldest method of fish preservation. Usually, the fish is dried after salting or brining, but sometimes it is dried without prior salting. The most common method used for fish products is the air-drying process, by using either solar heaters or mechanical dryers. In the first case, air flows naturally, while in the latter case there is a provision for fans or other means of mechanically moving the air (1).

Stockfish (dressed, split and dried gadoid species) are traditionally made in cold climates. They are an important dried product, still made without prior salting on a large scale. In Scandinavian countries, the fish is dried naturally in a relatively cold environment, and a fine product is obtained. Stockfish is extensively used in Portugal, Italy and Spain (5). In some coastal areas of Portugal, some fish species such as moray eel and varieties of dog fish, are sun-dried and, after frying, used as an appetiser. Dried octopus is also a very common dish in the interior of the country. Stockfish products have a golden brown colour and are very hard. Provided the product is kept in dry and fairly cool conditions, it will last for many years (5). Shark pectoral fins, a very fine delicacy for Oriental food lovers, are generally air-dried at sea. Other products, such as squid, jellyfish, sea cucumbers and seaweeds, are commonly dried in Asian countries. Dried shrimps are widely used in Asia, South America and the Caribbean.
When the fish is sun-dried in tropical conditions, the quality is more difficult to control. It is important to balance the drying rates and quality/safety issues. The amount of moisture transfer from the fish to the air is dependent on the relative humidity - the lower the humidity, the better the moisture transfer. However, if the rate of water removal is too high in comparison with the diffusion rate, the skin will form a hard case (case-hardening), making a water barrier and consequently, the diffusion of the bulk water to the surface of the fish is blocked. Thus, spoilage and possibly *C. botulinum* growth might occur in the bulk of the product. To prevent this, the protein must be set or denatured with low-temperature drying before applying higher temperatures. In conclusion, the fish must be dried slowly enough to avoid case-hardening, but fast enough to avoid spoilage caused by bacterial and enzymic activities (1).

A highly demanded dried fish product in Mediterranean countries is dried salted cod. After dry salting, the ‘green cured’ cod is rinsed or washed with water and then hung to dry either by the sun and breeze or, more commonly nowadays, by warm air circulating in a drying chamber. The product is dried to a final water content of about 25 to 38% (3). Dried salted products must have a water activity below 0.75 (1). When no more water can be removed from the fish, the product can be held for months at room temperature. The fish must be stored in reasonably dry conditions to avoid moisture absorption, which leads to mould growth (aflatoxins, toxic secondary metabolites of the moulds *Aspergillus flavus* and *Aspergillus parasiticus* are not produced at aw <0.83 (9)). They must be soaked in water, skin up, and the water must be changed several times before cooking.

Owing to restriction of fishing areas for Spanish and Portuguese vessels since the 1970s, dried salted cod (bacalão) is now mainly produced in Norway, Iceland and Canada, and it is exported to Portugal, Spain, France, Italy and South America. Portuguese and Spanish fisheries cannot satisfy their own countries’ demand for cod.

Whilst the production of dried fish products is tightly controlled in industrialised countries, and has the primary objective of creating a product with specific taste and textural characteristics, there are many regions of the world where air drying is the only means of ensuring that a day’s catch can be preserved for later use, typically in stews and soups. In many such artisanal fisheries the drying is simply conducted by spreading the fish on the beach, tarpaulin, or wooden drying rack to dry in the sun. Although controls are in place to ensure that products processed in such relatively uncontrolled conditions do not enter the supply chain, there is an increasing demand from people that have emigrated to industrialised nations for traditional products, and increasing amounts of such products are making their way into the market place.

Traditional processing conditions provide ample opportunity for product contamination from a wide range of hazards and sources, some of which are listed in Table 5.III. Whilst the low aw of the final product will inhibit microbial growth, neither it, nor subsequent cooking operations will affect the presence of toxins (e.g. *Staph. aureus*) or spores (e.g. *C. botulinum*), that may have contaminated the product during the drying process.
CURED, SMOKED AND DRIED FISH

TABLE 5.III
Hazards and sources of contamination associated with air drying

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Source</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteria</td>
<td>Sewage</td>
<td>Human, Insect</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Reptile</td>
<td>Birds, Humans</td>
</tr>
<tr>
<td>Staph. aureus (toxin)</td>
<td>Humans</td>
<td>Human</td>
</tr>
<tr>
<td>C. botulinum (spores)</td>
<td>Environment</td>
<td></td>
</tr>
<tr>
<td>Pesticides / Chemicals</td>
<td>Airborne</td>
<td>Applied to combat insect infestation</td>
</tr>
<tr>
<td>Physical Glass / sand etc.</td>
<td>Environment</td>
<td></td>
</tr>
</tbody>
</table>

5.3.5 Smoking

Smoking today, as referred to above, is mainly a process for flavouring fish rather than a preservation technique. The production of smoke by wood or sawdust burning can be done in a mechanical kiln or in traditional chimney kilns. In the mechanical kiln, temperature and humidity can be automatically controlled and the process is faster because the directed air movement draws the smoke through the fish. It can be used for cold or hot smoking. The quality of the smoke is related to the type of wood used. Aroma and flavour are a blend of smoke components (3). Hardwoods are considered better because of the milder flavour they impart to the food. However, softwood colours the product quickly. Blends of various woods can be formulated to produce sawdust with specific flavours. It has been reported that the smoke from mahogany, white mangrove and abura has an inhibitory effect on some microorganisms, such as *Escherichia coli*, *Staph. aureus* and *Saccharomyces cerevisiae* (10).

Preventing the occurrence of carcinogens during smoking has been an issue for the past several years. Maintaining the temperatures of pyrolysis between 425 and 200 °C, using electrostatic filtration of the smoke, and the use of smoke generated by superheated steam or liquid smoke, are some of the ways of reducing the polycyclic aromatic hydrocarbon (PAH) compounds (10).

There are many fish species that are used for smoking purposes - salmon, trout, herring, cod, haddock, eel, mackerel, sprats, tuna, swordfish, cod roes, oysters, clams, mussels and many others from tropical countries. The smoking procedures vary widely, making full description impossible here.

Top-quality fish must always be used. Proper cleaning and preparation prior to smoking and washing of the fresh or frozen fish by water spray or a continuous
water flow system with chlorinated water (25 - 50 ppm) should always be done just before process initiation (3). If frozen fish is used, thawing must be done slowly for 12 - 18 hours at 4 °C. When frozen/thawed fish is used, it will take up salt twice as fast as fresh fish (10). Whether the product is prepared headed, gutted or filleted, skin off or on, the following step is salting. The fish can be dry-salted using a weight of salt 5 - 10% of the weight of the fish, covering the fish surface completely (10). The salting period is dependent on the size of the fish and its presentation (whole, filleted, skin on or off); the excess salt is removed with water. Sugar can be added together with the salt; it will reduce the salty flavour.

If wet salting is chosen, 70 – 80 º brine is commonly used, but this concentration will again depend on the type of product and the length of the brining stage. Also, low temperatures will slow the uptake of salt. Brining at 10 - 15 °C is recommended (10). Wet salting or brining is preferable to dry-salting when smoking fillets because, owing to the elution of soluble proteins, an attractive glossy pellicle on the fish fillet surface is achieved after draining, which is important for the appearance of these products (4). However, as it retains more moisture, it will double the drying time in the kiln to get the same total weight loss as for dry-salted fillets (11). Sugar, spices, permitted colourings or smoke flavours can also be added to the product in the brining step.

All the smoked products, whether cold- or hot-smoked, after being smoked need to be chilled immediately to 4 °C before being packed, or quick-frozen and stored at -18 °C (2). Depending on the type of product, they can be vacuum packaged, shrink packaged, canned or bottled, or packed in retortable foil pouches, wooden boxes or master cartons (10).

5.3.5.1 Cold-smoking

The major difference between cold- and hot-smoking is the temperature of the process. In cold-smoking, temperatures must be kept under 30 °C throughout the process. This difference is crucial for safety issues because the flesh is not cooked. Thus, all cold-smoked products should be cooked before consumption; however, the highly desirable cold-smoked salmon, as well as many other products prepared in the same way, such as smoked salmon-trout, tuna and swordfish, are not cooked prior to consumption. Some herring products, smoked cod fillets and finnan haddock are usually cooked before consumption. Cod roes, usually dry-salted and cold-smoked, are boiled before smoking (12).

The maintenance of temperatures below 30 °C avoids protein coagulation, and the cold smoke is denser, thus allowing the deposit of smoke on the fish surface, giving the product the desired appearance and flavour (4). Drying should be minimal at the start, but it will increase towards the end of the process (10). The length of the smoking process for such products is much greater than for the hot-smoked fish, but a ‘safety’ (i.e. pasteurisation) temperature is not achieved in any stage of the process. Thus, temperatures and times used in processing cold-smoked fish are very favourable for the proliferation of food-spoilage and food-poisoning microorganisms.
During cold-smoking, there is no point in the process that can fully assure the absence of *L. monocytogenes*. Neither the smoking temperature nor the salt content is enough to kill the organism. Furthermore, refrigerated storage and vacuum packaging still allow its growth (12). Although other human pathogens that can grow at refrigeration temperature, such as *Yersinia enterocolitica*, can be isolated from seafood, they are more sensitive to sodium chloride and unlikely to grow in smoked products (13). If the refrigeration chain is maintained, risk from the presence of *Bacillus cereus* and *Clostridium perfringens* can also be excluded as potential hazards because of the relatively high temperatures they require to grow to, in order to produce toxin, and their sensitivity to salt (13). Pathogenic vibrios, typically mesophilic, are of concern when smoking fish from tropical waters (13).

During smoking, an initial humidity of 70% seems to be preferable because it is a good compromise between maximum smoke adsorption and minimum case-hardening (1). Afterwards, the products are cooled, stored at 4 °C and packed.

To avoid growth of non-proteolytic *C. botulinum* type E the product must have at least 3% salt in the water phase and be stored below 5 °C, but the proteolytic types A and B, if present, can grow with salt levels of 10% in the water phase, which underlines the danger of storage at abuse temperatures of >10 °C (13).

Smoked salmon fillets are usually vacuum packed whole or after slicing. Vacuum packing extends the shelf life of the lightly smoked products by suppressing the growth of other bacteria and moulds, thus allowing any psychrotrophic *C. botulinum* type E present an even longer time than before to grow and produce toxin (14). Vacuum packing is recommended only for cold-smoked salmon, gravad salmon (also called gravad lax or gravlax), and hot-smoked salmon (kippered) (3). Smoked products can be also packed under controlled-atmosphere packaging, and the recommended gas mixtures are 60% carbon dioxide and 40% nitrogen (15). See Figure 5.2.

### 5.3.5.2 Hot-smoking

Hot-smoking must use temperatures between 70 and 80 °C at any stage of the process to ensure protein coagulation throughout the product. The protein must be set or denatured with lower temperatures before the application of higher temperatures to avoid case-hardening. A typical temperature set is as follows: firstly, a drying period at 30 °C; secondly, a partial cooking period at 50 °C; thirdly, a final cooking period at 80 °C (12). Length of the smoking process at each stage will depend on the species and the type of product.

Hot-smoked products require a longer cooling stage, but they should be cooled to a temperature of 10 °C within 3 hours, further cooled to 3 °C within 12 hours and then packed. This temperature should be maintained during storage and distribution (3). The exception is vacuum packed fish, as, if they are taken from a chill room at 3 °C and packed, condensation will occur inside the pack. These products should be vacuum packed at room temperature. On the other hand, if a
product is packed too warm without being cooled to room temperature, it will have a reduced shelf life, and moulds will grow on the product (12).

Fig. 5.2. Process of cold-smoked sliced salmon
There are several species that are usually hot-smoked and the process can vary markedly. Boiled or steamed clams, oysters and mussels are usually wet-salted in 50º brine for 5 minutes and hot-smoked at 80 °C for less than 1 hour (12). Eels, a very expensive and fashionable smoked product in the Netherlands, are usually hot-smoked after being immersed in 80º brine or dry-salted (6). Mackerel, chilled or freeze-thawed, are usually 80º brined for 5 - 6 hours and smoked for 3 - 3½ hours using the typical temperature set for hot-smoking referred to above (12).

Hot-smoked products made from white fish will usually keep better than those from fatty fish. When refrigerated at 3 °C, fatty fish will remain in good condition for 6 days, white fish about 8 days. They will keep well frozen; they can be stored for 6 months at -30 ºC and for even longer when frozen in vacuum packs (12). Vacuum packaging retards the onset of rancidity when fatty fish is used, but seems, apart from the improved appearance, to have no further advantage over other packing methods for other fish (12).

A type of dried hot smoked tuna called masmeen or masmin is produced in the Lakshadweep islands of the Indian Ocean. Masmeen is usually prepared from bonito tuna by eviscerating and filleting the fish, moulding it into long thick sections, cooking in brine, then hot smoking and finally sun drying it.

Katsuobushi, similar to masmeen, is a boiled smoked fish product that is used in Japanese cooking to impart flavour, including ‘umami’ (16, 17, 18). The production method is similar to masmeen, but mechanical drying is used instead of sun drying. The product is often allowed to go mouldy by incubating it in a chamber containing a natural fungal flora, including Eurotium repens and Eurotium rubrum, and many closely related Aspergilli (Aspergillus ochraceus, Aspergillus oryzae, Aspergillus ostianus, Aspergillus tamarii) and several Penicillium spp. (Penicillum cyclopium and Penicillium puterellii) (19). Growth of these moulds on the tuna results in the extraction of moisture, break down of fats and the moderation of smoke flavour, resulting in a characteristic flavour (20, 21, 22, 23). The product is removed from the drying cycle, rewetted and remoulded to give the desired shape and consistency.

Hon kare bushi is a form of Katsuobushi from which shavings are cut to make aromatic stocks for use in Japanese dishes (24).

In terms of safety, hot-smoked fish can be classified as a ‘heat-treated or pasteurised’ product (7). They are generally not cooked prior to consumption, which means that any bacterial contamination and growth after the heat treatment is a serious hazard that must be controlled by Good Manufacturing Practices (GMP) and factory hygiene/sanitation after the heat treatment (7).

5.3.5.3 Liquid smoke

Smoke-flavoured fish represents a small percentage of the total smoked fish industry output. Natural smoke extracts, synthetic smoke flavours, and substances unconnected with smoke but with a smoky flavour or smell, such as yeast derivatives, are used. The composition of commercial liquid smoke can vary widely depending on the method of manufacture. The classical liquid smoke is
smoke condensate that is dissolved in water or oil, or smoke extracts in organic solvents. In experiments comparing traditionally smoked fish with liquid smoke, it has been reported that the liquid smoke-treated product has less pronounced aroma and flavour (25). Smoke-flavoured fish processed using liquid smoke can be prepared by including liquid smoke in the brine, applying it as a dip after brining, injecting it into the fish flesh, or applying it as an atomised spray within a modern automatic smoke kiln. According to some recent research, spraying is preferable because it is easiest to control flavour and acceptable with this method (3).

Liquid smoke in combination with sodium chloride in a hot-process smoke flavoured fish was found to be effective in preventing growth and toxin production associated with the growth of *C. botulinum* types A and E spores in several fish species that were stored at 25 °C for 7 and 14 days (25). When liquid smoke was used, the salt level could effectively be reduced from 4.6 to 2.8% and still provide protection for 7 days (25). The synergistic effect of liquid smoke with other preservatives could reduce the amount of salt in the product when light diets are necessary whilst still meeting the necessary safety criteria (26). Liquid smoke was considered to provide advantages of safety, consistency of flavour and colour, nutritional neutrality and antioxidant activity (27). There are two groups of chemicals of concern in smoke: polycyclic aromatic hydrocarbons (PAHs) and nitrosamines, both of which are considered potential carcinogens. It has been shown that liquid smoke can reduce the formation of PAHs (3). The European Scientific Committee for Food, established in its guidelines that smoke flavourings added to the food product must assure a level lower than 0.03 μg/kg of benzopyrene, and less than 0.06 μg/kg of benzoanthracene in the final foodstuff (28).

### 5.3.5.4 Clear smoke treatments

The “Clear” or “Tasteless” smoking process is a processing technique whereby smoke is generated as in a normal food smoking process but is filtered to remove the characteristic smoke flavours before application on to otherwise untreated (uncured) tuna, so as to impart an unnatural red colouration to the flesh. The process relies on the generation of the smoke to produce elevated levels of carbon monoxide (CO) which interact with haemoglobin in the muscle to form the very stable bright pink/red carboxymyoglobin complex. Whilst neither the CO or the carboxymyoglobin complex are harmful in themselves, the result of this process is seen as a manipulation of the natural degradation of tuna colour that hides the true nature of the product quality and therefore has been made illegal in the EU.

### 5.4 Spoilage

Brining, drying, smoking, pickling and marinating fish were originally used to lengthen the short shelf life of fresh fish. Fresh fish spoilage may be measured by
grading schemes or quality indices, which may also be produced to describe the quality attributes and their loss over time for processed fish. Within the limits of safety, a product may become undesirable for consumption as a result of changes in its sensory characteristics. Once these changes are defined, the shelf life may be estimated and investigation made into the causes of the loss of sensory quality. For some products, spoilage is due to endogenous microorganisms and enzymes of the fish; for others, chemical oxidation; and, for some, contaminant bacteria from other materials used to produce the product.

The use of drying and/or salt to lower the water activity, and of smoke to add chemical preservatives, limits the types of microorganism capable of growing; also, the production process will modify the microbial flora present on the product; therefore, both of these will affect the spoilage patterns. Spoilage needs to be carefully defined for each product.

Endogenous marine fish bacteria prefer to grow in the presence of salt (marine concentration approximately 2.3%), and high concentrations are usually required to inhibit their growth fully. When acid marinating or smoking, the consequent reduction in the pH will help to inhibit spoilage organisms; otherwise, a very dry product is required to produce ‘shelf-stability’, or extended refrigerated storage. Even saturated brines allow some halophilic organisms to grow.

Most marinated and smoked fish products are first brined or salt-pickled. Brining, pickling and dry-salting all introduce bacteria to the fish, which are found in the salt. Salts used for brining have been found to carry a microbial load of between 10 and 1000 organisms per gram of wet salt; solar salts investigated carried the highest load and included halophilic organisms as a result of their production method (open-air evaporation of sea water); rock and manufactured salts did not usually contain halophilic bacteria (29).

When heavy brining or salting is important for product shelf life, these halophilic organisms may grow and cause spoilage. This is not usually the case for smoked fish as the residual salt concentration is not high enough to inhibit all the non-halophiles; however, the pickle and dry-salting preservation processes require further processing for significant shelf life increases (e.g. marination or drying), or their products require refrigerated storage.

In salts, the bulk of the microflora comprises *Bacillus* spp., *Micrococcus* spp., and coryneform bacteria. Once placed into brines, these bacteria tend to continue to dominate the flora in the liquid, but, as the fish are added, the fish flora are transferred to the brine mix. Some brines are re-used over a period of days and a load of 10,000 cfu/ml of brine may build up, mainly comprising corynebacteria and Gram-negative bacteria from the fish and water (29). The microflora attached to the fish is relatively unaffected by light brines used before smoking. Stronger and longer brining has an effect like that of pickling.

Pickling and/or the anchovy process uses solid salt extraction of fish fluids to form a brine solution (cure), a process similar in microbiological effect to that of saturated brining. Often, the fish are first cleaned (known as ‘rousing’) by a salt rub to remove any outer slime. Usually, the fish are cut and splayed to allow greater surface contact with the salt. Upon salt packing, contact with the bacteria
in the salt is made and the flora will develop accordingly in the blood pickle. Only halophilic microbes may grow, as the water activity of saturated brine solutions is low enough to inhibit most bacteria, yeasts and fungi.

Spoilage of dry-salted and pickled fish is mainly due to mesophilic bacteria (those that grow with optimum temperatures around human body temperature and tend to grow poorly or not at all at refrigeration temperatures). Therefore, spoilage of these pickled and dried fish in refrigerated storage tends to be due to chemical changes (textural changes due to proteolysis, protein breakdown, rancidity due to lipolysis, or fat breakdown) caused by enzymes rather than by microbial growth. The microbiology of heavily salted products is dominated by Gram-positive, halotolerant or halophilic micrococci, yeasts, spore-forming bacteria, lactic acid bacteria (LAB) and moulds. A number of spoilage organisms can grow and produce conditions such as ‘slimming’, ‘pink’ and ‘dun’.

‘Slimming’ or ‘putty’ fish is a condition that occurs in poorly or insufficiently salted cod (e.g. gaspè), although it may occur in other salted fish. Insufficient salt penetration into the flesh leaves areas where Gram-negative rod-shaped bacteria and micrococci grow and cause a putrefactive spoilage. This condition is often associated with case-hardening.

‘Pink’ describes a condition associated with surface growth of the pigmented halophilic bacteria *Halococcus* spp. and *Halobacterium* spp. The bacteria form coloured colonies on the surface of salted fish prior to drying, or after drying if the fish is stored in excessive humidity. The metabolism of the organisms will produce off-odours, including hydrogen sulphide (26). The bacteria are osmosensitive and will lyse in water. At the pre-drying stage, it is possible to wash off the infected parts, if not too contaminated, and still produce a reasonable dried product. Bacteria from these two genera also grow sufficiently in brine and even salt to cause pink discolouration; the best way of controlling the organisms is to refrigerate the fish, as little if any growth will occur below 5 °C on salted fish (29).

Unlike ‘pink’, ‘dun’ is not characterised by the production of offensive odours. The condition describes the growth of the halophilic moulds (*Wallemia sebi*, (syn., *Sporendonema sebi*)), which, like the ‘pink’ organisms, may also be found in the salt used in production. Their growth produces noticeable spoilage of the fish appearance but is also controlled by refrigeration. *W. sebi* is considered to be the main spoilage agent of dried fish produced in temperate regions (30).

*Basipetospora halophila* (syn *Oospora halophila*) and *Polypaecilum pisce* are two white halophilic moulds that are associated with spoilage of salted fish (31, 32, 33). Studies conducted on dried and salted fish from Indonesia (31, 34) have shown that *P. pisce* was the main fungus isolated; it was found in about 50% of samples examined, sometimes covering the whole of the fish with a whitish, powdery growth. *Eurotium amstelodami*, *E. repens* and *E. rubrum* are also common isolates from dried fish, together with a range of Aspergilli, including *A. flavus*, *A. niger*, *Aspergillus sydowii*, *Aspergillus wentii*, and *Aspergillus penicilloides*, though only the latter two species were considered likely to have grown on the fish (32). Several *Penicillium* spp. were also isolated, most notably *Penicillium citrinum* and *Penicillium thomii*. *B. halophilica* was isolated
infrequently at low levels; it was, however, able to grow on dried salted fish, with a lower $a_w$ limit for growth of 0.747, around the water activity of saturated sodium chloride.

In Vietnam, *Aspergillus* spp., including *A. flavus*, *Aspergillus clavatus* and *Aspergillus niger*, has been isolated from dried fish (35).

Technical studies demonstrate the ability of many of the xerophiles above to grow at typical water activities seen in dried salted fish (32, 33, 36).

Smoked fish products tend to have a light brine treatment, which does not create the salty conditions required for spoilage by those halophilic microorganisms. The brining to 3 - 5% sodium chloride in the water phase will inhibit some bacteria, but, as already stated, most marine bacteria are moderately halophilic and are not effectively inhibited by these low salt concentrations.

Fish processed by cold-smoking, or any smoking process where temperatures in the interior of the fish do not reach $>50{\degree}C$ for a significant period of time, will have a different residual post-smoking flora from 'hot-smoked' fish, where a high internal temperature is reached. The heat during hot-smoking will kill vegetative microorganisms but not all spores, so the most probable spoilage organisms will be spore-formers or post-smoking contaminants. Refrigeration of hot-smoked fish will greatly reduce the number of organisms able to grow and will lengthen the shelf life.

Cold-smoked fish will contain microbial flora representative of that of the raw fish. The salt in the water phase must be high enough to inhibit the growth of *C. botulinum* type E, but this will not prevent the growth of spoilage bacteria. The preservative effect of the smoke will inhibit some spoilage microorganisms, but often colour changes due to chemical oxidation of the smoked product will limit the shelf life, not the growth of spoilage organisms. Vacuum packing limits chemical oxidation, and microbial spoilage is more likely. Refrigeration is necessary, so the bacteria spoiling these products will be psychrotrophic or psychrophilic. Reports for vacuum packed cold-smoked salmon have identified more than one organism associated with spoilage; LAB, Pseudomonadaceae, Vibrionaceae and Enterobacteriaceae may all play some part in shelf life limitation. It has been reported that the type of salting (dry- or wet-salting) in the cold-smoked salmon process leads to different microflora growth at the onset of spoilage. Marine vibrios were the dominant flora in smoked salmon that was dry-salted, whereas a mixture of Enterobacteriaceae and LAB were prevalent in brine-injected salmon (37). For more information on spoilage see Table 5.IV. Table 5.V gives details of pH ranges and $a_w$ minima for the growth of some important spoilage microorganisms and pathogens (38).
## TABLE 5.IV
Spoilage of salted, marinated and smoked fish

<table>
<thead>
<tr>
<th>Process</th>
<th>Product</th>
<th>$A_w$</th>
<th>Spoilage controlled by</th>
<th>Probable spoilage organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Canned Fish</td>
<td>v'</td>
<td>v+++v-</td>
<td>None</td>
</tr>
<tr>
<td>Salt marinades</td>
<td>Gravad, Matjes</td>
<td>+</td>
<td>-</td>
<td>Fresh fish spoilage organisms</td>
</tr>
<tr>
<td>Salted then boiled</td>
<td></td>
<td>++</td>
<td>-</td>
<td>Halophilic spore formers and PPC</td>
</tr>
<tr>
<td>Brined then boiled</td>
<td>Pindang</td>
<td>+</td>
<td>-</td>
<td>Halophilic spore formers and PPC</td>
</tr>
<tr>
<td>Salted then dried</td>
<td>Bacalao</td>
<td>+++</td>
<td>-</td>
<td>Halophilic organisms: Pink-Halococcus spp. and Halobacterium spp.</td>
</tr>
<tr>
<td>Gaspé cure</td>
<td></td>
<td>++</td>
<td>-</td>
<td>Halophilic spoilage organisms</td>
</tr>
<tr>
<td>Anchovie</td>
<td>Anchovies</td>
<td>++</td>
<td>-</td>
<td>Halophilic spoilage organisms. Histamine producers</td>
</tr>
<tr>
<td>Saturated brined fish</td>
<td></td>
<td>+++</td>
<td>-</td>
<td>Halophilic spoilage organisms</td>
</tr>
<tr>
<td>Salted, boiled, smoked and dried</td>
<td>Jellied eels</td>
<td>+/</td>
<td>++ -</td>
<td>Spore formers and PPC</td>
</tr>
<tr>
<td>Salted, boiled, smoked and dried</td>
<td>Ikan kaju</td>
<td>+++</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Citrus marinade</td>
<td>Ceviche</td>
<td>-</td>
<td>+</td>
<td>Acid-tolerant spoilage organisms</td>
</tr>
<tr>
<td>Dried</td>
<td>Stockfish</td>
<td>+++</td>
<td>-</td>
<td>Xerophilic moulds</td>
</tr>
<tr>
<td>Brined or salted then liquid or cold smoked</td>
<td>Cold-smoked fish</td>
<td>++</td>
<td>+</td>
<td>Fresh fish spoilage organisms, LAB, Enterobacteria and PPC</td>
</tr>
<tr>
<td>Brined or salted then liquid or hot smoked</td>
<td>Hot-smoked fish</td>
<td>++</td>
<td>+</td>
<td>Spore formers and PPC</td>
</tr>
</tbody>
</table>

1 Antimicrobial compounds added  
2 Refrigeration applied  
3 v variable, - not used/unchanged, + to +++ strength of preservative effect  
4 Post-process contaminants
5.5 Pathogens: Growth and Survival

5.5.1 Clostridium botulinum

In the last few decades, popular demand for healthy foods has led to a change in the tastes preferred by the public and, with this change, pressure on the traditional methods of curing. Before, the products were heavily salted, smoked and dried, and *C. botulinum*, when present in the raw material, could not grow and produce toxin. A number of surveys have shown that fish from temperate waters is mainly contaminated with the psychrotrophic *C. botulinum* type E, but the presence of the mesophilic types A and B is of concern in fish from warmer waters (13). Examination of 165 outbreaks of botulism caused by fish products showed that smoked, salted and pickled products accounted for 10, 9 and 8 of the outbreaks, respectively, the fermented products being the most dangerous group (7). The presence of salt in cured products has a great effect on the growth of the bacteria, but the concentration of salt in smoked salmon or trout (usually varying from 1 - 4% salt) is not high enough to prevent growth. The concentration required to prevent growth at room temperature can vary from as low as 3.5% to 5%, although approximately 10% salt in the water phase is necessary to fully inhibit the mesophilic types A and B strains (14). Since neither the smoking nor the drying parts of this curing process are particularly severe, and as botulism is a serious hazard in foods, the importance of cold-chain integrity must be emphasised. It has been shown that, under optimal conditions, the non-proteolytic type E strains can grow in up to 5% sodium chloride, but if the fish products are stored at temperatures <10 °C, 3% sodium chloride in the water phase is enough to inhibit growth of type E for at least 30 days (39). However, although the combination of the two factors, temperature ≤5 °C and salt ≥3% in the water phase is sufficient to prevent any growth of type E, types A and B are not affected by 3 - 5% of salt in the water phase and great care must be taken to avoid any temperatures >10 °C (13).

Sodium nitrite in conjunction with sodium chloride is known for its ability to inhibit *C. botulinum* type E and production of toxin. In the United States, sodium

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### TABLE 5.V

**pH ranges and aw minima for growth of microorganisms (38)**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>aw</th>
<th>Organisms</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most Pseudomonadaceae</td>
<td>0.97</td>
<td>Most Pseudomonadaceae</td>
<td>5.5-10</td>
</tr>
<tr>
<td><em>C. botulinum</em> type E</td>
<td>0.97</td>
<td><em>C. botulinum</em></td>
<td>4.5-8.5</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>0.86</td>
<td><em>Staph. aureus</em></td>
<td>4.0-9.8</td>
</tr>
<tr>
<td>Most spoilage bacteria</td>
<td>0.9</td>
<td>Yeasts</td>
<td>1.5-8.5</td>
</tr>
<tr>
<td>Most spoilage moulds</td>
<td>0.8</td>
<td>Moulds</td>
<td>0-11</td>
</tr>
<tr>
<td>Halophilic bacteria</td>
<td>0.75</td>
<td>Lactic acid bacteria</td>
<td>3.5-10.5</td>
</tr>
<tr>
<td>Xerophilic moulds</td>
<td>0.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
nitrite can be added to the brine but this is not permitted in EU countries because nitrites might lead to formation of the carcinogenic nitrosamines. The FDA established that hot- or cold-smoked fish should have at least 3.5% salt in the water phase, but, if it contains sodium nitrite ≥100 ppm, it should contain not less than 3.0% salt in the water phase (30).

5.5.2 Staphylococcus aureus

This is a ubiquitous organism but its main reservoir and habitat is animals and, more importantly, the human nose, throat and skin. Thus, if the person handling the product is a carrier of Staph. aureus, the organism may contaminate the product. The human carrier rate can be as high as 60% of healthy individuals with an average of 25 - 30% of the population positive for enterotoxin-producing strains (41). The organism is mesophilic with a minimum growth temperature of 7 °C, but requires higher temperatures for toxin production (>10 °C). It is a halotolerant bacterium able to grow at water activities as low as 0.86 and 10% sodium chloride, and the minimum pH for growth is 4.0 - 4.5. In foodstuffs, these parameters change as a result of the interference of food components and other limiting factors that can act in an additive or synergistic way to prevent Staph. aureus growth. As they are poor competitors, they do not grow well in the raw material, but, after processing, when the bacterial load of the product is lower, if recontamination occurs and storage conditions are favourable, they will grow rapidly (7). Sliced smoked fish products, as they require more handling, are particularly susceptible to contamination and, if storage temperatures are over 10 °C, growth and toxin formation may occur. Once the product is contaminated, the organism can survive most of the light curing processes.

5.5.3 Enterobacteriaceae

The pathogens Salmonella, Shigella and E. coli all occur on fish products as a result of contamination from animal/human reservoirs. As with other organisms, the risk of infection can be eliminated by proper cooking. However, they can survive lightly cured processes when the sodium chloride content is less than 5% and water activities are higher than 0.94 (7). The degree of concern in cold-smoked salmon is low unless temperature abuse >10 °C occurs (13). An outbreak of Shigella in an Italian cruise ship, related to the consumption of a raw smoked fish meal, has been reported (42). Infection by Shigella sonnei has increased in the UK. Usually, its occurrence in a final product is due to contamination during processing by an infected asymptomatic carrier with poor personal hygiene.

5.5.4 Listeria monocytogenes

The psychrotrophic L. monocytogenes is a ubiquitous organism that has been detected in fresh fish skin, gills and guts as well as in several lightly preserved fish
products such as cold-smoked fish, marinades (ceviche) (43) and gravad fish (44). Its limiting conditions for growth are 0 - 45 °C, salt levels of 8 - 12%, pH 4.8 - 9.6 and water activities below 0.92 - 0.95 (45).

Unlike some European countries, which considered *L. monocytogenes* pathogenic for only specific segments at risk, the USA established ‘zero tolerance’ for the organism, which means that the process must conform to a level of ‘no detectable *L. monocytogenes*’ in 25 g of the finished product. The Health Protection Agency (HPA) in the UK considered that it was unacceptable for ready-to-eat foods (including all the products that are consumed without prior cooking) to contain any serogroup of *L. monocytogenes* in levels at or above $10^2$ per gram (46).

Until 1997, when an outbreak of nine cases of listeriosis was suspected to have been caused by gravad or cold-smoked trout (47), there were no reported outbreaks related to cured fish. As its presence has been reported in lightly preserved fish products whose processing chain cannot fully assure the absence of the organism, researchers are looking for other ways of preventing the pathogen’s occurrence or growth. Some LAB, widely used as starter cultures in food processes, have been shown to have an anti-listerial activity in many food products. Marine LAB is part of the natural flora of chill-stored vacuum packed salmon and cod. Vacuum packaging (VP) of sliced smoked fish creates a microaerophilic atmosphere, which, in combination with curing agents and refrigerated storage, favours growth of psychrotrophic LAB. The use of LAB in fish preservation is a topic that has been studied in projects (UP.2.514, in the EU Fisheries R&D-FLAIR program, the CT 3162-FAIR program, and in CT95-1207-FAIR).

5.5.5 *Parasites*

Despite the presence of parasites in fish being very common, most of them are of little concern to public health. They have complicated life cycles, which include intermediate hosts. As they are heat-sensitive, the only concern to public health is when eating raw or uncooked fish products. Thus, a number of fish products, such as ‘lightly preserved’ fish products, are considered unsafe. This includes matjes herring, gravad fish, cold-smoked fish, lightly salted caviar, and other local traditional products (7).

Of microbiological concern in the northern hemisphere are two parasites - the round worm *Anisakis* spp. from seawater fish, and the tape worm *Diphyllobothrium* spp. from freshwater fish, both being detected in cold-smoked salmon. Two outbreaks in the United States have been reported to be linked to salmon (10). *Pseudoterranova decipiens*, a round worm, has also been detected in cod (7).

The round worms, or nematodes, do not survive 1 min at 60 °C, which means that cooking a fillet 3 cm thick for 10 min at 60 °C will kill any worms present (48). As freezing to -20 °C and maintaining this temperature for at least 24 h will kill all the nematodes, a short period of freezing either of the raw material or of
the final product should therefore be included in the processing as a means of controlling parasites (7).

### 5.6 Published Microbiological Criteria

**TABLE 5.VI**

Guidelines for the microbiological quality of ready-to-eat smoked fish, taramasalata and cooked shellfish sampled at the point of sale (adapted from (11))

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Satisfactory (cfu/g unless stated)</th>
<th>Borderline - limit of acceptability (cfu/g unless stated)</th>
<th>Unsatisfactory (cfu/g unless stated)</th>
<th>Unacceptable/ potential hazard* (cfu/g unless stated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic plate count†</td>
<td>&lt;10⁰</td>
<td>10⁰ - &lt;10⁷</td>
<td>≥10⁷</td>
<td>N/A</td>
</tr>
<tr>
<td>(30 °C, 48h ± 2h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indicator organisms‡</td>
<td>&lt;100</td>
<td>100 - &lt;10⁴</td>
<td>&gt;10⁴</td>
<td>N/A</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> (total)</td>
<td>&lt;20</td>
<td>20 - &lt;100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Listeria</em> spp. (not <em>L. monocytogenes</em>)</td>
<td>&lt;20</td>
<td>20 - 100</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathogens</td>
<td>not detected in 25 g</td>
<td>present in 25 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> O157 &amp; other VTEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio. cholerae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>&lt;20</td>
<td>20 - &lt;100</td>
<td>200 - &lt;10⁰</td>
<td>≥10⁰</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>&lt;20**</td>
<td>20 - &lt;100</td>
<td>N/A</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>&lt;20</td>
<td>20 - &lt;100</td>
<td>100 - &lt;10⁴</td>
<td>≥10⁰</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. perfringens</em></td>
<td>&lt;10</td>
<td>10 - &lt;100</td>
<td>100 - &lt;10⁰</td>
<td>≥10⁰</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. cereus</em> and <em>Bacillus subtilis</em>†</td>
<td>&lt;10⁰</td>
<td>10⁰ - &lt;10⁷</td>
<td>10⁰ - &lt;10⁰</td>
<td>≥10⁰</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Prosecution based solely on high colony counts and/or indicator organisms in the absence of other criteria of unacceptability is unlikely to be successful.

†Guidelines for aerobic colony counts may not apply to certain fermented foods, for example, salami, soft cheese, and unpasteurised yoghurt. These foods fall into category 5. Acceptability is based on appearance, smell, texture, and the levels or absence of indicator organisms or pathogens.

‡On occasions some strains may be pathogenic.

# If the *Bacillus* counts exceed 10⁴/g, the organism should be identified.

N/A denotes not applicable.
CURED, SMOKED AND DRIED FISH

TABLE 5.VII
US Food and Drug Administration (FDA) guidelines related to ready-to-eat fishery products (45)

<table>
<thead>
<tr>
<th>Product</th>
<th>Organisms and/or toxins</th>
<th>Limit of tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready-to-eat fishery products (minimal cooking by consumer)</td>
<td>Enterotoxigenic <em>E. coli</em> (ETEC)</td>
<td>$1 \times 10^3$ ETEC/g, LT or ST positive</td>
</tr>
<tr>
<td></td>
<td><em>L. monocytogenes</em></td>
<td>Presence of the organism</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella spp.</em></td>
<td>Presence of the organism</td>
</tr>
<tr>
<td></td>
<td><em>Staph. aureus</em></td>
<td>Positive for enterotoxin or $\geq 10^4$ (Most Probable Number)</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio cholerae</em></td>
<td>Presence of toxigenic 01 or non-01 ≥ $1 \times 10^4$ (Kanagawa positive or negative)</td>
</tr>
<tr>
<td></td>
<td><em>V. parahaemolyticus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Vibrio vulnificus</em></td>
<td>Presence of the organism</td>
</tr>
<tr>
<td></td>
<td><em>C. botulinum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paralytic shellfish poisoning*</td>
<td>0.8 ppm (saxitoxin equivalent)</td>
</tr>
<tr>
<td></td>
<td>Amnesic shellfish poisoning *</td>
<td>20 ppm domoc acid, except in the viscera of dungeness crab, where 30 ppm is permitted</td>
</tr>
</tbody>
</table>

*These criteria are applied to all fish

5.7 References

FISH AND SEAFOOD


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5.8 Further Reading


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CURED, SMOKED AND DRIED FISH


6. FERMENTED FISH

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6.1 Definitions

As applied to fish products, the term ‘fermented’ describes a spectrum of processes that ranges from the largely autolytic degradation of fish protein, to those processes in which the activity of lactic acid bacteria (LAB) plays an important part. The relative importance of these two activities depends on the formulation of the product. Those to which only salt is added tend to be mainly autolytic, producing the fish sauces and pastes, whereas in those where a carbohydrate source such as rice or sugar is also added, lactic fermentation can play a significant role.

Fermented fish products are largely confined to east and south-east Asia, though some are produced elsewhere. As with most traditional products that are still produced principally on a cottage industry or domestic scale, there are numerous variants of some common themes and a host of local names used to describe them. Essentially, they can be divided into two categories: fish/salt products and fish/salt/carbohydrate products.

6.1.1 Fish/salt products such as the fish pastes and sauces tend to contain relatively high levels of salt, typically in the range 15 - 25% and are used mainly as a condiment. The fish sauces are produced on the largest scale; they are exported to European, North American and other markets and are, in economic terms, the most important of all fermented fish products. The principal fish sauces and pastes of south-east Asia and their local names are listed in Table 6.I.

Carbohydrates are added to some fish sauces during their preparation. For example, the best-known of the fish sauces (gyoshoyu) of Japan is shottsuru. It is produced from the sandfish Arctoscopus japonicus and has the rice-based enzyme preparation, koji, added during preparation. Sugar can also be added to the Indonesian fish sauce bakasang and the Thai product nam-budu.
TABLE 6.I
Fish sauces and pastes of south-east Asia

<table>
<thead>
<tr>
<th>Country</th>
<th>Sauce</th>
<th>Paste</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amber/brown liquid; salty</td>
<td>Red/brown salty paste</td>
</tr>
<tr>
<td></td>
<td>taste, cheese-like aroma</td>
<td></td>
</tr>
<tr>
<td>Burma</td>
<td>Ngapi</td>
<td>nga-ngapi</td>
</tr>
<tr>
<td>Indonesia</td>
<td>ketjap-ikan</td>
<td>trassi-ikan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trassi-udang (shrimps)</td>
</tr>
<tr>
<td>Kampuchea</td>
<td>nuoc-mam</td>
<td>prahoc</td>
</tr>
<tr>
<td></td>
<td>nuoc-mam-gau-ca (livers only)</td>
<td>mam-ruoc (shrimps)</td>
</tr>
<tr>
<td>Korea</td>
<td>myulchleot</td>
<td>jeots, jeotkals (shrimp)</td>
</tr>
<tr>
<td>Laos</td>
<td>nam-pla (pa)</td>
<td>padec</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Budu</td>
<td>Belachan (shrimps)</td>
</tr>
<tr>
<td>Philippines</td>
<td>Patis</td>
<td>bagoong</td>
</tr>
<tr>
<td>Thailand</td>
<td>nam-pla</td>
<td>kapi</td>
</tr>
<tr>
<td>Vietnam</td>
<td>nuoc-mam</td>
<td>mam-ca</td>
</tr>
<tr>
<td></td>
<td></td>
<td>man-tom (shrimps)</td>
</tr>
</tbody>
</table>

Even within the limitations of fish and salt as the sole ingredients, a variety of products can be produced within a single country. This is well exemplified by some of the fish/salt products of Thailand described in Table 6.II.

6.1.2 Fish/salt/carbohydrate products range from those that resemble the fish sauces and pastes in the sense that extensive autolysis has occurred, to products analogous to other lactic fermented foods such as salami, where the bacterial production of lactic acid is a major feature. A selection of Asian products is presented in Table 6.III.

Fermented fish products are of very minor importance outside Asia. In Europe, fish sauces known as liquamen or garum were ubiquitous condiments for the Romans, who had adopted them from the Greeks. These have long since fallen from use, although garos, made from fish livers in Greece, and pissala, made from whole fish in France, may be direct descendants, and the more widely known Worcestershire sauce a very distant relative.
## Fermented Fish

### Table 6.II
Fermented fish/salt products common in Thailand

<table>
<thead>
<tr>
<th>Name</th>
<th>Type of fish</th>
<th>Form</th>
<th>Approx. salt/fish ratio</th>
<th>Production period</th>
<th>Type of use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hoi-dong</em></td>
<td>Molluscs</td>
<td>Whole or without shells</td>
<td>-</td>
<td>-</td>
<td>Main dish</td>
</tr>
<tr>
<td><em>Kapi</em> (fish or shrimp paste)</td>
<td>Small fish, shrimps, crustaceans</td>
<td>Whole</td>
<td>1:4</td>
<td>3 - 4 months</td>
<td>Condiment and main dish</td>
</tr>
<tr>
<td><em>Nam-pla</em> (fish sauce)</td>
<td>Variety of fish</td>
<td>Whole</td>
<td>1:3</td>
<td>18 months</td>
<td>Condiment</td>
</tr>
<tr>
<td><em>Nam-budu</em></td>
<td>Variety of brackish or marine fish</td>
<td>Whole</td>
<td>1:3</td>
<td>After 3 - 12 months</td>
<td>Condiment; the mixture is then boiled and bottled</td>
</tr>
<tr>
<td><em>Nam-khoei</em></td>
<td>Shrimps, crustaceans</td>
<td>Whole</td>
<td>1:4</td>
<td>-</td>
<td>Condiment</td>
</tr>
<tr>
<td><em>Pla-thu-khem</em></td>
<td>Mackerel eviscerated fish</td>
<td>Whole</td>
<td>1:3</td>
<td>2 - 3 months</td>
<td>Main dish</td>
</tr>
<tr>
<td><em>Tai-pla</em></td>
<td>Variety of fish</td>
<td>Bowels</td>
<td>1:3</td>
<td>6 - 8 months</td>
<td>Main dish</td>
</tr>
</tbody>
</table>

### Table 6.III
Fish/salt/carbohydrate products of south-east and east Asia

<table>
<thead>
<tr>
<th>Country</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td><em>I-sushi</em>, e.g. <em>ayu-sushi</em>, <em>funa-suchi</em>, <em>tai-suchi</em></td>
</tr>
<tr>
<td>Kampuchea</td>
<td><em>phaak</em>, <em>mam-chao</em>, <em>mam-seeing</em></td>
</tr>
<tr>
<td>Korea</td>
<td><em>sikhae</em></td>
</tr>
<tr>
<td>Laos</td>
<td><em>som-kay-pa-eun</em>, <em>som-pa</em>, <em>mam-pa-kor</em>, <em>pa-chao</em>, <em>pa-khem</em>, <em>som-pa-keng</em></td>
</tr>
<tr>
<td>Malaysia</td>
<td><em>pekasam</em>, <em>cencalok</em></td>
</tr>
<tr>
<td>Philippines</td>
<td><em>burong-isdia</em>, e.g. <em>burong-ayungi</em>, <em>burong-dalag</em>, <em>burong-bangus</em></td>
</tr>
<tr>
<td>Thailand</td>
<td><em>pla-ra</em>, <em>pla-som</em>, <em>pla-chao</em>, <em>som-fak</em>, <em>som-fug</em></td>
</tr>
</tbody>
</table>
FISH AND SEAFOOD

In Northern Europe, some fermented fish products remain popular. Norwegian gravlaks, or buried salmon, is a traditional, relatively mild-tasting product, which has been increasing in popularity over the last 50 years. There are also more heavily fermented products, rakefish or surfisk, the most popular varieties of which are rakørret, fermented trout, in Norway and surstromming, made from herring, in Sweden. These are often described as fish/salt products, which employ lower levels of salt than Asian products. A modern account of their production, however, states that sugar is often included with the salt, and old descriptions of their production often refer to the addition of whey, malt or flour (1).

6.2 Initial Microflora

Composition of the initial microflora of fish is described in some detail elsewhere in this Handbook and will not be reiterated here. The diversity of fish used in fermented products (see, for example, Table 6.IV) and in the way in which they are handled prior to processing means that the initial microbial levels are far from uniform, and counts ranging from below $10^4$ /g to in excess of $10^7$ have been reported.

<table>
<thead>
<tr>
<th>Product names</th>
<th>Local</th>
<th>English</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burong ayungin</td>
<td>Ayungin</td>
<td>Silver perch</td>
<td>Therapon plumbeus</td>
</tr>
<tr>
<td>Burong bangus</td>
<td>Bangus</td>
<td>Milkfish</td>
<td>Chanos chanos</td>
</tr>
<tr>
<td>Burong dala</td>
<td>Dalag</td>
<td>Mudfish</td>
<td>Ophicephalus striatus</td>
</tr>
<tr>
<td>Burong gurami</td>
<td>Gurami</td>
<td>Goramy</td>
<td>Osphronemus goramy</td>
</tr>
<tr>
<td>Burong hito</td>
<td>Hito</td>
<td>Catfish</td>
<td>Clarias batrachus</td>
</tr>
<tr>
<td>Burong kanduli</td>
<td>Kanduli</td>
<td>Sea catfish</td>
<td>Arias manillensis</td>
</tr>
<tr>
<td>Burong tilapya</td>
<td>Tilapya</td>
<td>Tilapia</td>
<td>Tilapia nilotica</td>
</tr>
<tr>
<td>Balao balao</td>
<td>Tagunton</td>
<td>Shrimp</td>
<td>Macrobrachium spp.</td>
</tr>
<tr>
<td>Burong hipon</td>
<td>Suwahe</td>
<td>Shrimp</td>
<td>Penaeus indicu</td>
</tr>
</tbody>
</table>

Many of the fish used in fermentation are uneviscerated, especially in fish/salt products, and the initial microflora is likely to be considerably larger in these cases. Lactic fermented fish products are often associated with inland areas such as the Central Luzon region of the Philippines and the north-east of Thailand. Here, freshwater fish are the usual raw material, and their microflora tends to reflect their local environment more than that of marine species. This could lead to quite marked variations since the same fish can be obtained from a variety of sources. For example, in a survey of fermented fish products in north-east Thailand, it was found that several different sources were used for the fish - the flooded rice fields, paddy ponds beside rice fields used for collecting fish when the field has dried up, and a large local freshwater reservoir (2).
FERMENTED FISH

Where other ingredients are used, they can contribute to the initial microflora. When this is a simple carbohydrate source such as sugar, or boiled or roasted rice, its contribution is likely to be insignificant compared with that of the fish microflora in terms of both spoilage potential and public health concerns. In some cases, however, traditional starter culture preparations such as koji (Japan), ang-kak (Philippines and Thailand) and look-pang (Thailand) are used, either directly or as the partially saccharified rice they produce. These preparations contain a mixed microflora of moulds and yeasts, principally species of the genera Rhizopus, Mucor, Hansenula, Endomycopsis and Saccharomyces - organisms that play an important part in the production process but can also contribute to the ultimate spoilage of the product.

6.3 Processing and its Effects on the Microflora

6.3.1 Fish sauces and pastes

Detailed flow charts for several fish/salt fermented products are presented as Figures 6.1 – 6.4. Generally, the fish substrate is mixed with dry salt and the product is packed into containers and left to ferment for an extended period of weeks to months. The amount of salt used is often only vaguely defined but is generally sufficient to reduce the $a_w$ below levels at which most bacteria associated with the raw material will grow and, in many cases, sufficient to saturate the water phase present and produce an $a_w$ of 0.74 or below. This, and the anaerobic conditions that prevail in the bulk of the material, mean that microorganisms have been generally held to play little or no role in the process that follows. Studies have shown that the number of bacteria declines rapidly during the production of the Thai fish sauce nam pla and that, after one month, the product contained about 500 cfu/g comprising mainly Micrococcus and Bacillus spp. (3, 4). Similar results were seen with patis, the Philippine fish sauce, where the total counts dropped from above 10⁷ cfu/g to below 10⁴ cfu/g after 14 days and below 10² after 40 days. In another microbiological study of four fish sauces, which included nam-pla and patis, Bacillus spp. were found to be the predominant isolates, probably reflecting their ability as spore-formers to survive rather than any capacity to multiply under the prevailing conditions (5). It has also been shown that a perfectly acceptable patis could be produced from fish that had been sterilised with ionising radiation (6). Similarly microbial inactivation by irradiation has not been found to adversely affect the properties of Korean fermented squid (7).

More recently, however, work with nam-pla in Thailand has isolated Halobacterium and Halococcus spp. in numbers reaching 10⁸/ml after 3 weeks of fermentation but declining thereafter. It also demonstrated their significant proteolytic activity during the first month of fermentation, suggesting that halophilic bacteria do play an important role in the production of fish sauce (8). Other halophilic species have been identified from fish/salt products, and studies
have demonstrated the production of halophilic proteinases from fish sauce isolates (9 - 12).

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Fig. 6.1. Belacan (Condiment)

Ingredients: Shrimp, salt
Shelf life: many months
Analysis: pH 7.2-7.8, ash 20.0-27.6%, moisture 27-40%, salt 13.0-25.3%

Fig. 6.2. Patis (Condiment)

Ingredients: fish 70-80%, salt 20-30%, food colour, optional
Shelf life: 1 - 2 years
Analysis: pH 5.5 - 5.9, acidity as lactic acid 1%, ash 21.9%, salt 20-25%
The extensive proteolysis and liquefaction that occur during production are, however, thought to be largely the result of autolytic breakdown of the fish tissues. This is more rapid when whole fish are used since the head and viscera contain higher concentrations of proteolytic enzymes than other tissues, and muscle cathepsins have been shown to be inhibited at relatively low salt concentrations (13, 14). To produce semi-solid fish pastes as opposed to fish sauces, eviscerated fish are sometimes used to slow the autolytic process. In fish sauce production, the
FISH AND SEAFOOD

A high salt level used also assists in the osmotic extraction of a supernatant rich in amino acids, particularly glutamate, volatile fatty acids and nucleotides, which is eventually separated from the residual fish tissues, filtered and bottled.

6.3.2 Fish/salt/carbohydrate products of Asia

This category encompasses an even greater diversity of products than the fish sauces and pastes. Their main feature is that the provision of a source of carbohydrate allows a lactic acid bacterial fermentation to occur, and the low pH produced contributes a second protective barrier or hurdle against the growth/survival of the normal fish-spoilage organisms and pathogens. In contrast to free-swimming fish, shellfish such as mussels often have appreciable levels of carbohydrate in their tissues and in these cases addition of carbohydrate is not necessary for a lactic fermentation to occur (see, for example, Figure 6.5). Some examples of products and their formulation are presented in Table 6.V and Figures 6.6 – 6.8.

**Fig. 6.5. Hoi-Malangpu-Dong (side dish, snack)**

![Diagram](Mussel Salt → Mix well → Ferment at 21-45°C for 14 days → Hoi-malangpu-dong)

Ingredients: mussels 90%, salt 10%

Shelf life: 6 months

Analysis: pH 4.5 - 5.8, salt 4%, lactic acid 0.27 - 1.30%
### Table 6.V
Fish/salt carbohydrate products of Thailand

<table>
<thead>
<tr>
<th>Name</th>
<th>Region</th>
<th>Formulation</th>
<th>Preparation time</th>
<th>Shelf life</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Pla-ra</td>
<td>C, N, NE</td>
<td>Fish (whole or pieces): salt: roasted rice: 3:1:0.2-4</td>
<td>6 - 12 months</td>
<td>1 - 3 years</td>
<td>Condiment and main dish</td>
</tr>
<tr>
<td>2 Pla-jao</td>
<td>C, N, NE</td>
<td>Fish (pieces): salt:khao-mark (fermented rice): 3:1:1-3</td>
<td>10 - 20 days</td>
<td>2 - 3 months</td>
<td>Main dish</td>
</tr>
<tr>
<td>3 Pla-som</td>
<td>NE, C</td>
<td>Fish: salt: boiled rice: garlic 10:2:1:0.25-1</td>
<td>5 - 12 days</td>
<td>3 weeks</td>
<td>Main dish</td>
</tr>
<tr>
<td>4 Pla-jom</td>
<td>NE, C</td>
<td>Fish: salt: roasted rice: garlic 10:1:3:1</td>
<td>3 - 7 days</td>
<td>2 weeks</td>
<td>Main dish</td>
</tr>
<tr>
<td>5 Som-fak</td>
<td>NE, N</td>
<td>Fish (minced): salt: rice: garlic 10:0.5:1:5.2-3:1</td>
<td>5 - 10 days</td>
<td>2 weeks</td>
<td>Main dish</td>
</tr>
<tr>
<td>6 Pla-paeng-daeng</td>
<td>S</td>
<td>Fish: salt: boiled rice: ang-kak 3:1:3:0.03</td>
<td>5 days</td>
<td>6 - 12 months</td>
<td>Condiment and main dish</td>
</tr>
</tbody>
</table>

Note:

Products 1 - 4 are also produced using shrimps. In these cases, the prefix 'pla' is replaced by 'kung'. *Ang-kak*: red mould rice obtained by fermenting rice with *Monascus purpureus*.

C, central; N, north; NE, north-east; S, south of Thailand.

---

**Fermented Fish**

**Fig. 6.6. Kung-Chom (staple food, side dish)**
FISH AND SEAFOOD

The extent to which lactic fermentation occurs depends largely on the salt content. Generally, these products are salted at lower levels than the fish sauces and pastes, typically in the range 2 - 10%, but there is considerable variation in this. A survey of fish/salt/carbohydrate products in Thailand (15) noted that there were two empirical rules governing formulation:-

6.3.2.1 The use of higher salt levels results in a longer production phase but a better keeping quality product.

This is readily explained by the susceptibility of LAB to elevated salt concentrations. The LAB isolated from these products are those with more marked salt tolerance but, while they are able to grow at high salt levels, their growth and metabolism are nonetheless slowed, giving a reduced rate of acid generation. If acidity is a desired sensory property of the product, then this will take longer to develop than if lower salt levels are used. It also indicates that, of the two factors contributing to shelf life - salt and lactic acid, the salt is of far greater importance in determining the product’s long-term stability. This can be clearly seen from the data on reported shelf lives of a number of fish salt carbohydrate products and their respective pH ranges and salt contents (Table 6.VI).

Fig. 6.7. Red burong isda

(from Arroyo et al., Phillipines Journal of Food Science and Technology, 2, 106)
(from Arroyo et al., Philippines Journal of Food Science and Technology, 2, 106)

Fig. 6.8. White balao-balao

TABLE 6.VI

Shelf lives reported for fish salt carbohydrate products

<table>
<thead>
<tr>
<th>Product</th>
<th>Salt</th>
<th>pH</th>
<th>Shelf life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pla-som</td>
<td>2.3 - 5.9</td>
<td>4.0 - 4.6</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Pla-paeng-daeng</td>
<td>4.5 - 9.2</td>
<td>3.9 - 5.2</td>
<td>6 - 12 months</td>
</tr>
<tr>
<td>Pla-ra</td>
<td>7.8 - 17.9</td>
<td>4.7 - 6.2</td>
<td>1 - 3 years</td>
</tr>
<tr>
<td>Pla-chao</td>
<td>4.4 - 9.5</td>
<td>4.0 - 5.3</td>
<td>1 - 2 years</td>
</tr>
<tr>
<td>Pla-chom</td>
<td>3.8 - 4.8</td>
<td>5.0 - 6.1</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Som-fak</td>
<td>2.5 - 5.8</td>
<td>4.1 - 5.0</td>
<td>2 weeks</td>
</tr>
</tbody>
</table>

Source: A Concise Handbook of Indigenous Fermented Foods in the ASCA Countries (16).
6.3.2.2 *Inclusion of more carbohydrate gives a faster fermentation and a stronger acid taste*

Increasing the proportion of carbohydrate in the formulation decreases the product’s buffering capacity and provides the LAB with more substrate on which to act. The effect of this will be to decrease the product’s final pH and increase the amount of total acidity. Addition of carbohydrate will, of course, also lower the unit cost of the product and this is probably a significant factor in many cases.

Rice is the most common carbohydrate source used. When added as boiled or roasted rice alone, efficient lactic acid production will depend upon the presence of amylolytic LAB or saccharification by other microorganisms or enzymes that may be present. Although the ability to ferment starch is not widespread in the LAB, a number of amylolytic species have been described and an amylolytic strain of *Lactobacillus plantarum* has been isolated from *burong bangus* in the Philippines (17).

In several products, the rice may be added in a partially saccharified form such as *kao-mark* (Thailand); alternatively, a saccharifying agent such as *koji* (Japan) or *ang-kak* (the Philippines) may be added separately (Table 6.VII). Where they are used, they increase the amount of soluble sugars produced and thus the range of LAB that can grow. This results in a more active lactic fermentation and a lower pH, although the final population of LAB may be similar. For example, a survey of *burong-isda* in the Philippines reported consistently lower pH values for red *burong-isda* where *ang-kak* was used (pH 3.0 - 3.9) compared with the white product (pH 4.1 - 4.5) despite other factors, such as salt content, being similar (18, 19).

Garlic, a common ingredient in these products, also serves as a valuable source of the more readily fermented carbohydrate, inulin. In *som-fak* 9% of LAB isolated were able to ferment rice whereas 19% could ferment garlic and omission of garlic from the formulation resulted in lower acid production during fermentation (20, 21).

**TABLE 6.VII**  
Fish/salt/carbohydrate products in which saccharified rice or a saccharifying starter culture is used

<table>
<thead>
<tr>
<th>Product</th>
<th>Country</th>
<th>Saccharified rice</th>
<th>Starter culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>red burong-isda</td>
<td>Philippines</td>
<td>-</td>
<td>ang-kak</td>
</tr>
<tr>
<td>i-sushi, nare sushi</td>
<td>Japan</td>
<td>-</td>
<td>koji</td>
</tr>
<tr>
<td>pla-chao</td>
<td>Thailand</td>
<td>kao-mark</td>
<td>-</td>
</tr>
<tr>
<td>pla-paeng-daeng</td>
<td>Thailand</td>
<td>-</td>
<td>ang-kak</td>
</tr>
<tr>
<td>pa-chao</td>
<td>Laos</td>
<td>Kha-namak</td>
<td>-</td>
</tr>
</tbody>
</table>

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*Inclusion of more carbohydrate gives a faster fermentation and a stronger acid taste*

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*Sikhae*, the Korean fermented fish product, is something of an exception since millet is used along with garlic as the carbohydrate source. Cleaned pieces of eviscerated fish flesh are salted with 6% salt overnight before mixing with cooked millet, red pepper powder and garlic in the ratio fish:millet:pepper:garlic of 80:7.5:9:3.5. The mixture is allowed to ferment for 2 - 3 weeks at around 20 °C. In a successful fermentation, the pH drops from 6.7 to below 4.5 in the first 2 - 3 days, thereafter declining to about 4.2 at the end of fermentation.

The Thai product *pla-ra* exemplifies these basic rules as well as the difficulties associated with attempts to classify such products. The same product can range from a brown turbid liquid where the fish has been subject to extensive autolysis to brown partly dried pieces of fish, and there is a marked regional variation in the preferred type. Generally, the level of salt employed is on the high side for fish/salt/carbohydrate products, around 12%, which is sufficient to prevent lactic fermentation by all but the most halotolerant LAB. Some authors have claimed that the function of the roasted rice added during *pla-ra* production is to improve texture and slow the fermentation rather than to serve as a substrate for lactic fermentation (22). It seems that a lactic fermentation takes place in many types of *pla-ra* but is not significant in others. Results of a product survey were consistent with this, reporting the product’s pH to range from 4.7 up to 6.2, presumably correlating with the salt content, which ranged from 7.8 - 17.9%.

6.4 Spoilage

The ASCA handbook on fermented foods in the region ascribes shelf lives to various fermented fish products ranging from 2 weeks in the case of some of the low-salt lactic fermented products to 5 years for some of the high salt sauces and pastes (16). Clearly, the salt levels in the latter are sufficient to rule out microbiological spoilage in all but the most exceptional of cases. Any loss of acceptability must arise from chemical or physical changes occurring over prolonged storage.

There is little published information on the factors that limit the shelf life of the low-salt products, but they are likely to behave similarly to lactic fermented products produced from other commodities. Excessive growth and acid production by the LAB could render products too sour for acceptability, but in most cases it is probable that yeast and mould growth would be an important cause of spoilage. The growth of yeasts and moulds could, in turn, lead to an increase in pH, allowing other spoilage organisms to grow. One study found that any effective measure to exclude air from a fermenting fish/carbohydrate mix prevented yeast growth (23). Traditional packing techniques used with lactic fermented fish products, such as wrapping in banana leaves, will not reliably exclude air, and surface mould growth has been shown to be a cause of rejection in the Thai product *som-fak* (24). This suggests that use of oxygen-impermeable wrapping films could extend the shelf life of such products. A combination of irradiation and chill storage has been shown to prevent both over-acidification and fungal growth in *som-fak*, markedly extending the shelf life (25).
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Products where a substantial population of moulds and yeasts is deliberately added in the form of saccharified rice or a starter culture would spoil as a result of their continuing activity breaking down the rice and producing unacceptable levels of alcohol in the product.

6.5 Pathogens: Growth and Survival

Many fermented fish products are stored at ambient temperatures, typically 30 °C, and consumed without cooking. Their safety therefore is critically dependent on the quality of the raw materials used and the inhibitory effects of the fermentation process.

There is little in the way of documented information associating fermented fish products with foodborne illness. In the fish sauces and pastes, the high salt concentrations preclude the growth of all food-poisoning bacteria and those initially present would not survive well the prolonged exposure to high salt during production. Improper storage of fish prior to processing could result in the production of toxins capable of persisting through to the product, but we are unaware of any reports of this happening.

The fish/salt/carbohydrate products depend on the combination of salt and pH/lactic acid for their safety. After capture, fish can potentially be contaminated with almost any foodborne pathogen as a result of improper handling and storage, and failure to achieve a sufficient combination of inhibitory factors could lead to their growth or survival. The bacterial pathogens most commonly associated with the living environment of fish, however, are *Vibrio parahaemolyticus* and *Clostridium botulinum*.

*V. parahaemolyticus* has been reported growing down to a pH of 4.8 and at salt levels as high 10%, although these limits apply when all other factors are optimal (26). The combinations of pH and salt in many fermented products are likely to preclude growth of the organism completely and, under conditions inimical to growth and high ambient storage temperatures, viable numbers will probably decline quite rapidly.

Because of the severity of the illness it causes, outbreaks of botulism are the only well documented instances of illness associated with fermented fish products. The Scandinavian fermented fish product rakefisk was responsible for 16 (84%) of the outbreaks of botulism recorded in Norway in the period 1961 - 90. During fermentation, the pH decreases below 5 and safe processing is recognised to depend on proper salt levels and temperature control until the pH drops. The epidemiological evidence points to greater awareness of the importance of these factors since outbreaks have declined over recent years (27).

In Japan, fish products are the most common source of botulism, and the most frequently implicated product is the fish-salt-carbohydrate fermented product *I-zushi*, which accounted for 88% of outbreaks in the period 1952 - 87. Several measures have been suggested for reducing the risk of botulism, such as soaking the fish at temperatures below 5 °C before fermentation and the use of starter cultures to ensure a rapid and reliable decrease in pH. As in Norway, outbreaks of
botulism have become less frequent in recent years, probably reflecting greater awareness of the critical factors that determine the product’s safety (27).

Botulism has also been associated with a number of traditional foods of the peoples of northern Canada, Greenland and Alaska. Many of these products are described as fermented, although the absence of any source of fermentable carbohydrate means that the process is putrefactive, giving an alkaline fermentation. Raw materials subject to this process include seal flipper, whale meat, walrus nose, beaver tail and paw, and salmon heads and eggs. They are packed into containers such as buckets or pits in the ground and left at ambient temperature to putrefy. The more widespread current use of non-traditional containers such as glass or plastic jars is thought to be one contributing factor (28). Salting levels are often insufficiently low to prevent growth of \textit{C. botulinum}, and warm weather can result in rapid growth of \textit{C. botulinum} and the production of toxin, which will persist into the product, which is generally consumed raw (29).

Foodborne trematode infections are emerging as a major public health problem, affecting an estimated 40 million people, particularly in south-east Asia and the western Pacific regions (30). Freshwater fish are the most important intermediate reservoir hosts of foodborne trematodes, and these are often used in the production of fermented fish products. Where these products are consumed without final cooking, they could act as an important vehicle of transmission. Infection with the trematode \textit{Opisthorchis viverrini} is associated with cholangiocarcinoma. A case-control study conducted in northeast Thailand showed that elevated levels of antibodies against \textit{O. viverrini} were a strong risk indicator and that consumption of fermented fish products was an independent risk factor in the contraction of cholangiocarcinoma (31). Studies to determine the effect of traditional processing techniques on the infectivity of metacercariae was identified as a priority research area by a WHO study group (30). An investigation of survival of the fish parasitic nematode \textit{Anisakis} in marinated herring has given some indication of what these studies might find. With this particular organism, salt content was found to have the greatest impact on the viability of larvae, while increasing the acetic acid content of the formulation had little or no effect (32). One study with the trematode \textit{Haplorchis taichui}, showed that inclusion of fermented fish in the Thai dish \textit{lab-pla} improved inactivation of the metacercariae, though 27% survival was still recorded (33).

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FERMENTED FISH


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7. FISH AND SHELLFISH TOXINS

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7.1 Introduction

Shellfish and finfish toxins continue to be a threat to public health and local fisheries around the world. In the last decade seafood monitoring has intensified and detection methods have continued to develop in selectivity and sensitivity. While some of these toxins only occur in seafood from a restricted geographical region, the export of exotic seafood that may contain such toxins can lead to unexpected public health issues. Increasingly sophisticated instrumentation (e.g. Liquid Chromatography/ Mass Spectrometry/ Mass Spectrometry -LC/MS/MS) is employed to identify and confirm the presence of known toxins, and is often necessary in light of the complex toxin profiles that can occur in shellfish tissue. Another important development is the introduction of sensitive and selective antibody-based detection methods for a number of toxins. Although most of these antibody-based test kits have yet to receive international regulatory approval, several scientific reports have described their efficacy and potential. An emerging technology is the use of immunosensors (1 - 3) and some practical applications for detecting shellfish toxins have been reported (4, 5). It remains to be seen if this approach gains momentum in the future. Nevertheless, more traditional methods such as the mouse bioassay continue to be used in many countries to warn of the presence of toxins, known and unknown.

Since the original chapter in this series was published (6), a number of new toxins and toxin families have been identified, most notably the azaspiracids and the cyclic imines as well as some additional polyethers. The manual on harmful marine algae and their products, produced by the Intergovernmental Oceanographic Commission (IOC) of UNESCO (United Nations Educational, Scientific and Cultural Organisation) has been updated (7), and a second updated edition dealing with the pharmacology, physiology and detection of marine and freshwater toxins has also appeared (8). Another book dealing with the clinical effects of a variety of poisons and toxins, including the well known marine toxins
has been produced (9). There is a recommendation from a joint FAO (Food and Agriculture Organisation of the United Nations)/IOC/WHO (World Health Organisation) report that marine biotoxins be classified according to their structural chemistry group rather than the symptoms they induce (e.g. Saxitoxin Group instead of the Paralytic Shellfish Poisons). An account of this meeting detailing recommendations and scientific advice on marine biotoxins has been published (10). A brief report on the status of rapid detection methods for phycotoxins in the European Union (EU) has appeared (11), and another, dealing with the detection methods and regulatory requirements for many marine toxins has been published (12). The remarkable frequency of neurotoxins from marine dinoflagellates has been noted (13), and a review on the risk assessment of marine toxins has also been produced (14). In addition, a number of useful and comprehensive reviews and book chapters have appeared dealing with various topics related to individual toxin groups and these are referred to in each appropriate section.

7.2 Paralytic Shellfish Poisoning (PSP) Toxins

The PSP toxins are arguably the most recognised group of marine toxins and continue to be regarded among the most dangerous, with intoxications occurring in many countries even today (15). Saxitoxin (STX) and neosaxitoxin (neoSTX) are the parent members of the group and are the most toxic. Around twenty PSP toxins are known, all of which contain a tetrahydropurine skeleton (Figure 7.1). Not all the toxins found in shellfish extracts are microalgal products; some are the result of biotransformation processes by the filter-feeding host. PSPs are water-soluble and heat stable so cooking has little or no effect on PSP toxicity. The N-carbamoyl sulfates are readily hydrolysed to STX, neoSTX and Gonyautoxins (GTX) 1-4. All PSPs degrade rapidly in alkaline conditions.

7.2.1 Origins and distribution

The PSP toxins are produced by several dinoflagellate species belonging to the genera *Alexandrium*, *Gymnodinium catenatum*, and a tropical species *Pyrodinium bahamense* (16). Many of these species are extremely common and are found throughout the world in a variety of cold, temperate, and sub-tropical waters and habitats (15). Consequently, a great variety of shellfish from all latitudes, even crustaceans such as lobsters, can become contaminated with these toxins.
7.2.2 Toxicology

PSP toxins affect the central nervous system of mammals, and humans seem to be particularly susceptible. PSPs bind to site 1 of sodium channels reversibly blocking sodium conductance in nerve and muscle membranes by binding to a specific receptor site located on the outside surface of the membrane close to the ion channel opening. Neurophysiological studies have shown that PSP toxins all possess the same mode of action though some derivatives such as STX and neoSTX are considerably more potent than the C-toxins (15). However, the less potent C toxins are readily hydrolysed by acid to the more potent GTX toxins. Human symptoms begin with tingling and numbness of the lips, tongue, and fingers within 5 - 30 minutes of consumption, leading to paralysis within a few hours and in extreme cases death by asphyxiation due to respiratory paralysis. Cooking does not destroy these compounds and although some of the toxins may be extracted into the cooking water, this is never sufficient to render the seafood safe to consume.
7.2.3 Detection methods

The safe limit set by most countries for PSP toxins is 800 \( \mu g/kg \) (0.8 \( \mu g/g \)) of shellfish or seafood tissue. The mouse bioassay has been in place for decades and the method has been standardised, and while simple and relatively inexpensive to operate, the detection limit (4 \( \mu g/g \)) of the method is close to the safe level and does not provide any information on the profile of PSP toxins present in the extract. However it remains as the monitoring method of choice in many countries. There continues to be a significant effort applied to the development of alternative chemical and biochemical detection methods for these toxins. One approach has been to take advantage of the ready conversion of these toxins to fluorescent derivatives. Two approaches have been taken here, namely oxidative conversion to fluorescent derivatives after High Performance Liquid Chromatography (HPLC) analysis (post-column derivatisation), or conversion before HPLC separation (pre-column derivatisation) and these have recently been thoroughly reviewed (12, 15, 17). Early post-column methods were somewhat time-consuming and required three separate HPLC runs to complete the analysis. Alternative HPLC methods have subsequently reduced this to a single HPLC run. After some development and various trials, a pre-column method has received Association of Analytical Communities (AOAC) approval (18), and has also been evaluated by a number of EU laboratories. While the chemical detection methods are most promising for the future, a criticism has been that such methods are time consuming, and more standards of individual PSP toxins are required to validate the complete profile to toxins that could occur in contaminated shellfish tissue. An antibody-based kit that can detect STX in shellfish is commercially available (RIDASCREEN®), and a second rapid dipstick kit (MIST Alert™) is available for the qualitative detection of positive and negative samples (19). The latter test is accepted by the United States Food and Drug Administration (US FDA) for use in the United States National Shellfish Sanitation Program. This kit was found effective in all the samples tested in Europe (20) and was applied recently in a survey of Scottish shellfish (21).

7.3 Tetrodotoxin

Tetrodotoxin (TTX) possesses a unique chemical structure and was the first marine toxin to be chemically characterised. Since its discovery, other derivatives have been reported, and over ten analogs are known (22, 23, 24) (Figure 7.2).

7.3.1 Origins and distribution

Unlike many other marine toxins there is no temporal distribution of TTX episodes. Most intoxication events have occurred in South East Asia, including Japan, Thailand, Taiwan, China, as well as other parts, and islands of the South Pacific. TTX most commonly occurs in many species of puffer fish, specifically
in the liver, gonads and roe (24). In Japan, where puffer fish of the genus *Fugu* are a delicacy, it is served in speciality restaurants with qualified chefs to properly prepare the fish. However TTX has been found to occur in a remarkable variety of genetically unrelated species including gastropods, the blue-ringed octopus, molluscs, horseshoe crabs, and even newts, raising speculation that TTX is produced by a bacterial source. This was indeed confirmed and a number of marine *Vibrios* have been shown to be capable of TTX production (24, 25, 26). Since then, this has expanded to include bacteria within the *Alteromonas* and *Pseudomonas* genera, and more recently by an actinomycete *Norcardiopsis dassonvillei* (26).

![Fig. 7.2. Structure of tetrodotoxin](image)

**7.3.2 Toxicology**

Like the PSP toxins, TTX acts on the central nervous system by binding extracellularly to site 1 of voltage-gated sodium channels, and blocks diffusion of sodium through the sodium channel, preventing depolarisation and propagation of action potentials in nerve cells. All of the observed toxicity is secondary to the action potential blockade. The onset of symptoms usually occurs rapidly, and in mild cases results in distal muscle weakness, nausea and vomiting. Severe poisoning causes paralysis and respiratory failure, and ingestion of larger amounts leads to cardiovascular effects including bradycardia, hypotension, respiratory failure and coma. An oral dose of 1 - 2 mg of purified toxin can be lethal.

**7.3.3 Detection methods**

TTX can be extracted from suspect tissue using dilute acid conditions, and once again the mouse bioassay has been used to detect the presence of the toxin. A review of TTX detection methods has been published (12, 23, 27). Chemical methods for TTX detection have also been investigated and are mainly based on its smooth conversion by heating in an alkaline solution to a detectable fluorescent (C9) base. Typically this has been accomplished using HPLC followed by post
column oxidation to the fluorescent derivatives. However this method has been 
criticised for being complex, and more rapid LC-MS methods [(M+H)\(^+\) m/z 320] 
have been reported for the detection and identification of TTX and its various 
derivatives (27). Immunoassay methods have also been investigated, and while 
promising, need further development (24).

7.4 Amnesic Shellfish Poisoning (ASP) Toxins

The parent compound of this group is domoic acid (DA), a polar glutamate 
agonist. Since the original episode of human poisoning following consumption of 
DA-contaminated shellfish in Canada in 1987 (28), both the toxin and the diatoms 
capable of producing it have been found to be distributed worldwide. Currently 12 
isomers of DA have been reported (see Figure 7.3 for examples), but all of them 
are considerably less toxic than DA itself (29). These isomers arise as a result of 
isomerisation and location of the double bonds in the side chain of the molecule, 
but it is known that such congeners are less toxic than DA itself (30).

Fig. 7.3. Structure of domoic acid and three naturally occurring photoisomers

7.4.1 Origins and distribution

The cosmopolitan diatom *Pseudonitzschia multiseries* was identified as the culprit 
organism in the 1987 poisoning event (31). Surveys by a number of groups have 
identified over a dozen species of *Pseudonitzschia* capable of producing DA, but
only three strains (\textit{P. multiseries}, \textit{Pseudonitzschia australis}, and \textit{Pseudonitzschia seriata}) are considered to be high producers of DA (>10 pg/cell). Levels of DA in the other strains are generally <1 pg/cell. The original 1987 event was followed some years later by a more widespread episode along the western coast of the US and today toxin-producing strains of \textit{Pseudonitzschia} have been found in the Gulf coast of the US and along the western shores of the US and Canada. \textit{Pseudonitzschia} and DA have been detected in many other maritime locations in the north Atlantic and the North Sea where it appears around the coast of the UK, southern and western portions of Ireland, the Mediterranean, and in locations around New Zealand (32, 33). Recently, another diatom species \textit{Nitzschia navisvaringica}, found in the Pacific around Vietnam, the Philippines, Okinawa and more northerly parts of Japan, has been reported as a medium-level producer of DA (33).

To a great extent, the wide distribution of toxin-producing strains of the diatom have resulted in the frequent occurrence of DA in many filter feeding organisms such as mussels, scallops, and clams. Another factor is the movement of the toxin through the food chain, which has been well documented in studies along the west coast of the US. For example, herbivorous fish become toxic after ingesting strains of \textit{Pseudonitzschia} spp., and this has led to ASP toxicity in birds, cormorants and pelicans (34), and sea animals such as sea lions and even whales (35).

\textbf{7.4.2 Toxicology}

DA is an acidic amino acid and potent neurotoxin unaffected by cooking or steaming. Indeed, it has been reported that cooking or steaming contaminated mussels merely has the effect of spreading the toxin to other tissues of the shellfish as a result of disruption of the digestive gland. When DA was identified as a new marine toxin it was quickly determined to be a glutamate agonist that binds to a sub-class of neuronal receptors called kainite receptors, and review of the toxicologic pathology of DA has been published (36). The oral absorption of DA is around 5 - 10% of the administered dose, and is mainly distributed in the blood. Penetration of the blood brain barrier is poor in healthy subjects, but any impairment of the blood brain barrier results in additional risk, as does impaired renal function which increases serum concentration and residence time of the toxin. Victims display a variety of symptoms including headache, nausea, seizures, disorientation and coma. Four deaths were attributed to the Canadian incident and years later a few seriously affected individuals still display chronic neurological deficits, including persistent short term memory deficits (36 - 39). The effects of DA may be augmented by the excitotoxic effects of glutamate and aspartate in the sample.
7.4.3 Detection methods

DA is water soluble and can be extracted from shellfish tissue by the acidic PSP extraction method although this may result in some decomposition. Alternatively, shellfish tissue can be efficiently extracted with aqueous methanol (1:1), and this is typically the method of choice. Provided the concentrations in shellfish are high enough (>50 µg/g shellfish tissue), DA can be detected by the mouse bioassay but the regulatory limit for shellfish is set below this level at 20 µg/g (20 mg/kg) shellfish tissue. Fortunately DA is conveniently detected by a variety of LC-linked analytical methods and this is the recommended approach for DA monitoring in EU countries. The sensitivity of the LC/UV (ultra violet) method (detection limit ~ 1 µg/g) can be improved (20 - 30 ng/g) with a rapid clean-up step using a strong anion exchange resin, as can LC/MS, and these methods have been reviewed (40, 41). Detection levels can be further lowered by conversion of DA to the fluorescent fluoresceinmethoxycarbonyl (FMOC) derivative, though this method is most commonly used to analyse plankton and seawater (42). A rapid immunodiagnostic kit is now available to detect DA in phytoplankton and is reported to work satisfactorily in this application in the field (43). An Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (Biosense) for DA has been approved by the AOAC, and an immunosensor method has also been reported (4). Of additional interest is the use of molecular probes originally developed to detect the presence of cells of potentially toxic *Pseudonitzschia* spp., in Monterey Bay, California. The probes have been employed in trial studies in New Zealand (44) and Scotland (45) to detect *P. australis*, although in the latter study the probes for *Pseudonitzschia pungens*, *Pseudonitzschia fraudulenta* and *Pseudonitzschia delicatissima* were found to be less effective.

7.5 Diarrhetic Shellfish Poisoning (DSP) Toxins

The active agents responsible for DSP incidents are a group of polyethers that include okadaic acid (OA), dinophysistoxin-1 (DTX-1), and dinophysistoxin-2 (DTX-2) (Figure 7.4). In the context of this chapter, the term “DSP toxins” will only be used to refer to these three polyether compounds and any derivative based on them. Since their first characterisation it was believed that the only causative agents of this group were OA and DTX-1 (45), but just over 15 years ago a third toxin, DTX-2 was discovered in contaminated Irish shellfish (46). Since then, DTX-2 has been found to be extremely widespread throughout many European maritime countries and is often the major DSP toxin present. DSP toxins are produced by dinoflagellates of the genera *Prorocentrum* and *Dinophysis* (47, 48, 49). The benthic *Prorocentrum* spp. are amenable to laboratory culture and consequently are used in many experiments involving studies of DSP toxins. However, the heterotrophic dinoflagellates *Dinophysis* spp. are the more potent source of toxins – often very low cells/ml of seawater can result in human poisoning, and consequently were often overlooked as the real source of the toxins. To further complicate matters, *Dinophysis* spp., produce a variety of other...
lipophilic compounds that are toxic in the mouse bioassay, but do not have the same mechanism of action as DSP toxins nor induce the same symptoms. Lately these compounds, which include the pectenotoxins and the yessotoxins, have been referred to as lipophilic shellfish poisoning toxins (LSPs or LSTs) (49) because they are found in the same lipophilic fractions as the DSP toxins and are described in more detail later in this chapter. The parent acids are the only DSP congeners to be found in shellfish tissue, although occasionally fatty acyl esters linked through the 7-OH group (referred to as DTX-3 compounds) are also found and are likely a result of bioconversion in shellfish tissue. In comparison, analysis of Prorocentrum cultures have uncovered an additional series of diol esters of OA and sulphated diesters (called DTX-4, DTX-5, and DTX-6) all of which are rapidly converted to OA when the plankton cells are ruptured (48). While these esters are not toxic in vitro, they can be converted to the parent toxins by non-specific esterases or lipases either in the producing organism or in the host shellfish.

![Structure of okadaic acid and the two related DSP toxins](image)

**Fig. 7.4. Structure of okadaic acid and the two related DSP toxins**

### 7.5.1 Origins and distribution

The first member of the group was identified in contaminated Japanese shellfish in 1985 and DSP toxins continue to be a threat in those areas. In this case *Dinophysis* was suspected as the culprit organism. This was probably not the first DSP episode, and anecdotal evidence now suggests that several seafood poisoning episodes during the 1960s and 1970s in the Netherlands, Norway and Japan were likely due to DSP toxins produced by a *Dinophysis* spp. Today over ten *Dinophysis* spp. are recognised as DSP toxin producers, and include *Dinophysis acuminata*, *Dinophysis acuta*, *Dinophysis fortii*, *Dinophysis caudata*, and *Dinophysis norvegica* among others (47, 49). DSP toxins produced by benthic *Prorocentrum* spp., such as *Prorocentrum lima*, *Prorocentrum maculosum*, and *Prorocentrum belizeanum*, are also recognised as a threat to human health.
Perhaps as a consequence of these multiple sources of DSP toxins, DSP episodes have been reported worldwide and are included in many monitoring programs (47). Since the late 1970s, DSP episodes have been reported from Japan, various European countries, New Zealand and Chile.

7.5.2 Toxicology

The DSP toxins are potent inhibitors of the serine/threonine phosphatases PP1 and PP2A, both critical enzymes involved in many key metabolic pathways in mammalian cells (50) and consequently affect a host of other biological processes in a cell including tumor promotion. All the DSP toxins have around a thousand-fold greater affinity for PP2A than PP1, although a comparative study of in vitro phosphatase inhibition with mouse bioassay data has shown that OA is about twice as potent as DTX-2 (51). Human symptoms include diarrhoea, nausea, vomiting, and abdominal pain. The onset of symptoms can occur within 30 minutes of consuming contaminated shellfish and generally last about 2 - 3 days.

7.5.3 Detection methods

Once again the mouse or rat bioassay is relied upon to detect DSP toxins in contaminated seafood, and while this provides critical seafood safety information, it does not provide information on the DSP toxin profile, or indeed on the presence of other associated LSTs that can occur with DSP toxins and which result in a positive mouse bioassay result (vide infra). At the first Meeting of the EU National Reference Laboratories on Marine Biotoxins and Analytical Methods and Toxicity Criteria in 1996, it was agreed that the established mouse bioassay (52) with an observation time of 24 hours is currently the preferred method for the detection of the acute toxicity of acetone soluble DSP toxins. A regulatory level of 80 - 160 µg OA equivalents/kg of whole shellfish tissue has been implemented in some countries. This regulatory level includes the possibility of LSPs in the DSP fraction being tested. Due to the lipophilic nature of the toxins, some careful clean-up steps are required to avoid such matrix interferences and to simplify data interpretation (48). Preparation of a fluorescent 9-anthryldiazomethane (ADAM) derivative followed by HPLC offers reasonable sensitivity, but the method is complex and matrix effects as well as interferences with other lipophilic compounds such as the pectenotoxins compromise the method, and a detection limit of 100 µg/kg has been reported (12). In recent years, most progress in DSP toxin detection has been using new or improved LC/MS methods and a detection limit of 10 µg/kg has been reported, even with the minimal of clean up steps (48). Immunoassays have been developed for OA detection but an inherent problem of these methods has been the low cross reactivity with DTX-1, and DTX-2, which can lead to an underestimation of the total DSP content in a sample. The 7-O acyl derivatives (DTX-3) of OA that can form in shellfish also show poor cross reactivity and this further exacerbates the problem. DSP-Check is a commercially
available ELISA test kit that can detect both OA and DTX-1 with comparable sensitivity, with a claimed detection limit of 20 µg/kg, and another rapid antibody-based test kit for DSP toxins has also been reported (53). In yet another approach, fluorometric protein phosphatase inhibition assays have been found to perform better than colorimetric assays, with good agreement with the mouse bioassay and LC-based methods (54, 55). If a European collaborative study of the fluorometric protein phosphatase inhibition method is successful it may eventually be approved for regulatory purposes in the EU.

7.6 Lipophilic Shellfish Toxins (LST)

Lipophilic extraction of toxic shellfish for DSP toxins often results in the simultaneous extraction of other lipophilic polyethers such as the pectenotoxins (PTXs) and yessotoxins (YTXs). Indeed, for many years these compounds were included in the DSP toxin complex, but with the increased availability of pure toxins permitting detailed toxicology studies it is now recognised that both these groups of compounds possess a different toxic profile from the DSP toxins. As a result these compounds are now being referred to as lipophilic shellfish toxins (LSTs) (49). Within this group, over 20 PTXs are known (56) and more than 30 YTXs have been reported (57, 58). Some representative examples of both groups of toxins are shown in Figures 7.5 and 7.6. Some doubt remains as to the significance of these compounds as marine or shellfish toxins with respect to human health since the PTXs for example are co-extracted with the DSP toxins, and the latter group are usually found to be responsible for any reported shellfish toxicity (59). However, they are toxic in the mouse bioassay and can result in false positives and hence are included here for completeness.

Fig. 7.5. Structure of the principal pectenotoxins and one of the seco-acid derivatives
Fig. 7.6. Structure of the two parent yessotoxins, YTX and homo-YTX

7.6.1 Origins and distribution

It is now well recognised that PTXs are co-produced with DSP toxins by various strains of the dinoflagellate Dinophysis and hence the distribution of both families of toxins in shellfish is similar (49). The sources of YTXs have now been identified as the dinoflagellates Protoceratium reticulatum and Lingulodinium polyedrum, which may co-occur in blooms with other organisms. As is typical for many algal species, the toxin content and profile may vary among different strains, but nevertheless the toxins are extremely widespread and have been found in various shellfish in Norway and Scandinavia, many parts of Europe and the Mediterranean, eastern Canada, Japan, New Zealand and South America (49, 57, 58).

7.6.2 Toxicology

PTXs show varied toxicity in the mouse bioassay (59). PTX-1 and PTX-2 are frequently the most common derivatives to occur and PTX-2 is the most potent following intraperitoneal (IP) injection (230 μg/kg body weight). Oxidation of the methyl group at C-43 to a carboxyl function as in PTX-6 significantly reduces toxicity (500 μg/kg body weight) as does hydrolysis to PTX-2 seco acid (>5000 μg/kg body weight) a common metabolite found in shellfish extracts. While
demonstrating considerable toxicity following IP injection, the PTXs show considerably less toxicity when administered orally, and this is believed to be due to hydrolysis of PTX-2 to the seco acid derivative. Some of the PTXs are potent cytotoxins \textit{in vitro} \cite{60}. There are no clear cases of human illness resulting from ingestion of PTXs \cite{59}. The YTX group has not been reported to cause human illness, but various congeners are acutely toxic in the mouse bioassay following IP injection, but less so following oral dosing \cite{61}. Estimates of acute toxicity of the parent compound following IP injection vary considerably, which is further compounded by differences in the experimental design. Their toxicology and mechanism of action remain uncertain, though its cell effects may be linked to modulation of calcium channels \cite{62}.

### 7.6.3 Detection methods

Typically these toxins are co-extracted with DSP toxins following acetone or aqueous methanol extraction of the shellfish tissue. The crude extract may be used directly in the mouse bioassay. The EU has recommended an extraction and solvent partition process to separate the polar YTX compounds from the more lipophilic PTX and DSP compounds. Acetone extracts of shellfish tissue are dried and re-suspended in 60% aqueous methanol before partitioning against dichloromethane. The YTXs are retained in the aqueous methanol fraction while the PTX and DSP toxins are concentrated in a dichloromethane fraction. However, the PTXs are susceptible to bioconversion in shellfish tissue and consequently it is often desirable to determine the profile of toxins present. While LC/UV detection is possible for PTXs, it may be compromised by other biological materials in the matrix. The regulatory level of 160 µg/kg shellfish tissue has been established and the mouse bioassay is often the primary detection method although LC/MS methods are preferred and this requires some simple clean-up of the crude extract prior to chemical analysis. The structures and chemical data including the molecular weight of each member have been collated \cite{56}.

Many YTX toxins contain a conjugated diene system, which aids detection by LC/UV, but a fluorescent derivative has been prepared for others. Because of the number and complexity of the YTX group, some of which are found as glycosides and sulfates, chemical analysis is best carried out by LC/MS and several approaches have been described \cite{57,58}. Although there is no evidence that yessotoxins are responsible for human illness, a regulatory level of 1 mg/kg shellfish tissue has been set.

#### 7.7 Neurotoxin Shellfish Poisoning (NSP) Toxins

NSP episodes are caused by a family of fused polyether compounds known as the brevetoxins \cite{63,64}. There are two skeletal types known as PbTx-1 (or brevetoxin A) and PbTx-2 (brevetoxin B) (Figure 7.7). For many years it was believed that
NSP toxins only occurred in the Gulf of Mexico and along the western coast of Florida, but in 1992/1993 they were identified as the culprits in a human poisoning event in New Zealand (65, 66). They have also been associated with fish kills along the eastern mid-Atlantic coast of the US. A complicating factor both in terms of establishing the profile and toxicity of brevetoxin-contaminated shellfish has been the discovery of the so-called “brevetoxin metabolites”. These are biotransformation products of principally PbTx-2 by addition of certain amino acids to the conjugated aldehyde function (66).

![Structure of the two parent brevetoxins, PbTx-1 and PbTx-2, and one of the brevetoxin metabolites BTX-B2](image)

**Fig. 7.7. Structure of the two parent brevetoxins, PbTx-1 and PbTx-2, and one of the brevetoxin metabolites BTX-B2**

### 7.7.1 Origins and distribution

The brevetoxins are produced by the dinoflagellate *Karenia brevis* (formerly known as *Gymnodinium breve* and *Pytochodiscus brevis*) a dinoflagellate found in the Gulf of Mexico, and off the coast of New Zealand (65). Interestingly, the same toxins have been reported to be produced by three genera of Raphidophytes, namely *Chatonella marina*, *Fibrocapsa japonica*, and *Heretosigma akashiwo*, which are found in Japanese waters (66). Many of these species are associated with massive fish kills, and recently the major toxin associated with an ichthyotoxic unialgal bloom of *Chatonella cf. verruculosa* off the mid Atlantic coast of the U.S. was chemically characterised as PbTx-2 (67). In the Gulf of Mexico, brevetoxins are found in mussels, oysters, whelks, and cockles (65, 66),
although recently it has been shown that herbivorous fish can store brevetoxins in their flesh (68).

7.7.2 Toxicology

Brevetoxins bind to site 5 of voltage-gated sodium channels, which can result in a cascade of associated events in a cell (66). The human health implications following exposure to NSPs have been reviewed (69). Ingestion of brevetoxins results in a variety of symptoms including parathesia, vertigo, malaise, nausea, and diarrhoea, and in severe cases seizure. The mean time to onset of symptoms is around 3 hours, with a mean duration of symptoms of around 1 day. Exposure to brevetoxins can also occur through inhalation of brevetoxin aerosols resulting in irritation of the upper respiratory tract (65, 66, 69). The brevetoxin metabolites are reported to be less toxic than the parent compound (70).

7.7.3 Detection methods

Three basic approaches for the detection of NSP toxins have been recently reviewed (71). The mouse bioassay is used as the primary detection method, and an action level has been set at 800 µg/kg (or 80 µg PbTx-2 equivalents/100 g or 20 MU/100 g or 4 µg/mouse) of shellfish tissue to trigger regulatory action. While the mouse bioassay continues to be used, considerable progress has been made in the development of LC-MS methods to routinely monitor for brevetoxins in shellfish. In this application, pre-treatment or clean-up of the shellfish sample is essential in order to obtain reproducible results, but this method is now used routinely in New Zealand and the US to monitor for the presence of brevetoxins (68). A second generation competitive ELISA assay has been developed that has successfully quantified brevetoxins in a variety of biological and environmental matrices including seawater, shellfish homogenates, and mammalian body fluids without pretreatment or dilution (72). In a comprehensive multi laboratory evaluation of five different detection methods as possible replacements for the mouse bioassay, the competitive ELISA method correlated most closely with the mouse bioassay results, and it has also been used in studies to quantify occupational and recreational exposure to aerosolised brevetoxin exposure (73).

7.8 Ciguatoxins

Known for centuries and originally referred to as ciguatera by Spanish explorers in the Indo-Pacific ocean and Caribbean, this family of toxins has proven to be a challenge both to identify and monitor. Outbreaks of ciguatera poisoning are confined to tropical and sub-tropical regions of the ocean, with hot spots in the coral reef areas of the Caribbean and French Polynesia. Ciguatera toxins (CTXs) are extremely potent and occur in finfish rather than shellfish, beginning with herbivores that feed on benthic dinoflagellates associated with reefs and which are
transferred through the food chain to the larger carnivores. CTXs are large, complex, lipid soluble polyethers, and only a few structures have been identified, but more are suspected to exist, most likely a consequence of biotransformation of the parent toxins as they pass up the food chain. Indeed even the chemistry of CTXs from the Pacific differs subtly from those found in the Caribbean (Figure 7.8). The diversity of benthic phytoplankton that exist on reefs further complicates the picture. Culprit species producing CTXs may simultaneously produce unrelated toxins such as maitotoxin, and other genera of neighbouring reef dinoflagellates can further contribute to an extremely complex profile of toxins in finfish. A detailed comprehensive review of ciguatoxins has recently been published (74).

Fig. 7.8. Structure of the Pacific variant of ciguatera toxin (P-CTX-1) and the Caribbean variant (C-CTX-1)

7.8.1 Origins and distribution

It is recognised that several species of benthic dinoflagellates can produce CTXs and the gambiertoxins, a group of closely related polyethers that are considered the source of ciguatoxins. These include species belonging to the genera Ostreopsis and Coolia, although the dinoflagellate Gambierdiscus toxicus is considered to be the major source of these toxins. Such microalgae contribute to the primary production of tropical and sub-tropical reefs and live in epiphytic association with bushy red, green and brown seaweeds and also occur in sediments and coral debris. Distribution through the food chain begins with
grazing by herbivorous fish including the sturgeon fish and parrotfish, which in turn are consumed by carnivores such as snappers, groupers, barracudas, sharks, and moray eels. Local island populations attempt to avoid poisoning by catching only smaller fish, but fish toxicity can vary widely depending on where the fish are caught and how long they have been feeding in that area. Nevertheless CTX poisoning continues to be a threat to local and visiting populations, and there are suggestions that such incidents are increasing and spreading worldwide (74).

7.8.2 Toxicology

Ciguatoxins (and maitotoxin which is often co-produced with CTXs) are extremely poisonous, although not all CTXs are equipotent, and it is estimated that ingestion of as little as 0.1 µg of ciguatoxin can result in human illness (74, 75). Like the brevetoxins, they bind to voltage-dependant sodium channels in the cell membrane causing them to open, and inducing membrane depolarisation resulting in prolonged symptoms indicative of damage to nerve tissue. Differences in potency among the CTXs correlate directly with their binding affinity for the sodium channel. The pharmacological effects following CTX exposure are numerous and include severe gastrointestinal distress and neurological symptoms, all of which can be linked to the effect of CTX on excitable membranes. The onset of symptoms can occur rapidly within 24 hours, and although the gastronomical problems may dissipate within a day or so, the neurological symptoms can persist for days, weeks or even months (74 - 76). These include paresthesias (numbness and tingling of the extremities), and temperature sensation reversal, where cold objects feel hot and vice versa. Other symptoms include a metallic taste, pruritus, arthralgia, myalgia, and the sensation of loose teeth (77). Another symptom that can develop over time is extreme fatigue, which can be confused with chronic fatigue syndrome. Patients have reported symptoms lasting weeks and even months, which can return following the consumption of alcohol and certain foods such as oily fish (74, 75, 78).

7.8.3 Detection methods

As a consequence of their potency, only trace amounts of CTXs can cause illness following consumption of contaminated fish, and in fact CTX poisoning is often only identified after consumption of contaminated fish. Even when suspect samples are available, the absence of a strong chromophore means that simple detection methods based on UV or fluorescence-based detection methods are ineffective. Globally, there are neither standards nor an official testing program, though the mouse bioassay continues to be relied upon as the most common method of detecting CTXs. The recommended method to extract fish tissue has been described in detail (74) and the initial warm acetone extract is partitioned against hexane and diethyl ether where the CTXs are concentrated. A good knowledge and careful observation of the symptoms in the bioassay (Minimal
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Lethal Dose (MLD) 0.25 µg/kg) can provide useful clues as to the toxins present but more specific and informative assays are required. Immunoassays may be an alternative detection method (79), although specificity and sensitivity remain major hurdles (74) and the presence of DSP toxins affects the test (80). A sodium channel-specific assay using mouse neuroblastoma cells has been described although this approach has still to be developed in a routine, high-throughput screen (74). Recently, another in vitro sodium channel-based assay, specific for CTX activity, has been developed, but this only has application in a clinical setting (81). Chemical detection methods are severely compromised by the lack of standards, and the separation of these polyethers from the lipid-soluble fraction of fish provides an additional challenge. Consequently, only a few laboratories are able to undertake such analyses, which are based on an LC-MS/MS approach. Acetone extracts of fish tissue are subjected to solvent partitioning, followed by clean-up on silica and aminopropyl cartridges. This is then subjected to LC-MS/MS analysis, which features characteristic fragment ions corresponding to sequential losses of three molecules of water. In this way Caribbean and Pacific CTX-1 compounds can be distinguished (74).

7.9 Azaspiracids

Azaspiracid (AZA1) was originally identified in 1995 as the causative agent for a poisoning episode in Ireland, following consumption of contaminated mussels Mytilus edulis (82). Recent reviews of this new group of toxins have appeared (83, 84), and another dealing with the chemistry and detection methods has been published (85). The original structure proposed revealed an entirely new class of shellfish toxin, and following synthesis of the molecule some time later, minor revisions of a few stereocenters were made, and the corrected structure was published in 2004 (86) (Figure 7.9). Since the original discovery and identification of the toxin, almost 20 additional derivatives have been reported (83 - 85). The majority of these have been proposed based on LC/MS analytical data, and only a few structures (AZA 1 - 5) have been fully characterised by Nuclear Magnetic Resonance (NMR) and MS analysis. Usually the major AZA derivatives found in shellfish are AZA1 (50 - 80%), with AZA2 (10 - 30%) and AZA3 (5 - 20%) present in lesser amounts (Figure 7.9). Usually the additional AZA derivatives found in shellfish tissue are hydroxylated derivatives (e.g. at C-3 and C-23), and it is likely that most of these compounds are biotransformation products formed in the shellfish tissue.
7.9.1 Origins and distribution

The azaspiracids were first isolated from contaminated shellfish, and to date this has been the only source from which these toxins have been found at toxic levels. However, as in other cases of shellfish toxins, these molluscs were never considered to be the biogenetic source. There has been considerable debate over the source of azaspiracids, and some time after the original episode in Ireland, it was reported that the heterotrophic dinoflagellate Protoperidinium cassipes was the planktonic source of the toxins (87). While this result is not necessarily in debate, it has been impossible to establish that P. cassipes produces AZAs in culture, and variable field results have led to suggestions that the actual producing organisms are Dinophysis spp. which are ingested by P. cassipes (83). In fact the routes of trophic transfer of AZAs through the food chain ending in contaminated shellfish remain unknown. Following the original episode, AZA occurrence has been reported by several European countries including France, Portugal, Scandinavia, and off the coast of Morocco (83, 84, 88, 89). The bivalves affected include mussels (Mytilus edulis, Mytilus galloprovincialis), oysters (Crassostrea gigas, Ostrea edulis), scallops (Pecten maximus), clams (Tapes philippinarum, Ensis siliqua, Donax spp.), cockles (Cerastoderma edule), and most recently in a crustacean (Cancer pagurus).

7.9.2 Toxicology

Consumption of AZA contaminated shellfish can result in acute symptoms that include nausea, vomiting, diarrhoea and stomach cramps, which can persist for 2 - 3 days, and to date no long-term effects have been recorded (83, 90). These symptoms are not unlike those experienced following the consumption of DSP toxins, but current data indicates that unlike the DSP compounds, AZA toxins do
not inhibit the phosphatases PP1 and PP2, though the possibility exists that they may inhibit other phosphatases. The limited amounts of AZA toxins available continue to hinder a complete understanding of their mechanism of action. Injection of a lethal dose of AZA1 in mice (>150 µg/kg) caused vacuole formation and fatty acid accumulation in hepatocytes, parenchymal cell pyknosis in the pancreas, abnormalities in the thymus and spleen, and erosion and bleeding in the stomach. Importantly these pathological outcomes were reported as being different from those caused by DSP, PSP, and ASP. Oral administration of AZAs at substantially higher doses than those used in IP injection studies did not result in any mouse mortalities after 24 hours, although autopsies conducted after 4 and 8 hours revealed various gastrointestinal (GI) abnormalities including accumulation of fluid in the ileum, and necrosis of epithelial cells on the microvilli (91). Based on IP minimum lethal dose data, it is suggested that the order of toxicity is AZA2 (110 µg/kg) > AZA3 (140 µg/kg) > AZA (150 µg/kg). Using the same approach, it is believed that the hydroxylated derivatives AZA4 (470 mg/kg) and AZA5 (<1000 mg/kg) are considerably less toxic. Once again, the lack of sufficient material and other logistical problems have prevented complete studies of any chronic effects arising from long term exposure to low levels of the AZA toxins. AZAs also display cytotoxicity towards various mammalian cell lines (83, 91, 92).

7.9.3 Detection methods

The regulatory level for AZAs in shellfish has been set by the EU at 160 µg AZA equivalents/kg shellfish tissue. The mouse bioassay has been used to monitor shellfish extracts, but unlike DSP monitoring methods which use only the hepatopancreas of the shellfish for extraction, it is recommended that the entire shellfish be used for AZA analysis since it has been shown that these toxins can migrate to other tissues. Currently LC/MS and LC-MS/MS analysis is the preferred chemical analytical detection method for AZAs, which are particularly amenable to detection by positive ion electrospray mass spectrometry. Key fragment ions are useful in identifying the various AZA compounds that can occur in shellfish tissue (83, 85). However, interferences can arise when analysing a matrix as complex as a crude shellfish extract and it is recommended that a simple solid phase extraction (SPE) step be introduced prior to LC/MS analysis (93).

7.10 Cyclic imines

These toxins are represented by several distinct structural types, but all contain a spirocyclic or cyclic imine function within their structure. This common cyclic imine feature is believed to be a common pharmacophore of this group of toxins which comprises the gymnodimines, the spirolides, the pinnatoxins, and closely related pterotoxins (Figures 7.10 – 7.12). Due to their relatively recent discovery and limited knowledge of their toxicological properties and impact on human
health, they are sometimes referred to as emerging toxins of uncertain human health concern, but they are included here for completeness. Cyclic imines are often referred to as fast acting toxins due to their “all or nothing” effect in the mouse bioassay over a very narrow dose range. In only two cases (gymnodimines and spirolides) has a planktonic source been identified. Reviews of the current status of the cyclic imine group were published by Molgo and Cembella (94, 95).

7.10.1 Gymnodimines

The observation of neurotoxic symptoms in the mouse bioassay of lipophilic extracts of dredge oysters (*Tiostrea chilensis*) collected from around the South Island of New Zealand, led to the identification of a new marine toxin with an unprecedented structure named gymnodimine (96) (Figure 7.10). The structure is novel in that it contains a spirocyclic imine group and a single tetrahydrofuran ring embedded within a macrocyclic structure. Some time later, two additional congeners; gymnodimine B and C were isolated from the dinoflagellate *Karenia selliformis* (formerly *Gymnodinium selliformis*). The gymnodimines are the smallest members of the cyclic imine group with a molecular weight range of 500 - 520 Da (94, 95).

![Gymnodimine structure](image)

Fig. 7.10. Structure of the cyclic imine gymnodimine

7.10.1.1 Origins and distribution

The original observation of toxicity in oysters led to a wider survey of other shellfish species in New Zealand when it was discovered that the toxin was present in a variety of bivalve molluscs including mussels, scallops, cockles, Pacific oyster, surf clam and New Zealand abalone. At the time of the initial observation of gymnodimine, it was proposed that a dinoflagellate identified as *Gymnodinium* spp., a species closely related to *Gymnodinium mikimoitoi* was the planktonic source of the toxin. Subsequent taxonomic studies revised this again to a new dinoflagellate *K. selliformis* (97).
7.10.1.2 Toxicology

A review of the toxicology of all cyclic imines has been published (98). The original observation of toxicity in New Zealand followed from IP injection of lipophilic extracts in the mouse bioassay. A later study using purified toxin established an LD₅₀ dose (Lethal Dose, 50%) of 96 µg/kg. There is a rapid onset of symptoms including respiratory distress, paralysis of the hind quarters and eventually complete immobility, abdominal breathing, and lack of responsiveness to stimuli. The time lapse between ingestion and death is between 5 and 15 minutes. Reduction of the imine group eradicated the toxicity, and short-acting cholinesterase inhibitors such as neostigmine or physostigmine protected mice against the toxic effects of gymnodimine. This led to the suggestion that gymnodimine acts by blocking nicotinic acetylcholine receptors at the neuromuscular junction. Oral administration of the toxin to mice by gavage resulted in about an 8- to 10-fold reduction of toxicity (LD₅₀ 600 - 900 µg/kg), and there was a further ten-fold reduction in toxicity when the toxin was supplied with food. Based on this diminished oral toxicity, together with a lack of evidence of human intoxication, it has been stated that regulation against gymnodimine is not required (98).

7.10.1.3 Detection methods

The characteristic “fast-acting, all or nothing” mouse bioassay response to gymnodimines and cyclic imines in general continues to be a useful monitoring approach. Most cyclic imines lack an extended chromophore, and so spectroscopic detection by UV methods is not favoured. However, cyclic imines are readily observed by electrospray mass spectrometry in the positive ion mode, and this is the detection method of choice. Whether isolated from dinoflagellate cells directly or from shellfish tissue, the first step in the process is typically an initial clean-up step using a short C18 cartridge and elution with water containing increasing amounts of methanol, finishing with 100% methanol. Fractions are then dried and re-suspended in methanol or acetonitrile in preparation for LC/MS analysis (94, 95).

7.10.2 Spirolides

The spirolides comprise the largest group of cyclic imine compounds. They were originally discovered in extracts of mussels (M. edulis) and scallops (Placopectin magellanicus) from eastern Canada that displayed abnormal symptoms in the mouse bioassay. In particular, extracts exhibited the fast acting symptoms that became associated with cyclic imines following IP injection. Since their original discovery which resulted in the characterisation of spirolides A-F (99,100), almost a dozen derivatives in total have been identified to date (94, 95) (Figure 7.11). The structure of the spirolides bears a strong resemblance to both gymnodimine and
the pinnatoxins (*vide infra*). Structural differences among the various spirolides result from variations in the number of methyl groups and the sequence of spiroketal rings 6-5-5 in one group and 6-6-5 in the other.

![Fig. 7.11. Structure of four spirolide toxins A-D](image)

**7.10.2.1 Origins and distribution**

At the time of the original discovery of the spirolides in shellfish from the coastal waters of eastern Nova Scotia, their localisation in the digestive gland of mussels and scallops led to the suspicion that plankton were the real source of the toxins. This proved to be the case and eventually the dinoflagellate *Alexandrium ostenfeldii* was identified as the culprit organism (101). Since then the spirolides have been found to be globally distributed and are found in shellfish or plankton fractions along eastern Canada, the gulf of Maine, in fjords in Denmark and Norway, southern Ireland, the western coast of Scotland, the Bay of Biscay and most recently from Mediterranean shellfish and plankton in the Adriatic (102). They have also been reported in the southern hemisphere in Chilean mussels and in New Zealand shellfish and plankton (94, 95).

**7.10.2.2 Toxicology**

The spirolides are the most studied group of the cyclic imines. The LD$_{50}$ values vary considerably depending upon whether the compound is administered by IP injection (5 - 8 µg/kg), by gavage (87 - 166 µg/kg; fasted mice), or by oral ingestion (381 - 707 µg/kg; fasted mice). All data reported here are for desmethyl spirolide C. While oral toxicity is considerably less than by IP injection, those spirolides possessing the vicinal dimethyl grouping (see Figure 7.11; R$_1$ = CH$_3$) of the cyclic imine ring are the most toxic of the group (98). Interestingly, these are the toxins that are also most resistant to acid hydrolysis of the imine function.
While the mechanism of action remains to be clearly defined, experiments have shown upregulation of muscarinic and nicotinic receptors in mice following dosing with spirolide C, consistent with modulation of acetyl cholinergic receptors (103).

7.10.2.3 Detection methods

As discussed for gymnodimine, the mouse bioassay can be used to detect these fast acting toxins. However, more useful information on the amount and distribution of toxins present in a shellfish extract is obtained by LC-MS analysis usually following a simple clean-up step. Once again this relies on the sensitivity of these cyclic imines to detection by positive ion electrospray mass spectrometry. A number of methods following this procedure have been described (94, 95, 102).

7.10.3 Pinnatoxins and pteriatoxins

Pinnatoxins A - B contain a spiro imine moiety similar to that found in the spirolides, and in fact these compounds share a number of other structural features. The compounds are zwitterionic and therefore somewhat more polar than the spirolides. To date, four members of the group have been identified – two of them being the C-34 diastereomers. The pteriatoxins were isolated from a different mollusc but bear a strong structural resemblance to the pinnatoxins (Figure 7.12). Three members of this group have been reported, which includes two diastereomers.

Fig. 7.12. Structure of the pinnatoxin group and the closely related pteriatoxins
7.10.3.1 Origins and distribution

Shellfish of the genus *Pinna*, which occupy tropical waters around Okinawa and the south east coast of China, have long been suspected of causing human illness after ingestion. This prompted a chemical investigation of *Pinna muricata* that ultimately led to the identification of the pinnatoxins in 1995 (104), and later the closely related pteriatoxins were isolated from the digestive glands of another Okinawan bivalve *Pteria penguin* (105). There is strong circumstantial evidence to suspect that these toxins, like the gymnodimines and spirolides, arise from an algal source - most likely a dinoflagellate, but this remains to be conclusively established (106). However, there is no compelling evidence to suggest that they have resulted in human toxicity (98).

7.10.3.2 Toxicology

There is little information describing the toxicology of these compounds (98). Only the acute toxicity in mice following IP injection has been reported. The LD$_{99}$ (Lethal Dose, 99%) values for pinnatoxins A-C are in the range (22 - 180 µg/kg), while pinnatoxin D was essentially inactive. The LD$_{99}$ dose for the pteriatoxins is reported to be in the range (8 - 100 µg/kg).

7.10.3.3 Detection methods

No formal detection methods for these compounds have been reported, but the mouse bioassay data and LC-MS can be used to monitor suspect seafood for these compounds.

7.11 Conclusion

There has been considerable progress in the detection of known marine toxins, which in many cases are now found to be more widespread than previously believed. This is generally a result of increased monitoring and continually improving detection technologies and methods. Coupled with this, new toxin groups such as the azaspiracids and cyclic imines have also been reported, once again reflecting improvements in purification and spectroscopic methods permitting structural identification of new and complex structures. Despite this progress, research and certified standards of the key toxins are often unavailable or in short supply and in the future considerable logistical effort will be required to address this issue.

With increasing knowledge of the chemistry of toxins, and more advanced means of detection, has come the realisation that a “toxin profile” can be a complex mixture of many different classes of toxins, and in some cases it is known that one toxin can potentiate or augment the toxicity of the other. While this is recognised as a real possibility for a wide range of toxins, to date action or
regulatory levels for toxins continue to be assessed only for individual purified toxins. In most cases this is due to the paucity of material that is usually available for any single group of toxins, never mind the amounts required of several groups, in order to fully study the synergistic effects of multiple toxin types.

While the mouse bioassay continues to be the mainstay of most monitoring programs, an encouraging development is the use of antibody-based methods to detect the presence of marine toxins. The challenge facing such approaches is the variety of matrices that must be examined, and in particular the ruggedness of such methods, but these are gradually being overcome. Even if they do not completely replace other methods such as the mouse bioassay or chemical analysis that require sophisticated chemical analytical instrumentation, they can provide a valuable “yes/no” filter for shellfish and finfish extracts, thus significantly reducing the number of mouse or chemical analyses that have to be performed.

7.12 References

FISH AND SHELLFISH TOXINS


FISH AND SHELLFISH TOXINS


FISH AND SHELLFISH TOXINS


FISH AND SEAFOOD


8. HACCP IN FISH AND SEAFOOD PRODUCT MANUFACTURE

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8.1 Introduction

The Hazard Analysis Critical Control Point (HACCP) system is a structured, preventative approach to ensuring food safety. HACCP provides a means to identify and assess potential hazards in food production and establish preventive control procedures for those hazards. A critical control point (CCP) is identified for each significant hazard, where effective control measures can be defined, applied and monitored. The emphasis on prevention of hazards reduces reliance on traditional inspection and quality control procedures and end-product testing. A properly applied HACCP system is now internationally recognised as an effective means of ensuring food safety.

The HACCP concept can be applied to new or existing products and processes, and throughout the food chain from primary production to consumption. It is compatible with existing standards for quality management systems such as the ISO 9000-2000 series, and HACCP procedures can be fully integrated into such systems. The new ISO 22000 food safety standard formally integrates HACCP within the structure of a quality management system. HACCP is fully integrated into the British Retail Consortium (BRC) Global Standard for Food Safety, and is one of the ‘fundamental’ requirements of that system.

The application of HACCP at all stages of the food supply chain is actively encouraged, and increasingly required, worldwide. For example, the Codex Alimentarius advises that “the application of HACCP systems can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety”.

In many countries, there is a legal requirement for all food business operators to have some form of hazard analysis based on HACCP as a means of ensuring food safety. For example, within the European Union, Regulations 852/2004 and
853/2004 require a fully operational, and maintained HACCP system, according to Codex, to be in place.

8.2 Definitions

Control (verb) - To take all necessary actions to ensure and maintain compliance with criteria established in the HACCP plan.

Control (noun) - The state wherein correct procedures are followed and criteria are met.

Control measure - An action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action - An action to be taken when the results of monitoring at the CCP indicate a loss of control.

Critical Control Point (CCP) - A step at which control can be applied and is essential to prevent or eliminate a food safety hazard, or reduce it to an acceptable level.

Critical limit - A criterion which separates acceptability from unacceptability.

Deviation - Failure to meet a critical limit.

Flow diagram – A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

HACCP - A system which identifies, evaluates and controls hazards which are significant for food safety.

HACCP Plan – A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for safety in the segment of the food chain under consideration.

Hazard - A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis - The process of collecting and evaluating information on hazards and the conditions leading to their presence to decide which are significant for food safety and therefore should be addressed by the HACCP plan.

Monitoring – The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.
Step - A point, procedure, operation or stage in the food chain including raw materials, from primary production to final consumption.

Validation - Obtaining evidence that the elements of the HACCP plan are effective.

Verification - The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan.

8.3 Stages of a HACCP Study

The HACCP system consists of the following seven basic principles:

1. Conduct a hazard analysis.

2. Determine the CCPs.

3. Establish the critical limit(s).

4. Establish a system to monitor control of the CCP.

5. Establish the corrective action to be taken when monitoring shows that a CCP is not under control.

6. Establish procedures for verification to confirm that the HACCP system is working effectively.

7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

It is recommended by the Codex Alimentarius that the practical application of the HACCP principles be approached by breaking the seven principles down into a 12-stage logic sequence. Each stage is discussed below in detail. Figure 8.1 is a flow diagram illustrating this 12-stage logic sequence.

8.3.1 Assemble HACCP team

HACCP requires management commitment of resources to the process. An effective HACCP plan is best carried out as a multidisciplinary team exercise to ensure that the appropriate product-specific expertise is available. The team should include members familiar with all aspects of the production process as well as specialists with expertise in particular areas such as production, hygiene managers, quality assurance or control, ingredient and packaging buyers, food microbiology, food chemistry or engineering. The team should also include
personnel who are involved with the variability and limitations of the operations. If expert advice is not available on-site, it may be obtained from external sources.

The scope of the plan should be determined by defining the extent of the production process to be considered and the categories of hazard to be addressed (e.g. biological, chemical and/or physical).

Fig. 8.1. Logic sequence for application of HACCP
8.3.1.1 Fish and seafood

The HACCP team should ideally have access to expertise on the appropriate fisheries, including practices onboard fishing vessels, or in aquaculture operations. These factors are likely to have a significant effect on some potential hazards, for example contamination by foodborne pathogens as a result of time/temperature abuse or unsatisfactory handling practice. In addition, there may be species-specific hazards related to certain fish and shellfish, such as enteric viruses in shellfish, marine toxins associated with particular fish and shellfish species, aquaculture drug residues and potentially pathogenic *Vibrio* spp. naturally present on fish from warm waters (> 15 °C). Expertise on such inherent hazards, challenge testing and inoculation studies for evaluation of safety aspects are therefore essential for an effective HACCP team.

8.3.2 Describe the product

It is important to have a complete understanding of the product, which should be described in detail. The description should include information such as the product name, composition, physical and chemical structure (including water activity ($a_w$), pH, etc.), processing conditions (e.g. heat treatment, freezing, fermentation, etc.), packaging, shelf life, storage and distribution conditions and instructions for use.

8.3.3 Identify intended use

The intended use should be based on the expected uses of the product by the end-user or consumer (e.g. is a cooking process required?). It is also important to identify the consumer target groups. Vulnerable groups of the population, such as children or the elderly, may need to be considered specifically.

8.3.3.1 Fish and seafood

Certain products may be contaminated or carry pathogenic organisms as a part of the natural flora. If the processing does not include a kill step, the only CCP that can render the product safe from pathogenic organisms is adequate heat treatment during preparation. However, it must be noted here that certain toxins (algal and shellfish) are not destroyed by heat treatments; contaminated fish and shellfish should not be used.

8.3.4 Construct a flow diagram

The flow diagram should be constructed by the HACCP team and should contain sufficient technical data for the study to progress. It should provide an accurate representation of all steps in the production process from raw materials to the end-
product. It may include details of the factory and equipment layout, ingredient specifications, features of equipment design, time/temperature data, cleaning and hygiene procedures and storage conditions. Ideally, it should also include details of CCP steps, once determined.

8.3.4.1 Fish and seafood

Examples of flow diagrams for specific fish products may be found in the appropriate product chapters. Many fish-processing operations have relatively few steps and the flow diagrams appear simple. However, it is essential that the details of each step are fully appreciated and recorded. For example, adequate chilling of finfish immediately after capture is vital for the control of several potential hazards, and therefore a detailed description of the procedure applied must be available.

8.3.5 On-site confirmation of the flow diagram

The HACCP team should confirm that the flow diagram matches the process that is actually being carried out. The operation should be observed at all stages, and any discrepancies between the flow diagram and normal practice must be recorded and the diagram amended accordingly. It is also important to include observation of production outside normal working hours such as shift patterns and weekend working, or circumstances of any reclaim or rework activity. It is essential that the diagram is accurate, because the hazard analysis and decisions regarding CCPs are based on these data. If HACCP studies are applied to proposed new process lines or products, then any pre-drawn HACCP plans must be reviewed once the lines/products are finalised.

8.3.6 List all potential hazards associated with each step; conduct a hazard analysis; and identify any measures to control identified hazards

The HACCP team should list all hazards that may reasonably be expected to occur at each step in the production process.

The team should then conduct a hazard analysis to identify which hazards are of such a nature that their elimination or reduction to an acceptable level is essential to the production of safe food.

The analysis is likely to include consideration of:

- the likely occurrence of hazards and the severity of their adverse health effects;
- the qualitative and/or quantitative evaluation of the presence of hazards;
- survival or multiplication of pathogenic microorganisms;
The HACCP team should then determine what control measures exist that can be applied for each hazard.

Some hazards may require more than one control measure for adequate control and a single control measure may act to control more than one hazard. One control measure may be relevant to several process steps, where a hazard is repeated.

Note: it is important at this stage that no attempt is made to identify CCPs, since this may interfere with the analysis.

8.3.6.1 Fish and seafood

The term 'fish and seafood' includes an extremely varied group of products, and there are an equally varied range of potential hazards associated with them. Hazards specific to certain types of product are detailed in the appropriate chapters of this manual. For example, there are particular hazards associated with contamination of shellfish by microorganisms from human sewage, and the potential growth of *Listeria monocytogenes* on smoked fish.

Many of the microbiological hazards associated with fish and seafood products are derived from the raw materials. Pathogens may be part of the resident microflora of the living animal (e.g. *Vibrio* spp.), or may originate from polluted water or from post-capture contamination (e.g. *Salmonella* spp. and viruses). The incidence of psychrotrophic, non-proteolytic *Clostridium botulinum* on fresh fish is also sufficiently high that its presence may be assumed.

There may also be inherent hazards from parasites such as the roundworm *Anisakis simplex* and from marine toxins such as ciguatera in reef fish (derived from microalgae) and scombrotxin (biogenic amines) development in fish containing high levels of histidine.

Hazards introduced during processing of fish products depend very much on the characteristics of the process. For example, modified-atmosphere packaging (MAP) of chilled raw fish may provide conditions suitable for the growth of psychrotrophic *C. botulinum*, or poor control of batter mixes in frozen battered fish portions may allow growth of *Staphylococcus aureus* and production of enterotoxin. Therefore it is not possible, or desirable, to generalise about expected hazards and the reader is once again referred to the appropriate product chapter for additional advice on specific hazards.
8.3.7 Determine CCPs

The determination of CCPs in the HACCP system is facilitated by a decision tree (Figure 8.2) to provide a logical, structured approach to decision making. However, application of the decision tree should be flexible, and its use may not always be appropriate. It is also essential that the HACCP team has access to sufficient technical data to determine the CCPs effectively.

If a significant hazard has been identified at a step where control is required for safety, but for which no control exists at that step or any other, then the process must be modified to include a control measure.

8.3.7.1 Fish and seafood

Again, given the enormous variety of fish products and processes in use, it is not possible to generalise on likely CCPs, and the reader is referred to the appropriate product chapter. However, it can be said that effective control measures are likely to include the following:

- Careful selection of sources for raw materials
- Adequate temperature control
- Effective sanitation
- Food handler hygiene
- Prevention of cross-contamination

8.3.8 Establish critical limits for each CCP

Critical limits separate acceptable from unacceptable products. Where possible, critical limits should be specified and validated for each CCP. More than one critical limit may be defined for a single step. For example, it is necessary to specify both time and temperature for a thermal process. Criteria used to set critical limits must be measurable and may include physical, chemical, biological or sensory parameters.

It is prudent to set stricter limits (often-called target or process limits/levels) to ensure that any trend towards a loss of control is noted before the critical limit is exceeded.

8.3.8.1 Fish and seafood

Specific product chapters provide information on criteria that may be used to set critical limits. Some examples relevant to fish products are:

- Time and temperature limits for pasteurised and cooked products
- Temperature limits for chilled fish
- Brine concentration in cured fish
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- Water activity ($a_w$) values for dried fish
- pH values in fermented fish
- Microbiological quality of water in shellfish production areas

### 8.3.9 Establish a monitoring system for each CCP

Monitoring involves planned measurement or observation of a CCP relative to its critical limits. Monitoring procedures for target levels must be able to detect loss of control of the CCP, and should provide this information with sufficient speed to allow adjustments to be made to the control of the process before the critical limits are violated. Monitoring at critical limits should be able to rapidly detect when the critical limit has been exceeded. Monitoring should therefore either be continuous, or carried out sufficiently frequently to ensure control at the CCP. Therefore, physical and chemical on-line measurements are usually preferred to lengthy microbiological testing. However, certain rapid methods, such as ATP assay by bioluminescence, may be useful for assessment of adequate cleaning, which could be a critical limit for some CCPs, for example pre-start-up hygiene.

Persons engaged in monitoring activities must have sufficient knowledge, training and authority to act effectively on the basis of the data collected. These data should also be properly recorded.

### 8.3.10 Establish corrective actions

For each CCP in the HACCP plan, there must be specified corrective actions to be applied if the CCP is not under control. If monitoring indicates a deviation from the critical limit for a CCP, action must be taken that will bring it back under control. Actions taken should also include proper isolation of the affected product, repair of defective equipment, and an investigation into why the deviation occurred. A further set of corrective actions should relate to the target levels, if process drift is occurring. In this case, only repair of the process defect and investigation of the fault are required. All corrective actions should be properly recorded.

### 8.3.11 Establish verification procedures

Verification usually involves auditing and testing procedures. Auditing methods, procedures and tests should be used frequently enough to determine whether the HACCP system is being followed, and is effective at controlling the hazards. These may include random sampling and analysis, including microbiological testing. Although microbiological analysis is generally too slow for monitoring purposes, it can be of great value in verification, since many of the identified hazards are likely to be microbiological.

In addition, reviews of HACCP records are important for verification purposes. These should confirm that CCPs are under control and should indicate the nature
of any deviations and the actions that were taken in each case. It is also useful to review customer returns and complaints regularly.

8.3.12 **Establish documentation and record keeping**

Efficient and accurate record keeping is an essential element of a HACCP system. The procedures in the HACCP system should be documented.

- Determination of critical limits
- The completed HACCP plan

Examples of documented procedures include:

- The hazard analysis
- Determination of CCPs

Examples of recorded data include:

- Results of monitoring procedures
- Deviations from critical limits and corrective actions
- Records of certain verification activities, e.g. observations of monitoring activities, and calibration of equipment.

The degree of documentation required will depend partly on the size and complexity of the operation, but it is unlikely to be possible to demonstrate that an effective HACCP system is present without adequate documentation and records. The length of time that records are kept will be as per company policy, but should not be less than one year beyond the shelf life of the product. Three to five years is typical for many food companies.

8.4 **Implementation and review of the HACCP plan**

The completed plan can only be implemented successfully with the full support and co-operation of management and the workforce. Adequate training is essential and the responsibilities and tasks of the operating personnel at each CCP must be clearly defined.
Finally, it is essential that the HACCP plan be reviewed following any changes to the process, including changes to raw materials, processing conditions or equipment, packaging, cleaning procedures and any other factor that may have an effect on product safety. Even a small alteration to the product or process may invalidate the HACCP plan and introduce potential hazards. Therefore, the implications of any changes to the overall HACCP system must be fully
considered and documented, and adjustments made to the procedures as necessary.

Triggered reviews/audits should occur as a result of changes, whereas a scheduled review/audit should be annual, as a minimum.

8.5 References


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9. EC FOOD HYGIENE LEGISLATION

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9.1 Introduction

Hygiene is an important aspect of ensuring food safety, and one that plays an important role in most countries’ food legislation. Hygiene is a general concept that covers a wide subject area, from structural conditions in the factory or process facility, to personnel requirements, final product specifications, including microbiological criteria, transport and delivery vehicles requirements, and conditions of raw materials.

Microbiological standards have a useful role and help establish requirements for the microbiological safety and quality of food and raw materials. A number of standards are provided in food legislation; however, the existence of microbiological standards alone cannot protect consumer health. It is generally considered that the principles of Good Manufacturing Practice (GMP) and application of Hazard Analysis Critical Control Point (HACCP) systems are of greater importance.

A new package of European Commission (EC) hygiene measures became applicable on 1 January 2006 to update and consolidate the earlier 17 hygiene directives, with the intention of introducing consistency and clarity throughout the food production chain from primary production, to sale or supply to the final consumer. The general food hygiene Directive 93/43/EEC and other Directives on the hygiene of foodstuffs and the health conditions for the production and placing on the market of certain products of animal origin intended for human consumption, have been replaced by several linked measures on food safety rules and associated animal health controls.

The new legislation was designed to establish conditions, under which food is produced to optimise public health and to prevent, eliminate or acceptably control pathogen contamination of food. Procedures under the new legislation are based on risk assessment and management and follow a ‘farm to fork’ approach to food
safety with the inclusion of primary production in food hygiene legislation. Prescribed are detailed measures to ensure the safety and wholesomeness of food during preparation, processing, manufacturing, packaging, storing, transportation, distribution, handling and offering for sale or supply to the consumer.

9.2 Legislative Structure

From 1 January 2006, the following European Union (EU) hygiene regulations have applied:

- Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs

The general hygiene requirements for all food business operators are laid down in Regulation 852/2004. Regulation 853/2004 supplements Regulation 852/2004 in that it lays down specific requirements for food businesses dealing with foods of animal origin. Regulation 854/2004 relates to the organisation of official controls on products of animal origin, and sets out what those enforcing the provisions have to do.

N.B. A number of more detailed implementing and transitional measures have been adopted at EC level.

Subsequently, existing hygiene Directives including those below were repealed:

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The EU hygiene regulations apply to all stages of food production including primary production. As regulations, the legislation is directly applicable law, and binding in its entirety on all member states from the date of entry into force.

Although the regulations have the force of law, national legislation in the form of a Statutory Instrument (S.I.) in England, and equivalent legislation in Scotland, Wales and Northern Ireland, is required to give effect to the EU regulations, e.g. setting offences, penalties and powers of entry, revocation of existing implementing legislation, etc.

The Food Hygiene (England) Regulations 2006 (S.I. 2006 No.14, as amended) came into force on 11 January 2006 (separate but similar national legislation also came into force on that day in Scotland, Wales and Northern Ireland). The national legislation in all four UK countries also applied the provisions of the EU Microbiological Criteria Regulation No. 2073/2005.

Although EU food hygiene regulations are directly applicable in the individual Member States there are some aspects where Member States are required or allowed to adopt certain provisions into their national laws.


Food business operators must ensure that all stages of production, processing and distribution of food under their control satisfy the relevant hygiene requirements laid down in Regulation (EC) No. 852/2004.

This Regulation lays down general rules for food business operators on the hygiene of foodstuffs, particularly taking into account a number of factors ranging from ensuring food safety throughout the food chain to begin with primary production, right through to the implementation of procedures based on HACCP principles.

There are some exemptions, for example, with primary production, domestic preparation or handling, food storage that is for private or domestic consumption, and also if the producer supplies small amounts of primary product to the final consumer or local retail establishments supplying the final consumer. Likewise Regulation 852/2004 will not apply to collection centres and tanneries meeting the definition of food business because they handle raw material for the production of gelatine or collagen.

The regulation lays down general hygiene provisions for which food business operators carrying out primary production must comply with as laid down in Part A of Annex 1. Primary products are defined as products of primary production including products of the soil, of stock farming, and of hunting and fishing.

Additionally the requirements of EC Regulation 853/2004 must be complied with, which will be covered later in this chapter.
9.3.1 Annex I – Primary production

Annex I (Part A) relates to general hygiene provisions for primary production and associated operations covering:

(a) the transport, storage and handling of primary products at the place of production,

(b) the transport of live animals,

(c) for fishery products - transport operations to deliver primary products (which have not been substantially altered) from the place of production to an establishment

Food business operators have the responsibility to ensure primary products are protected against contamination. Any community and national legislation relating to the control of hazards in primary production such as measures to control contamination resulting from surroundings e.g. air, soil, water etc., and measures relating to animal health and welfare, and plant health that may impact on human health should be complied with.

Requirements for record keeping are also laid down. This relates to animal feed (nature and origin), veterinary medicines administered to animals (date given and withdrawal periods), any diseases, analysis of samples from other animals that might impact on human health as well as reports on animal checks performed.

Part B of Annex I contains recommendations for guides to good hygiene practice.

9.3.2 Annex II – Stages other than primary production

Annex II of the regulation lays down additional general hygiene requirements that must be met by food business operators carrying out production, processing and distribution of food at all stages following primary production. A summary of Chapters I to IV of Annex II is provided as follows:

9.3.2.1 Chapter I

Chapter I applies to all food premises, except premises to which Chapter III applies.

- Food premises must be kept clean and maintained in good repair and condition. Their layout should allow for this.
- The environment should allow good hygiene practices and provide temperature-controlled handling and storage conditions where necessary, and enable foods to be kept at correct temperatures and be monitored.
LEGISLATION

- Additionally, there are requirements for provision of adequate lavatories, basins, ventilation, lighting and drainage.

9.3.2.2 Chapter II

Chapter II applies to all rooms where food is prepared, treated or processed, except dining areas and premises to which Chapter III applies.

- The design and layout of rooms should allow for good hygiene practices between and during operations. Therefore floor and wall surfaces, ceilings and windows should be constructed to prevent dirt accumulating.
- Surfaces where food is handled must be well-maintained and allow easy cleaning and disinfection, preferably using smooth, washable corrosion-resistant and non-toxic materials.
- There should be facilities for cleaning or disinfecting, and for storing working utensils or equipment. Clean potable water and adequate provision for washing food is needed.

9.3.2.3 Chapter III

Chapter III applies to temporary premises (e.g. marquees, market stalls, and mobile sales vehicles), premises used primarily as a private dwelling-house but where foods are regularly prepared for placing on the market, and vending machines.

- Premises and vending machines should be practically sited, designed, constructed and kept clean and maintained in good repair and condition so as to avoid the risk of contamination, in particular by animals and pests.
- Facilities should allow adequate personal hygiene, and surfaces in contact with food should be easy to clean. Enough potable water and storage arrangements for hazardous or inedible substances are required as well as adherence to food safety requirements.

9.3.2.4 Chapter IV

Chapter IV applies to all transportation.

- Conveyances and/or containers used for transporting foodstuffs are to be kept clean and maintained in good repair and condition to protect foodstuffs from contamination and are, where necessary, to be designed and constructed to permit adequate cleaning and/or disinfection.
- Food should be maintained at appropriate temperatures.
9.3.2.5 Chapter V

Chapter V refers to equipment requirements.

- Adequate cleaning and disinfection is to be carried out frequently for articles, fittings and equipment in contact with food where contamination needs to be avoided.
- Equipment should be installed to allow adequate cleaning, and be fitted with the required control device.

9.3.2.6 Chapter VI

Chapter VI refers to food waste.

- Food waste, non-edible by-products and other refuse is to be removed from rooms where food is present as quickly as possible to avoid accumulation. Such waste is to be deposited in closable containers, to allow easy cleaning.
- Refuse stores should allow easy cleaning and be free of pests.
- Waste must be eliminated hygienically in accordance with European Community legislation.

9.3.2.7 Chapter VII

Chapter VII refers to the water supply.

- There must be an adequate supply of potable water, which should be used whenever necessary to ensure foodstuffs are not contaminated. There are also requirements for recycled water, ice in contact with food, use of steam and for the water used in the cooling process for heat-treated foods in hermetically sealed containers.
- Specific requirements are that clean water may be used with whole fishery products. Clean seawater, may be used for live bivalve molluscs, echinoderms, tunicates and marine gastropods; and for external washing. If it is used, there must be adequate facilities to ensure the supply does not create a source of contamination for the foodstuff.
- Recycled water used in processing or as an ingredient must not present a contamination risk. It must be of the same standard as potable water, unless the competent authority is satisfied that the quality of water cannot affect the wholesomeness of food in the finished form.

Additionally, ice that comes into contact with food or which could possibly contaminate it needs to be made from potable water or, in the case of chilling whole fishery products using clean water. Ice must be made, handled and stored under conditions that protect it from contamination.
Chapter VIII sets out personal hygiene requirements for those working in a food handling area including clean protective clothing and the requirement that those carrying or suffering from a disease are not permitted to handle food.

Chapter IX covers provisions applicable to foodstuffs.

- A food business operator should not accept raw materials or ingredients, other than live animals, or any other material used in processing products, if they are known to be contaminated with parasites, pathogenic microorganisms or foreign substances, to be toxic or decomposed to such an extent that, even after the business operator applied normal hygienic processing, the product would be inedible.
- Raw materials must be kept under appropriate conditions throughout production, processing and distribution. In particular, temperature control (i.e. cold chain and food thawing) requirements are laid down.

Chapter X lays down provisions applicable to the wrapping and packaging of foodstuffs to avoid contamination of any form.

Chapter XI lays down heat treatment requirements for food that is placed on the market in hermetically sealed containers. The main relevant process parameters (particularly temperature, pressure, sealing and microbiology) must be checked by the food business operator to ensure that the required heat treatment has been achieved.

Also, the process used should comply with an internationally recognised standard (e.g. pasteurisation, ultra high temperature or sterilisation).

Chapter XII states training requirements for food business operators to ensure that food handlers are trained in food hygiene matters and in the application of HACCP principles.
9.3.3 Registration

The Regulation requires that food business operators must notify the relevant competent authority of each establishment under their control that carries out any of the stages of production, processing and distribution of food, with a view to the registration of each establishment.

Food business operators must also ensure that the competent authority always has up-to-date information on establishments, including the notification of significant changes in activity, and closure of an existing establishment.

Food business operators must ensure that establishments are approved by the competent authority, following at least one on-site visit, when approval is required by the national law of the Member State in which the establishment is located, or under Regulation (EC) No. 853/2004, or by a separate decision adopted.

Separate rules apply for businesses producing products of animal origin.

9.3.4 HACCP

Food business operators, other than at the level of primary production, and associated operations must put in place, implement and maintain a permanent procedure or procedures based on principles of the system of hazard analysis and critical control points (HACCP). Emphasis is placed on risk-related control, with responsibility placed on the proprietor of the food business to ensure that potential hazards are identified and systems are developed to control them. Under HACCP, food business operators must amongst other considerations, identify hazards to be prevented or eliminated or reduced to acceptable levels, identify critical control points (CCP) at which control is essential to prevent, eliminate or reduce hazards and establish critical limits at these points, implement effective monitoring procedures, and establish corrective actions in the case where a CCP is out of control. Procedures must be followed to confirm the above is in place and up-to-date, as well as providing the competent authority with documents and records as evidence, when required.


9.4.1 Definitions

The following definitions apply for fishery products and live bivalve molluscs:
Fishery products means all seawater or freshwater animals (except for live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, and all mammals, reptiles and frogs) whether wild or farmed and including all edible forms, parts and products of such animals.

Factory vessel means any vessel on board which fishery products undergo one or more of the following operations followed by wrapping or packaging and, if necessary: chilling or freezing: filleting, slicing, skinning, shelling, shucking, mincing or processing.

Freezer vessel means any vessel on board in which freezing of fishery products is carried out, where appropriate, after preparatory work such as bleeding, heading, gutting and removal of fins and, where necessary, followed by wrapping or packaging.

Mechanically separated fishery product means any product obtained by removing flesh from fishery products using mechanical means resulting in the loss or modification of the flesh structure.

Fresh fishery products means unprocessed fishery products, whether whole or prepared, including products packaged under vacuum or in a modified-atmosphere that have not undergone any treatment to ensure preservation other than chilling.

Prepared fishery products means unprocessed fishery products that have undergone an operation affecting their anatomical wholeness, such as gutting, heading, slicing, filleting, and chopping.

Bivalve molluscs means filter-feeding lamellibranch molluscs

Marine biotoxins means poisonous substances accumulated by bivalve molluscs, in particular as a result of feeding on plankton containing toxins.

Conditioning means the storage of live bivalve molluscs coming from class A production areas, purification centres or dispatch centres in tanks or any other installation containing clean seawater, or in natural sites, to remove mud, slime or sand, to preserve or to improve organoleptic qualities and to ensure they are in a good state of vitality before wrapping or packing.

Purification centre means an establishment with tanks fed by clean seawater in which live bivalve molluscs are placed for the time necessary to reduce contamination to make them fit for human consumption.
9.4.2 Fishery product requirements

9.4.2.1 Identification marking

An identification mark needs to be applied following the requirements of Annex II, Section I of Regulation (EC) 853/2004. An identification mark can only be applied provided the relevant requirements as stated above in relation to Regulation (EC) 852/2004 have been met in particular for the registration and approval of establishments.

Extracts of the requirements of Annex II, Section I of Regulation (EC) 853/2004 are given as follows:

9.4.2.1.1 Application of the identification mark

(i) The identification mark must be applied before the product leaves the establishment of production.

(ii) However, when a product's packaging and/or wrapping is removed or is further processed in another establishment, a new mark must be applied to the product. In this case, the new mark must show the approval number of the establishment where this occurred.

(iii) Food business operators must have in place systems and procedures to identify food business operators from whom they have received and to whom they have delivered products of animal origin.

9.4.2.1.2 Form of the identification mark

(i) The mark must be legible and indelible, and the characters easily decipherable and be clearly displayed.

(ii) The mark must indicate the name of the country in which the establishment is located and written out in full or shown as a two-letter code in accordance with the relevant ISO standard. For the UK, the code would be UK; for other codes, please refer to Regulation (EC) 853/2004.

(iii) Additionally, the mark must show the approval number of the establishment. If an establishment manufactures both food to which Regulation 853/2004 applies and food to which it does not, the food business operator may apply the same identification mark to both types of food.

(iv) If the identification mark is applied in an establishment within the Community, the mark must be oval shaped and include the abbreviation CE, EC, EF, EG, EK, EO, EY, ES, EÜ, EK, EB or WE.
9.4.2.1.3 Method of marking

(i) The mark is to be applied directly to the product, the wrapping or the packaging, or be printed on a label affixed to the product, its wrapping or the packaging. It may also be a tag made of a resistant material affixed in such a way that it cannot be removed.

(ii) For products of animal origin that are placed in transport containers or large packages, and are intended for further handling, processing, wrapping or packaging in another establishment, the mark may be applied to the outer surface of the container or packaging.

(iii) In the case of liquid, granulate and powdered products of animal origin carried in bulk, and fishery products carried in bulk, an identification mark is not necessary if accompanying documentation contains the name of the country or its two-letter code, the approval number of the establishment and the abbreviation for the community where appropriate.

(iv) If products of animal origin are placed in a package destined for direct supply to the final consumer, it is sufficient to apply the mark to the exterior of that package only.

(v) When the mark is applied directly to products of animal origin, the colourings used must be EC authorised colours.

9.4.2.2 Hygiene requirements for fishery products

The regulation details specific hygiene requirements for fishery products. Extracts of the requirements of Annex III, Section VIII of Regulation 853/2004, as amended, specifically relating to fishery products are given below; for full requirements, reference should be made to the actual Regulation.

This section does not apply to bivalve molluscs, echinoderms, tunicates and marine gastropods when placed on the market live. With the exception of Chapters I and II, it applies to those animals when not placed on the market live, i.e. they would need to be obtained following Section VII on live bivalve molluscs.

Chapter III, Parts A, C and D, Chapter IV, Part A and Chapter V apply to retail. The requirements of this section accompany those in Regulation (EC) 852/2004. Therefore,

a) requirements for establishments, including vessels, involved in primary production and such operations accompany the needs of Annex I of Regulation (EC) 852/2004

b) for other establishments including vessels, the requirements accompany those of Annex II of Regulation (EC) 852/2004
c) for the water supply, this supplements the requirements of Annex II, Chapter VII of Regulation 852/2004; clean seawater may be used for the handling and washing of fishery products, the production of ice used to chill fishery products and the rapid cooling of crustaceans and molluscs after their cooking.

There is a derogation from point a, in that record keeping requirements stated by point 7 in Part A of Annex I of Regulation 852/2004 do not apply to operators involved in small-scale coastal fishing (as defined through Regulation (EC) 1198/2006) if the activity is under 24 hours.

The definition of fishery products covers primary production as well as its associated operations, i.e.

(a) primary production covers the farming, fishing and collection of live fishery products with a view to their being placed on the market; and

(b) related operations include any of the following operations if done on board fishing vessels: slaughter, bleeding, heading, gutting, removing fins, refrigeration and wrapping; they also include

   (i) the transport and storage of fishery products that have not been substantially altered (including live fishery products, within fish farms on land)

   (ii) the transport of fishery products that have not been substantially altered (including live fishery products, from the place of production to the first establishment of destination.)

9.4.2.2.1 Chapter I - Requirements for vessels

Food business operators are required to ensure that they meet the structural and equipment requirements as well as hygiene requirements required for vessels used to harvest fishery products from their natural environment, or to handle or process them after harvesting.

A. Structural and equipment requirements refer to the following points

1. Requirements for all vessels
Vessels should be made in a way that does not cause contamination with substances such as fuel and oil etc.; surfaces that contact fishery products need to be made from cleanable corrosion-resistant material; if a vessel has water coming in that is used with fishery products, it must be placed somewhere that will not contaminate the water supply.

2. Requirements for vessels designed and equipped to preserve fresh fishery products for more than 24 hours
Such vessels need to have holds, tanks or containers for the storage of fishery products at the temperatures laid down in Chapter VII.

Holds must be separated from the engine compartments and from the crew quarters by partitioning that prevent any contamination of the stored fishery products. Holds and containers used for storing fishery products must allow its preservation under adequate hygiene conditions and, where necessary, to ensure that melt water does not stay in contact with the products.

In vessels equipped for chilling fishery products in cooled clean seawater, tanks must incorporate devices that allow a uniform temperature throughout the tanks. Such devices must achieve a chilling rate that ensures that the mix of fish and clean seawater does not exceed 3 °C six hours after loading, and not more than 0 °C after 16 hours, and allow the monitoring and, where necessary, recording of temperatures.

3. Requirements for freezer vessels

These need to contain freezing equipment in order to achieve a core temperature of no more than -18 °C.

Refrigeration equipment needs to keep fishery products in storage holds at not more than -18 °C. A temperature recording device must be present and placed where the temperature in the hold is the highest.

The above requirements for holds in vessels designed and equipped to preserve fresh fishery products for more than 24 hours must be met.

4. Requirements for factory vessels

a) Factory vessels

These must have at a receiving area reserved for taking fishery products on board so each catch can be separated

There must be a hygienic system for conveying fishery products from the receiving area to the work area

Large work areas are needed to allow fishery products to be prepared and processed hygienically. Storage areas for the finished products also need to be sufficiently large and cleanable. Also, any waste processing unit needs to have a separate hold to store the waste.

There needs to be somewhere for storing packaging materials and this is to be separate from product preparation and processing areas.

Special equipment for disposing of waste/ inedible fishery products into the sea or a watertight tank and allocation of separate areas.
A water tank needs to be placed where there is no contamination of the water supply and hand washing taps should be designed to prevent contamination.

b) Factory vessels on board which crustaceans and molluscs are cooked, chilled and wrapped, do not have to meet the above requirements if no other form of handling or processing takes place on board such vessels.

c) Factory vessels that freeze fishery products must have equipment meeting the requirements for freezer vessels as stated above.

B. Hygiene requirements

Hygiene requirements refer to the following:

a) Parts of vessels used for storage of fishery products must be cleaned and maintained in a good state with no contamination.

b) Fishery products are to be handled and stored in a way that prevents the flesh bruising. Handlers may use spiked instruments to move large fish or fish that might injure them, provided no damage occurs to the flesh.

c) Fishery products, other than those kept alive, must be chilled or, if this is not possible, landed as soon as possible.

d) Any gutting or heading of fish on board is to be done hygienically and quickly after capture, and products washed thoroughly. Parts that may present a danger to public health are to be removed quickly and kept away from products for human consumption. Livers and roes for human consumption must be preserved under ice (temperature to approach that of melting ice) or be frozen.

e) In cases where whole fish intended for canning is frozen in brine, the product must be kept at no more than -9 °C. The brine must not be a source of contamination.

9.4.2.2 Chapter II - Requirements during and after landing

(i) Unloading and landing equipment that comes into contact with fishery products needs to be made of material that is easily cleanable. The unloading and landing process is to be done quickly to avoid contamination (but at the same time not causing bruising to the fish) and to ensure fishery products are placed in a protected environment at the temperatures stated in Chapter VII.

(ii) In the case of food business operators responsible for auction and wholesale markets etc. where fishery products are displayed for sale, there need to be lockable facilities to refrigerate detained fishery products and separate lockable facilities to store fishery products that are unfit for human consumption.
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Consumption. Additionally, there are requirements that such premises should be well lit and vehicles emitting fumes should not be able to access the premises. Food business operators need to co-operate with the relevant competent authorities so as to allow them to carry out official controls as laid down in Regulation (EC) No. 854/2004.

9.4.2.2.3 Chapter III - Requirements for establishments, including vessels, handling fishery products

A. Requirements for fresh fishery products

(i) Where chilled or unpackaged products are not distributed, dispatched, prepared or processed immediately after arrival at an establishment on land, they must be stored under ice in appropriate facilities. Packaged fresh fishery products must be chilled to a temperature approaching that of melting ice.

(ii) Heading and gutting must be carried out hygienically. Gutting should ideally be done quickly once products have been caught or landed and afterwards be washed thoroughly.

(iii) Whole and gutted fresh fishery products can be transported and stored in cooled water on board vessels. They may also continue to be transported in cooled water after landing and be transported from aquaculture establishments until they reach the first establishment on land that carries out an activity other than transport or sorting.

(iv) Filleting and cutting is to be carried out in a way that avoids contamination or spoilage of fillets or slices. Fillets and slices must then be removed from work tables as soon as possible after preparation. They should be wrapped, and where necessary, packaged and chilled as quickly as possible after preparation.

(v) Containers for dispatching and storing unpackaged prepared fresh fishery products kept under ice should ensure that melt water does not remain in contact with the products.

B. Requirements for frozen products

An establishment on land that freezes fishery products needs to have equipment that meets that for freezer vessels as stated in Section VIII, Chapter I, part I. C, points 1 and 2.

C. Requirements for mechanically separated fishery products

(i) Raw materials used must satisfy the following
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Only whole fish and bones after filleting may be used to produce mechanically separated fishery products, raw materials must have no guts.

(ii) The manufacturing process must satisfy the following

Mechanical separation is to be carried out immediately after filleting, and whole fish must be gutted and washed beforehand. Once produced, mechanically separated fishery products must be frozen as quickly as possible or incorporated into a product intended for freezing, or a stabilising treatment.

D. Requirements concerning parasites

(i) The fishery products below must be frozen at no more than -20 °C in all parts of the product for at least 24 hours (this treatment must be applied to the raw product or the finished product):

(a) fishery products to be consumed raw or almost raw;
(b) fishery products from the following species, if they are to undergo a cold smoking process in which the internal temperature of the fishery product is not more than 60 °C:
   (i) herring;
   (ii) mackerel;
   (iii) sprat;
   (iv) (wild) Atlantic and Pacific salmon; and
(c) marinated and/or salted fishery products, if the processing is insufficient to destroy nematode larvae.

(ii) Compliance with the above is not necessary if epidemiological data indicates that the fishing grounds of origin do not present a health hazard in relation to the presence of parasites and this has been authorised by the competent authority.

(iii) A document from the manufacturer, stating the type of process they have undergone, must accompany fishery products referred to above when placed on the market, except when supplied to the final consumer.

9.4.2.2.4 Chapter IV - Requirements for certain processed fishery products

A. Requirements for cooking of crustaceans and molluscs

After crustaceans and molluscs are cooked, they must be cooled rapidly. If no other method of preservation is used, cooling must continue until a temperature
approaching that of melting ice is reached. Shelling/shucking is to be done hygienically, and after this, cooked products are to be frozen or chilled to the temperatures stated in Chapter VII.

B. Requirements for fish oil intended for human consumption

(i) In relation to the raw materials used in their preparation and their transport, raw materials need to be chilled as soon as possible and should meet the temperature requirements in Chapter VII. However, there is an exception: food business operator may refrain from chilling the fishery products when whole fishery products are used directly in the preparation of fish oil for human consumption, and the raw material is processed within 36 hours after loading, provided that the freshness criteria are met and the total volatile basic nitrogen (TVB-N) value of the unprocessed fishery products does not exceed the limits set out in point 1 of Chapter I of Section II of Annex II to Regulation (EC) No. 2074/2005.

(ii) The production process for fish oil must ensure that all raw material intended for the production of crude fish oil is subject to a treatment including (where relevant) heating, pressing, separation, centrifugation, processing, refining and purification steps before being placed on the market for the final consumer.

9.4.2.2.5 Chapter V - Health standards for fishery products

In addition to compliance with microbiological criteria laid down in Regulation (EC) No. 2073/2005, the following conditions need to be met:

A. Organoleptic properties of fishery products

An organoleptic examination of fishery products must be carried out to ensure that fishery products comply with any freshness criteria (such criteria have yet to be set).

B. Histamine

Histamine limits must not be exceeded (limits are set in Regulation (EC) No. 2073/2005).

C. Total volatile nitrogen

Unprocessed fishery products cannot be placed on the market if chemical tests reveal that TVB-N or TMA-N limits are exceeded (limits are set in Regulation (EC) No. 2074/2005).
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D. Parasites

Fishery products are to be visually examined so that visible parasites are identified before being placed on the market. Fishery products contaminated with parasites may not be placed on the market for human consumption.

E. Toxins harmful to human health

Fishery products derived from poisonous fish of the following families must not be placed on the market: Tetraodontidae, Molidae, Diodontidae and Canthigasteridae. Fresh, prepared and processed fishery products belonging to the family Gempylidae, in particular *Ruvettus pretiosus* and *Lepidocybium flavobrunneum*, may only be placed on the market in wrapped/packaged form and must be appropriately labeled to provide information to the consumer on preparation/cooking methods and on the risk related to the presence of substances with adverse gastrointestinal effects. The scientific name must accompany the common name on the label.

The requirements for histamine and parasites do not apply to whole fishery products being used directly for preparing fish oil intended for human consumption.

9.4.2.2.6 Chapter VI - Wrapping and packaging of fishery products

(i) Containers in which fresh fishery products are kept under ice must be water-resistant and ensure that melt water does not remain in contact with the products.

(ii) Frozen blocks prepared onboard vessels must be adequately wrapped before landing, and when fishery products are wrapped on board fishing vessels, food business operators must ensure that wrapping material is not contaminated and is stored in such a way that it is not exposed to contamination and is easy to clean where necessary.

9.4.2.2.7 Chapter VII - Storage of fishery products

(i) Fresh fishery products, thawed unprocessed fishery products, and cooked and chilled products from crustaceans and molluscs are to be kept at a temperature close to that of melting ice.

(ii) Frozen fishery products must be kept at a temperature of not more than -18 °C in all parts of the product. In the case of whole frozen fish in brine intended for the manufacture of canned food, it should be kept at no more than -9 °C.
(iii) Fishery products kept alive are to be kept at a temperature which will not adversely affect food safety or its viability.

9.4.2.2.8 Chapter VIII - Transport of fishery products

The above points for Chapter VII apply in relation to transport. The temperature of frozen fishery products must not fluctuate above -15°C. The required temperature for whole frozen fish in brine intended for the manufacture of canned food during transport is not stated.

Note:
(i) Frozen fishery products do not need to be kept at a temperature of -18 °C if they are being transported from a cold store to an approved establishment to be thawed on arrival for the purposes of preparation and/or processing, provided that the journey is short and the competent authority allows this.
(ii) If fishery products are kept under ice, melt water must not remain in contact with the products.

9.4.2.3 Other information:

Rules relating to visual inspections for the detection of parasites in fishery products are stated in Annex II (obligations on the competent authorities) of Regulation (EC) No. 2074/2005. This covers Total volatile basic nitrogen (TVB-N) limits and how to determine them. The following in relation to TVB-N limits is extracted from the regulation:

- Unprocessed fishery products shall be regarded as unfit for human consumption where organoleptic assessment has raised doubts as to their freshness and chemical checks reveal that the following TVB-N limits are exceeded:

(a) 25 mg of nitrogen/100 g of flesh for the species: Sebastes spp., Helicolenus dactylopterus, Sebastichthys capensis;
(b) 30 mg of nitrogen/100 g of flesh for the species belonging to the Pleuronectidae family (with the exception of halibut: Hippoglossus spp.);
(c) 35 mg of nitrogen/100 g of flesh for the species: Salmo salar, species belonging to the Merlucciidae family, species belonging to the Gadidae family;
(d) 60 mg of nitrogen/100 g of whole fishery products used directly for the preparation of fish oil for human consumption as referred to in the second sub-paragraph of Part B(1) of Chapter IV of Section VIII of Annex III to
Regulation (EC) No 853/2004; however, where the raw material complies with points (a), (b) and (c) of Part B(1) of that Chapter, Member States may set limits at a higher level for certain species pending the establishment of specific Community legislation.

The reference method to be used for checking the TVB-N limits involves distilling an extract deproteinised by perchloric acid as set out in Chapter III.

- For distillation apparatus and routine methods which may be used to check the TVB-N limits, please refer directly to the regulation.

- The sample must consist of about 100 g of flesh, taken from at least three different points and mixed together by grinding.

9.4.3 Live bivalve mollusc requirements

The regulation details specific hygiene requirements for bivalve molluscs. Extracts of the requirements of Annex III, Section VII of Regulation 853/2004, as amended, specifically relating to bivalve molluscs are given below; for full requirements, reference should be made to the actual regulation. Apart from the provisions on purification, this Section also applies to live echinoderms, tunicates and marine gastropods.

The section is split into 9 chapters for which a summary is provided as follows: Chapters I to VIII apply to animals harvested from production areas that the competent authority has classified in accordance with Regulation (EC) No 854/2004. Chapter IX applies to pectinidae harvested outside those areas. Chapters V, VI, VIII and IX, and point 3 of Chapter VII applies to retail.

The requirements of this Section accompany those laid down in Regulation (EC) No 852/2004:

(a) In the case of operations that take place before live bivalve molluscs arrive at a dispatch or purification centre, they supplement the requirements of Annex I to that Regulation.

(b) In the case of other operations, they supplement the requirements of Annex II to that Regulation.

9.4.3.1 Chapter I - General requirements for the placing on the market of live bivalve molluscs

Live bivalve molluscs can only be put on the market for retail sale if done through a dispatch centre. A food business operator can only accept batch of such bivalve molluscs provided correct documentary requirements are presented. The information required in the registration document is given in point 4 and includes the health status of the production area, shellfish species etc. The copy of
document must be kept for at least 12 months post dispatch or receipt or longer as stated by the competent authority.

9.4.3.2 Chapter II - Hygiene requirements for the production and harvesting of live bivalve molluscs

A. Requirements for production areas

(i) The competent authority classes (classed A, B or C) production areas with fixed locations and boundaries for where live bivalve molluscs may be harvested and this must be complied with.

(ii) Provided the requirements of chapter V are met, collection from:

- Class A means they can be placed directly on the market
- Class B means treatment in a purification centre or after relaying is done before it can be placed on the market
- Class C means relaying over a long time before it can be placed on the market

For bivalve molluscs not subject to Class B or C treatments, they need to be sent to a processing establishment and undergo specified treatments such as sterilisation in hermetically sealed containers or specific heat treatments such as immersion in boiled water so the internal temperature of the mollusc flesh reaches at least 90 °C and this temperature is maintained for at least 90 seconds.

B. Requirements for harvesting and handling following harvesting

This includes requirements that any further handling should not cause contamination or excess damage to shells/tissues of the live bivalve molluscs that might affect their suitability for purification, processing or relaying. Transport needs to allow sufficient drainage for best survival conditions.

C. Requirements for relaying live bivalve molluscs

Only areas approved by the competent authority can be used for relaying live bivalve molluscs with a requirement that buoys, poles or other fixed means are to identify the boundaries of the sites. Other requirements such as conditions for relaying in order to allow optimal conditions for purification and for permanent record keeping are also specified.
Chapter III - Structural requirements for dispatch and purification centres

Premises on land should not be subject to flooding by tides etc. and there are requirements for tanks and water storage containers.

Chapter IV - Hygiene requirements for purification and dispatch centres

A. Requirements for purification centres

There are requirements for live bivalve molluscs to be washed, the purification system to allow them to resume/maintain filter-feeding activity, requirements if there are many batches of live bivalve molluscs, containers and a label to identify purification.

B. Requirements for dispatch centres

Handling should not result in contamination to any degree. Before dispatch, shells of live bivalve molluscs are to be washed throughout with clean water and the molluscs are to come from one of the following:

(i) Class A production area
(ii) Relaying area
(iii) Purification centre
(iv) Another dispatch centre

Chapter V - Health standards for live bivalve molluscs

In addition to microbiological criteria and organoleptic characteristic requirements, the levels of marine biotoxins should not exceed the following limits, for:

(i) paralytic shellfish poison (PSP), 800 micrograms per kilogram;
(ii) amnesic shellfish poison (ASP), 20 milligrams of domoic acid per kilogram
(iii) okadaic acid, dinophysistoxins and pectenotoxins together, 160 micrograms of okadaic acid equivalents per kilogram;
(iv) yessotoxins, 1 milligram of yessotoxin equivalent per kilogram;
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(v) azaspiracids, 160 micrograms of azaspiracid equivalents per kilogram.

(Note: testing methods for detecting marine biotoxins are detailed in Annex III of Regulation (EC) 2074/2005)

9.4.3.6 Chapter VI - Wrapping and packaging of live bivalve molluscs

(i) Oysters must be wrapped or packaged with the concave shell downwards.

(ii) Individual consumer-size packages of live bivalve molluscs must be closed and remain closed after leaving the dispatch centre and until presented for sale to the final consumer.

9.4.3.7 Chapter VII - Identification marking and labelling

In addition to general identification marking (contained in 9.4.2.1), the species of bivalve mollusc and date of packaging (at least the date and month) are required. Also, by way of derogation from directive 2000/13/EC, the date of minimum durability may be replaced by the words ‘these animals must be alive when sold’. Finally, the label, including the identification mark, must be waterproof.

9.4.3.8 Chapter VIII - Other requirements

(i) The temperature at which live bivalve molluscs are stored/transported in should be at one that does not adversely affect food safety or their viability.

(ii) Once the live bivalve molluscs have been packed for retail sale and have left the dispatch centre, they cannot be re-immersed in or sprayed with water.

9.4.3.9 Chapter IX - Specific requirements for pectinidae harvested outside classified production areas

Requirements for harvesting and handling following harvesting and health standards for live bivalve molluscs must be met. The requirements for production areas as stated earlier apply to pectinidae and these can only be placed on the market through a fish auction, dispatch centre or processing establishment. Requirements of Chapters III and IV are to be complied with. Registration document requirements also apply.

Regulation (EC) 854/2004 gives requirements for official controls on products of animal origin and states requirements for those enforcing the provisions.

In this regulation, general principles for official controls in respect of all products of animal origin falling within the scope of the regulation are given. It is a requirement that food business operators give assistance to ensure that official controls carried out by the competent authority can be done properly. The official controls include audits of good hygiene practices and hazard analysis and critical control point (HACCP)-based procedures.

Fishery products in particular must comply with the requirements of Annex III of the regulation. This refers to official controls or production and placing on the market of fishery products. The requirements refer to hygienic conditions and regular inspections both on land and at port to ensure compliance of temperature and other requirements. Therefore the health standards for fishery products as previously stated in Annex III, Section VIII, Chapter V of Regulation 853/2004 apply.

9.5.1 Regulation (EC) 854/2004 Annex III

Chapter III states that fishery products are to be declared unfit for human consumption if:

- organoleptic, chemical, physical or microbiological checks or checks for parasites have shown that they are not in compliance with the relevant Community legislation;

- they contain in their edible parts contaminants or residues in excess of the limits laid down in Community legislation or at levels where the calculated dietary intake would exceed the acceptable daily or weekly intake for humans;

- they derive from:
  (i) poisonous fish,
  (ii) fishery products not complying with the requirement concerning biotoxins, or
  (iii) bivalve molluscs, echinoderms, tunicates or marine gastropods containing marine biotoxins in total quantities exceeding the limits referred to in Regulation (EC) No 853/2004; or
9.5.2 Regulation (EC) 854/2004, Annex II

This states official controls for the production and placing on the market of live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods. A summary is given as follows:

9.5.2.1 Chapter II - Official controls concerning live bivalve molluscs from classified production areas

A. Classification of production and relaying areas:

- This section reinforces that specified in Annex III, section VII, chapter II on hygiene requirements for the production and harvesting of live bivalve molluscs in Regulation (EC) 853/2004, as previously stated. Also, chapter V is reinforced.

- Live bivalve molluscs from class A areas may be collected for direct human consumption provided health standards are met.

- Live bivalve molluscs from Class B areas must not exceed, in 90% of the samples, 4,600 $E. coli$ per 100 g of flesh and intravalvular liquid. In the remaining 10% of samples, live bivalve molluscs must not exceed 46,000 $E. coli$ per 100 g of flesh and intravalvular liquid. The reference method for this analysis is the five-tube, three dilutions Most Probable Number (MPN) test specified in ISO 16649-3.

- Live bivalve molluscs from Class C areas must not exceed 46,000 $E. coli$ per 100 g of flesh and intravalvular liquid. The reference method for this analysis is the five-tube, three dilutions MPN test specified in ISO 16649-3.

- Requirements for the competent authority are stated in relation to the classification or a production or relaying area is stated.

B. Monitoring of classified relaying and production areas

This is to ensure there is no malpractice, that microbiological criteria and chemical contaminants amongst other requirements are met. Sampling plans and frequency are required.
C. Decisions after monitoring

There are specific requirements whereby if sampling results indicate that health standards for molluscs are exceeded or there is a risk to human health, the competent authority is to close the production area. Procedures for re-opening a closed production area are stated.

D. Additional monitoring requirements

This concerns the competent authority in relation to monitoring of classified production areas where bivalve mollusc harvesting is forbidden and of reelaying and production zones to ensure there is no malpractice etc.

E. Recording and exchange of information

Requirements are stated for competent authorities in relation to establishing and maintaining a current list of approved production and reelaying areas and their role to inform interested parties of any changes of location, boundaries or class of a production area or its closure.

F. Food business operators' own checks

Controls that food business operators have done are considered by the competent authority when classifying, opening or closing a production area.

9.5.2.2 Chapter III - Official controls concerning pectinidae harvested outside classified production areas

These are to be carried out in fish auctions, dispatch centres and processing establishments in order to confirm that health standards and other specific requirements are met.

9.5.3 Imports

By way of derogation from Article 11(1) of Regulation (EC) No 854/2004, the Member States may permit the import of bivalve molluscs and fishery products from the countries listed respectively in Annex I and Annex II to regulation (EC) 2076/2005.


Regulation (EC) No. 2073/2005, which has applied since 1 January 2006, establishes microbiological criteria for a range of foods.
The aim of this legislation is to complement food hygiene requirements, ensuring that foods being placed on the market do not pose a risk to human health, and the legislation applies to all businesses involved in food production and handling.

The definition of ‘microbiological criterion’ means a criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of microorganisms, and/or on the quantity of their toxins or metabolites, per unit(s) of mass, volume, area or batch;

Two kinds of criteria have been established: food safety criteria, applying to products placed on the market and process hygiene criteria that are applied during the manufacturing process.

9.6.1 Food safety criteria

Chapter 1 of the regulation focuses on food safety criteria that cover foods such as ready to eat foods and fishery products and live bivalve molluscs. The relevant criteria are outlined in Table 9.I.

9.6.2 Process hygiene criteria

Chapter 2 focuses on process hygiene criteria, with chapter 2.4 referring to fishery products. The criteria are outlined in Table 9.II.
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</tr>
</thead>
<tbody>
<tr>
<td>1.1 Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes (4)</td>
<td><em>L. monocytogenes</em></td>
<td>10</td>
<td>5</td>
<td>Absence in 25 g</td>
<td>EN/ISO 11290-1</td>
</tr>
<tr>
<td>1.2 Ready-to-eat foods able to support the growth of L. monocytogenes, other than those intended for infants and for special medical purposes</td>
<td><em>L. monocytogenes</em></td>
<td>5</td>
<td>0</td>
<td>100 cfu/g</td>
<td>EN/ISO 11290-2 (5)</td>
</tr>
<tr>
<td>1.3 Ready-to-eat foods unable to support the growth of L. monocytogenes, other than those intended for infants and for special medical purposes (4) (8)</td>
<td><em>L. monocytogenes</em></td>
<td>5</td>
<td>0</td>
<td>Absence in 25 g</td>
<td>EN/ISO 11290-1</td>
</tr>
<tr>
<td>1.16 Cooked crustaceans and molluscan shellfish</td>
<td><em>Salmonella</em></td>
<td>5</td>
<td>0</td>
<td>Absence in 25 g</td>
<td>EN/ISO 6579</td>
</tr>
<tr>
<td>Food category</td>
<td>Microorganisms</td>
<td>Sampling plan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Limit&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Analytical reference method&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Stage where the criterion applies</td>
</tr>
<tr>
<td>------------------------------------------------------------------------------</td>
<td>----------------</td>
<td>---------------------------</td>
<td>------------------</td>
<td>----------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>1.17 Live bivalve molluscs and live echinoderms, tunicates and gastropods</td>
<td><em>Salmonella</em></td>
<td>n = 5, c = 0</td>
<td>Absence in 25 g</td>
<td>EN/ISO 6579</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td>1.25 Live bivalve molluscs and live echinoderms, tunicates and gastropods</td>
<td><em>E. coli</em>&lt;sup&gt;(16)&lt;/sup&gt;</td>
<td>n = 1&lt;sup&gt;(16)&lt;/sup&gt;, c = 0</td>
<td>230 MPN/100 g of flesh and intra-valvular liquid</td>
<td>ISO TS 16649-3</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td>1.26 Fishery products from fish species associated with a high amount of histidine&lt;sup&gt;(17)&lt;/sup&gt;</td>
<td>Histamine</td>
<td>n = 9&lt;sup&gt;(18)&lt;/sup&gt;, c = 2</td>
<td>100 mg/kg, 200 mg/kg</td>
<td>HPLC&lt;sup&gt;(19)&lt;/sup&gt;</td>
<td>Products placed on the market during their shelf life</td>
</tr>
<tr>
<td>1.27 Fishery products which have undergone enzyme maturation treatment in brine, manufactured from fish species associated with a high amount of histidine&lt;sup&gt;(17)&lt;/sup&gt;</td>
<td>Histamine</td>
<td>n = 9, c = 2</td>
<td>200 mg/kg, 400 mg/kg</td>
<td>HPLC&lt;sup&gt;(19)&lt;/sup&gt;</td>
<td>Products placed on the market during their shelf life</td>
</tr>
</tbody>
</table>
1. $n$ = number of units comprising the sample; $c$ = number of sample units giving values between $m$ and $M$

2. For point 1.1 - 1.25, $m = M$.

3. The most recent edition of the standard shall be used.

4. Regular testing against the criterion is not required in normal circumstances for the following ready-to-eat foods:
   - those which have received heat treatment or other processing effective to eliminate *L. monocytogenes*, when recontamination is not possible after this treatment (for example, products heat treated in their final package),
   - fresh, uncut and unprocessed vegetables and fruits, excluding sprouted seeds,
   - bread, biscuits and similar products,
   - bottled or packed waters, soft drinks, beer, cider, wine, spirits and similar products,
   - sugar, honey and confectionery, including cocoa and chocolate products,
   - live bivalve molluscs.

5. This criterion shall apply if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf life. The operator may fix intermediate limits during the process that must be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf life.

6. 1 ml of inoculum is plated on a Petri dish of 140 mm diameter or on three Petri dishes of 90 mm diameter.

7. This criterion shall apply to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf life.

8. Products with pH ≤ 4.4 or aw ≤ 0.92, products with pH ≤ 5.0 and aw ≤ 0.94, products with a shelf-life of less than five days shall be automatically considered to belong to this category. Other categories of products can also belong to this category, subject to scientific justification.

15. *E. coli* is used here as an indicator of faecal contamination.

16. A pooled sample comprising a minimum of 10 individual animals.

17. Particularly fish species of the families: *Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombresoxidae*.

18. Single samples may be taken at retail level. In such a case the presumption laid down in Article 14(6) of Regulation (EC) No 178/2002, according to which the whole batch is to be deemed unsafe, shall not apply.

plaice (Pleuronectes platessa) and whiting (Merlangus merlangus. J. AOAC Internat. 1999, 82, 1097-101.

Interpretation of the test results relating to Table 9.I
The limits given refer to each sample unit tested, excluding live bivalve molluscs and live echinoderms, tunicates and gastropods in relation to testing E. coli, where the limit refers to a pooled sample.

The test results demonstrate the microbiological quality of the batch tested.

L. monocytogenes in ready-to-eat foods intended for infants and for special medical purposes:
— satisfactory, if all the values observed indicate the absence of the bacterium,
— unsatisfactory, if the presence of the bacterium is detected in any of the sample units.

L. monocytogenes in ready-to-eat foods able to support the growth of L. monocytogenes before the food has left the immediate control of the producing food business operator when he is not able to demonstrate that the product will not exceed the limit of 100 cfu/g throughout the shelf life:
— satisfactory, if all the values observed indicate the absence of the bacterium,
— unsatisfactory, if the presence of the bacterium is detected in any of the sample units.

L. monocytogenes in other ready-to-eat foods:
— satisfactory, if all the values observed are ≤ the limit,
— unsatisfactory, if any of the values are > the limit.

Salmonella in different food categories:
— satisfactory, if all the values observed indicate the absence of the bacterium,
— unsatisfactory, if the presence of the bacterium is detected in any of the sample units.

Histamine in fishery products from fish species associated with a high amount of histidine:
— satisfactory, if the following requirements are fulfilled:
1. the mean value observed is ≤ m
2. a maximum of c/n values observed are between m and M
3. no values observed exceed the limit of M,
— unsatisfactory, if the mean value observed exceeds m or more than c/n values are between m and M or one or more of the values observed are > M.

(1) The test results may be used also for demonstrating the effectiveness of the hazard analysis and critical control point principles or good hygiene procedure of the process.
### TABLE 9.II

**Process hygiene criteria**

<table>
<thead>
<tr>
<th>Food category</th>
<th>Microorganisms</th>
<th>Sampling plan$^{(1)}$</th>
<th>Limit$^{(2)}$</th>
<th>Analytical reference method$^{(3)}$</th>
<th>Stage where the criterion applies</th>
<th>Action in case of unsatisfactory results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.1 Shelled and shucked products of cooked crustaceans and molluscan shellfish</td>
<td>E. coli</td>
<td>5 2</td>
<td>1 cfu/g</td>
<td>10 cfu/g</td>
<td>ISO TS 16649-3</td>
<td>End of the manufacturing process</td>
</tr>
<tr>
<td></td>
<td>Coagulase-positive staphylococci</td>
<td>5 2</td>
<td>100 cfu/g</td>
<td>1000 cfu/g</td>
<td>EN/ISO 6888-1 or 2</td>
<td>End of the manufacturing process</td>
</tr>
</tbody>
</table>
LEGISLATION

1. \( n \) = number of units comprising the sample; \( c \) = number of sample units giving values between \( m \) and \( M \)

2. The most recent edition of the standard shall be used.

Interpretation of the test results relating to Table 9.II

The limits given refer to each sample unit tested.

The test results demonstrate the microbiological quality of the process tested.

\( E. \ coli \) in shelled and shucked products of cooked crustaceans and molluscan shellfish:
— satisfactory, if all the values observed are \( \leq m \),
— acceptable, if a maximum of \( c/n \) values are between \( m \) and \( M \), and the rest of the values observed are \( \leq m \),
— unsatisfactory, if one or more of the values observed are \( > M \) or more than \( c/n \) values are between \( m \) and \( M \).

Coagulase-positive staphylococci in shelled and cooked crustaceans and molluscan shellfish:
— satisfactory, if all the values observed are \( \leq m \),
— acceptable, if a maximum of \( c/n \) values are between \( m \) and \( M \), and the rest of the values observed are \( \leq m \),
— unsatisfactory, if one or more of the values observed are \( > M \) or more than \( c/n \) values are between \( m \) and \( M \).

(Hygiene requirements specific to the U.K)

9.7.1 Temperature control requirements


The regulations prescribe a chilled food holding temperature of 8 °C or less, but there is also a general requirement that foods must not be kept at temperatures that would result in a risk to health, and particularly that perishable foodstuffs must not be kept at above the maximum recommended storage temperature, which overrides the 8 °C requirement. Hot-held foods (food having been cooked or reheated and is for service or on display for sale) must not be kept below 63 °C.

The regulations provide for defences in relation to upward variations of the 8 °C temperature, tolerance periods for chill-holding of foods and hot-holding variations. The defendant may be required to produce well-founded scientific proof to support his claims. For example, with chill holding tolerance periods, the defendant will need to prove that the food was on service or display, had not been
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previously put on display at more than 8 °C and had been kept there for less than four hours. Alternatively, it would need to be proved that the food was being transferred to or from a vehicle used for the activities of a food business, to or from premises (including vehicles) at which the food was to be kept at or below 8 °C or the recommended temperature, or, was kept at above 8 °C or the recommended temperature for an unavoidable reason, such as that below, and was kept at above 8 °C or the recommended temperature, for a limited period consistent with food safety. The permitted reasons are given below:

- to facilitate handling during and after processing or preparation
- the defrosting of equipment, or
- temporary breakdown of equipment

For Scotland there are separate provisions to include requirements to hold food under refrigeration or in a cool ventilated place, or at a temperature above 63 °C and to reheat food to a temperature of at least 82 °C (The Food Hygiene (Scotland) Regulations 2006, S.S.I. 2006 No. 3).

Schedule 4 of the Food Hygiene (England) Regulations 2006 contains several definitions, including:

Shelf life: where the minimum durability or 'use by' indication is required according to Regulation 20 or 21 of the Food Labelling Regulations 1996 (form of indication of minimum durability and form of indication of 'use-by date') the period up to and including that date. For other food, the period for which it can be expected to remain fit for sale when kept in a manner consistent with food safety.

Recommended temperature: a specified temperature that has been recommended in accordance with a food business responsible for manufacturing, preparing or processing the food recommending that it be kept at or below a specified temperature between 8 °C and ambient temperatures.

It should be noted that the temperature control requirements as detailed in Schedule 4 of the Food Hygiene (England) Regulations 2006 (S.I. 2006 No. 14) do not apply to any food covered by EU Regulation 853/2004 on hygiene of products of animal origin or any food business operation carried out on a ship or aircraft.

9.8 Guidance

In the U.K, the Food Standards Agency has published guidance notes on the requirements of the EU hygiene and microbiological criteria regulations which can be accessed at the following link:
http://www.food.gov.uk/foodindustry/guidancenotes/hygguid/fhlguidance/
9.9 Other Relevant Legislation

The following regulations about fish designations need to be considered:

- Regulation (EC) No 104/2000 on the common organisation of the markets in fishery and aquaculture products


Note: Requirements for preserved fish are laid down elsewhere, namely, Regulation (EEC) 2136/89, as amended, laying down common marketing standards for preserved sardines, and Regulation (EEC) 1536/92 laying down common marketing standards for preserved tuna and bonito; these are not be discussed here.

9.10 Further Reading


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10. PATHOGEN PROFILES

10.1 Aeromonas spp.

10.1.1 Morphology
Gram-negative rods, 0.3 - 1.0 x 1.0 - 3.5 µm

10.1.2 Oxygen requirements
Facultative anaerobe.

10.1.3 Temperature
*Aeromonas* grows at temperatures of 2 - 45 ºC (1). At 5 ºC, a 10 to 1,000-fold increase in number is observed after one week in food systems (1). The optimum growth temperature is 28 ºC (2).

10.1.4 Heat resistance
Aeromonads are sensitive to moderately high temperatures, and D-values at 45 and 51 ºC have been reported as 29.5 minutes, and 2.3 minutes, respectively (1).

10.1.5 pH
Under otherwise optimal conditions, *Aeromonas* has the potential to grow over a pH range of ca 4 - 10 (3). The minimum pH value for growth is thought to be pH 5.5, under optimal incubation temperatures. Some death will occur at this pH if the temperature is 4 ºC. At pH 4.5, no growth is observed (1).
10.1.6 *Aeromonas* sodium chloride

Growth of *Aeromonas* is optimal in the presence of 1 - 2% sodium chloride (NaCl) (1), and is sensitive to > 4.5% NaCl (4).

10.2 *Clostridium botulinum*

Seven different types of *C. botulinum* are known, forming at least seven different toxins, A to G. Types A, B, E and, to a lesser extent, F are the types that are responsible for most cases of human botulism (5, 6). All type A strains are proteolytic and type E strains are usually non-proteolytic; types B and F can be either. There are four main groupings of the organism, and groups I and II are those responsible for the botulism cases.

10.2.1 Morphology

Gram-positive, spore-forming rods; 0.5 - 2.4 x 1.7 - 22.0 µm.

10.2.2 Oxygen requirements

Although *C. botulinum* is an obligate anaerobe, many foods that are not obviously ‘anaerobic’ can provide adequate conditions for growth. Thus an aerobically packed product may not support the growth of the organism on the surface, but the interior of the food may do so. It is also important to note that the inclusion of oxygen as a packaging gas cannot ensure that growth of *C. botulinum* is prevented.

10.2.3 Temperature

All strains of *C. botulinum* grow reasonably well in the temperature range of 20 - 45 °C, but the minimum temperatures required to inhibit Groups I (proteolytic group) and II (non-proteolytic group) are different. Group I will not grow at temperatures of 10 °C or less, but Group II strains are psychrotrophic, being capable of slow growth and toxin production at low temperatures - even as low as 3 °C (7).

10.2.4 Heat resistance

The vegetative cells of *C. botulinum* are not particularly heat resistant, but the spores of this organism are more so. All *C. botulinum* types produce heat labile toxins, which may be inactivated by heating at 80 °C for 20 - 30 minutes, at 85 °C for 5 minutes, or at 90 °C for a few seconds.
10.2.5 pH

The minimum pH for the growth of proteolytic and non-proteolytic strains is pH 4.6 and pH 5.0, respectively (8, 9).

10.2.6 $a_w$/Sodium chloride

The minimum water activity ($a_w$) for growth of *C. botulinum* depends on solute, pH and temperature, but under optimum growth conditions 10% (w/w) NaCl is required to prevent growth of Group I, and 5% (w/w) NaCl is necessary to prevent growth of Group II organisms. These concentrations correspond to limiting $a_w$ of 0.94 for Group I and 0.97 for Group II (5). These values have been established under carefully controlled laboratory conditions. In commercial situations, safety margins must be introduced to allow for process variability. Note also that if humectants other than salt are used, the minimum $a_w$ for growth may be lower.

10.2.7 Characteristics of *C. botulinum* spores

The most heat resistant spores of Group I *C. botulinum* are produced by type A and proteolytic B strains ($D_{121^\circ C}=0.21$ minutes) (9).

The spores of Group II (non-proteolytic/psychrotrophic) strains are less heat resistant than Group I strains. However, they may survive mild heat treatments (70 - 85 °C) and their ability to grow at refrigeration temperatures necessitates their control in foods capable of supporting their growth (e.g. vacuum packed, par-cooked meals with pH value > 5.0 and $a_w > 0.97$) (10, 11)).

10.3 *Clostridium perfringens*

10.3.1 Morphology

Gram-positive spore-forming rods; 0.3 - 1.9 x 2.0 - 10.0 µm.

10.3.2 Oxygen requirements

*C. perfringens* - like other clostridia - is an obligate anaerobe. It will not, therefore, grow on the surface of foods unless they are vacuum or gas-packed. The organism will grow well in the centre of meat or poultry dishes, where oxygen levels are reduced, particularly by cooking.
10.3.3 Temperature

The most significant characteristic of *C. perfringens* in relation to food safety is the organism’s ability to grow extremely rapidly at high temperatures. Its optimum temperature for growth is 43 - 45 °C, although *C. perfringens* has the potential ability to grow within the temperature range 15 - 50 °C, depending on strain and other conditions. While some growth can occur at 50 °C, death of the vegetative cells of this organism usually occurs rapidly above this temperature (12, 13). At cold temperatures, 0 - 10 °C, vegetative cells die rapidly (12).

10.3.4 Heat resistance

Exposure to a temperature of 60 °C or more will result in the death of vegetative cells of *C. perfringens*, although prior growth at high temperatures or the presence of fat in a food will result in increased heat resistance. (It is unusual for spores to be formed in foods after the growth of this organism (14)). In addition, the enterotoxin is not heat resistant - it is destroyed by heating at 60 °C for 10 minutes (14, 15, 16). The D-value for *C. perfringens* in roast beef at 60 °C is 14.5 minutes (17).

10.3.5 pH

*C. perfringens* is not a tolerant organism with respect to pH. It grows best at pH values between 6 and 7 (the same pH as most meats). Under otherwise ideal conditions, very limited growth may occur at pH values over the range pH ≤ 5 and ≥ 8.3. Spores, however, will survive greater extremes of pH (and aw) (12, 13).

10.3.6 aw/Sodium chloride

*C. perfringens* is not tolerant of low water activities. As in the case of other factors limiting the growth/survival of this organism, the limits for aw are affected by temperature, pH, type of solute, etc. The lowest aw recorded to support the growth of *C. perfringens* appears to be 0.93 and 0.97 (glycerol and sucrose respectively) depending on the solute used to control aw (13, 18). Salt concentrations of 6 - 8% inhibit growth of most *C. perfringens* strains; lower concentrations may be effective in combination with other factors. Some studies indicate that the presence of 3% NaCl delays growth of *C. perfringens* in vacuum packed beef (18).

10.3.7 Characteristics of *C. perfringens* spores

The spores of *C. perfringens* can vary quite considerably in their heat resistance, and their heat resistance is also affected by the heating substrate. Recorded heat
resistance values at 95 °C (D-values) range from 17.6 - 64.0 minutes for heat resistant spores to 1.3 - 2.8 minutes for heat sensitive spores (12).

10.4  Listeria spp.

10.4.1 Morphology
Gram-positive short rods; 0.4 - 0.5 x 0.5 - 2.0 µm.

10.4.2 Oxygen requirements
Aerobe or microaerophilic.

10.4.3 Temperature
Listeria monocytogenes is unusual amongst foodborne pathogens in that it is psychrotrophic, being potentially capable of growing - albeit slowly - at refrigeration temperatures down to, or even below 0 °C. However, -0.4 °C is probably a more likely minimum for growth in foods (19). Its optimum growth temperature, however, is between 30 and 37 °C; growth at low temperatures can be very slow, requiring days or weeks to reach maximum numbers. The upper temperature limit for the growth of L. monocytogenes is reported to be 45 °C (20).

10.4.4 Heat resistance
L. monocytogenes is not a particularly heat resistant organism; it is not a spore-former, so can be destroyed by pasteurisation. It has been reported to have slightly greater heat resistance than certain other foodborne pathogens. D-values for L. monocytogenes in crawfish tail meat at 55, 60 and 65 °C were reported as 10.23, 1.98 and 0.19 minutes, respectively (21).

10.4.5 pH
The ability of Listeria to grow at different pH values (as with other bacteria) is markedly affected by the type of acid used, and temperature. Under ideal conditions, the organism is able to grow at pH values well below pH 5 (pH 4.3 is the lowest value where growth has been recorded, using hydrochloric acid as an acidulant). In foods, however, the lowest limit for growth is likely to be considerably higher - especially at refrigeration temperatures, and where acetic acid is used as an acidulant; pH < 5.2 has been suggested as the lowest working limit (22).
10.4.6 A<sub>W</sub>/Sodium chloride

*L. monocytogenes* is quite tolerant of high NaCl/low water activities. It is likely to survive, or even grow, at salt levels found in foods (10 - 12% NaCl or more). It grows best at a<sub>W</sub> of ≥ 0.97, but has been shown to be able to grow at an a<sub>W</sub> level of 0.90. The bacterium may survive for long periods at a<sub>W</sub> values as low as 0.83 (20).

10.5 *Plesiomonas*

10.5.1 *Morphology*

Gram-negative, 0.8 - 1.0 x 1 - 3 μm.

10.5.2 *Oxygen requirements*

Facultative anaerobe.

10.5.3 *Temperature*

*Plesiomonas* can grow in the temperature range of 8 - 45 ºC, the optimum being 30 ºC (23).

10.5.4 *Heat resistance*

Pasteurisation at 60 ºC for 30 minutes has been reported as being effective in killing *Plesiomonas shigelloides* (24).

10.5.5 *pH*

*Plesiomonas* has a pH range for growth of pH 4.5 - 8.5 (24, 25).

10.5.6 A<sub>W</sub>/Sodium chloride

*Plesiomonas* can grow well at NaCl concentrations up to 4% (24, 25).

10.6 *Salmonella* spp.

10.6.1 *Morphology*

Gram-negative short rods; peritrichous flagella; 0.5 - 0.7 x 1.0 - 3.0 μm.
10.6.2 Oxygen requirements

Facultative anaerobe.

10.6.3 Temperature

Salmonellae can grow in the temperature range of 7 - 48 ºC. However, some strains are able to grow at temperatures as low as 4 ºC (26). Growth is slow at temperatures below about 10 ºC, the optimum being 35 - 37 ºC.

Salmonellae are quite resistant to freezing.

10.6.4 Heat resistance

Salmonella is not a spore-forming organism. It is not, therefore, a heat resistant organism; pasteurisation and equivalent treatments will destroy the organism under normal circumstances. $D_{60}$ values normally range from about 1 to 10 minutes, with a z-value of 4 - 5 ºC. However, high fat or low moisture levels (low $a_w$) will reduce the effectiveness of heat treatments, and appropriate heat treatments must be determined experimentally for low-$a_w$ foods. Furthermore, strains vary in their ability to withstand heating; Salmonella senftenberg 775W is about 10 - 20 times more heat resistant than the average strain of Salmonella at high $a_w$ (27).

10.6.5 pH

Salmonella has a pH range for growth of pH 3.8 - 9.5, under otherwise ideal conditions, and with an appropriate acid. Some death will occur at pH values of less than about 4.0, depending on the type of acid and temperature. The optimal pH for Salmonella growth is between 6.5 - 7.5.

10.6.6 $a_w$/Sodium chloride

Where all other conditions are favourable, Salmonella has the potential to grow at $a_w$ levels as low as 0.945, or possibly 0.93 (as reported in dried meat and dehydrated soup), depending on serotype, substrate, temperature and pH. Salmonellae are quite resistant to drying.

The growth of Salmonella is generally inhibited by the presence of 3 - 4% NaCl, although salt tolerance increases with increasing temperature (28).
10.7  *Staphylococcus aureus*

10.7.1  **Morphology**

Gram-positive cocci; 0.7 - 0.9 µm diameter.

10.7.2  **Oxygen requirements**

Facultative anaerobe.

NB: The growth of *Staph. aureus* is more limited under anaerobic than aerobic conditions. The limits for toxin production are also narrower than for growth. The following relate to limits for growth only.

10.7.3  **Temperature**

Under otherwise ideal conditions, *Staph. aureus* can grow within the temperature range 7 – 48.5 ºC, with an optimum of 35 - 37 ºC (29). It can survive well at low temperatures.

Freezing and thawing have little effect on *Staph. aureus* viability, but may cause some cell damage (30).

10.7.4  **Heat resistance**

Heat resistance depends very much on the food type in which the organism is being heated (conditions relating to pH, fat content, a_w, etc.). As is the case with other bacteria, stressed cells can also be less tolerant of heating.

Under most circumstances, however, the organism is heat sensitive and will be destroyed by pasteurisation. In meat macerate, the D_60 ºC value is 2 - 20 minutes, depending on a_w.

10.7.5  **pH**

The pH at which a staphylococcal strain will grow is dependent upon the type of acid (acetic acid is more effective at destroying *Staph. aureus* than citric acid), a_w, and temperature (sensitivity to acid increases with temperature).

Most strains of staphylococci can grow within the pH range 4.2 to 9.3 (optimum 7.0 - 7.5), under otherwise ideal conditions (29, 31).
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10.7.6 $A_w$/Sodium chloride

_Staph. aureus_ is unusual amongst food-poisoning organisms in its ability to tolerate low water activities. It can grow over the $a_w$ range 0.83 - >0.99 aerobically under otherwise optimal conditions. However, an $a_w$ of 0.86 is the generally recognised minimum in foods (32).

Staphylococci are more resistant to salt present in foods than other organisms. In general, _Staph. aureus_ can grow in 7 - 10% NaCl, but certain strains can grow in 20% NaCl. An effect of increasing salt concentration is to raise the minimum pH of growth.

10.7.7 Limits permitting toxin production

**Temperature:** 10 - 46 ºC (optimum between 35 and 40 ºC) (very little toxin is produced at the upper and lower extremes) (31)

**pH:** 5.2 - 9.0 (optimum 7.0 - 7.5) (29, 31)

**$A_w$:** between 0.87 and > 0.99

**Atmosphere:** little or no toxin production in anaerobically packed foods, especially vacuum packed foods (33)

**Heat Resistance:** Enterotoxins are quite heat resistant. In general, heating at 100 ºC for at least 30 minutes may be required to destroy unpurified toxin (31, 34).

* dependent on temperature, pH, atmosphere, strain, and solute.

10.8 _Vibrio cholerae_

10.8.1 Morphology

Gram-negative curved rods; 0.5 - 0.8 x 1.4 - 2.6 µm

10.8.2 Oxygen requirements

Facultative anaerobe.

10.8.3 Temperature

The optimum temperature for growth has been reported as 30 ºC, with a temperature range for growth of 10 - 43 ºC (35).
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Also, there is evidence that *V. cholerae* may persist on frozen fish for >180 days (36).

10.8.4 *Heat resistance*

*V. cholerae* is sensitive to heat at temperatures of >45 °C. The D-value for *V. cholerae* in crab meat homogenate at 60 °C is 2.65 minutes (35).

10.8.5 *pH*

This organism is tolerant of high pH values, but not acid conditions; it has a pH range for growth of pH 5 - 9.6, with optimum at 7.6 (35, 37, 38).

10.8.6 *a_w/Sodium chloride*

*V. cholerae* can grow over the *a_w* range 0.97 - 0.998, with an optimum of 0.984 (35). It can grow in the presence of 0.1 - 4.0% NaCl (35).

10.9 *Vibrio parahaemolyticus*

10.9.1 *Morphology*

Gram-negative curved rods.

10.9.2 *Oxygen requirements*

Facultative anaerobe.

10.9.3 *Temperature*

The minimum and maximum temperatures reported for growth in laboratory media, under otherwise favourable conditions, are 5 and 43 °C, respectively, with an optimum of 37 °C (39).

*V. parahaemolyticus* is easily destroyed by drying, and it is sensitive to refrigeration temperatures (0 - 5 °C), declining in number during storage, but only moderately sensitive to freezing (40).
10.9.4 Heat resistance

*V. parahaemolyticus* are not heat resistant, and are readily destroyed by cooking. The D-values at 55 °C for clam and crab homogenate are 0.02 - 0.29 minutes, and 2.5 minutes, respectively (39).

10.9.5 pH

The pH range for growth is 4.8 - 11, with an optimum pH of 7.6 - 8.6 (41).

10.9.6 A_w/Sodium chloride

*V. parahaemolyticus* will grow at a_w levels as low as 0.922, depending on solute. The optimum concentration of NaCl equates to a_w of approximately 0.98 (35, 41).

*V. parahaemolyticus* is unable to grow in the absence of salt; its upper and lower limits for growth are 8 (although some reports suggest 10) and 0.5% NaCl, respectively, but the optimum concentration is 2 - 4% (39, 41).

10.10 *Vibrio vulnificus*

10.10.1 Morphology

Gram-negative curved rods.

10.10.2 Oxygen requirements

Facultative anaerobe.

10.10.3 Temperature

The optimum temperature for growth has been reported at 37 °C, with a temperature range for growth of 8 - 43 °C (42).

The organism can survive for long periods at freezer temperatures (42).

10.10.4 Heat resistance

*V. vulnificus* is heat sensitive; it has been reported that cooking oysters for 10 minutes at 50 °C should ensure destruction of the organism (43).
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**10.10.5 pH**

The pH range for growth is 5 - 10, with an optimum pH of 7.8 (42).

**10.10.6 A. w/Sodium chloride**

*V. vulnificus* grows over the range 0.5 - 5.0% NaCl, with an optimum of 2.5% (42).

**10.11 References**


PATHOGEN PROFILES


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Useful Web Sites

- Gateway to Government food safety information (US)
  http://www.FoodSafety.gov/
- World Health Organization (WHO): Food safety programmes and projects
  http://www.who.int/foodsafety/en/
- European Commission: Activities of the European Union food safety
  http://europa.eu/pol/food/index_en.htm
- Centre for Disease Control and Prevention (CDC) (US)
  http://www.cdc.gov/foodsafety/
- Food Safety Authority of Ireland
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