# Microbiological Spoilage of Fish and Seafood Products

**Lone Gram**

## Introduction

Fish and seafood products are some of the most important protein sources in human nutrition. At the same time, these products are perishable and, if left unpreserved, spoil rapidly. Some fish products are heavily cured (salted, dried) and shelf stable at ambient temperature. An increasing number of fish products are preserved by low levels of salt, cooling, packaging in modified atmosphere, and/or addition of low levels of preservatives. The microflora of these products is often complex; however, spoilage is mostly caused by microbial action.

Production of fish has increased over many years and the increase in the last two decades has mostly been due to a dramatic growth in the aquaculture sector (Fig. 1). Catches from wild fish populations have stagnated at approximately 90–100 million metric tons and today (2005), 40% of the fish used for human consumption are aquaculture-reared species. Also, our processing of fish has changed. The increase in fish production has mostly been utilized as "fresh fish," whereas cured and canned products have become, proportionally, less popular (Fig. 2).

The raw materials for seafood products are bivalve mollusks, crustaceans, cephalopods, or finfish. These organisms have very different lifestyle and different gross composition, which influence subsequent spoilage patterns. Bivalve mollusks such as oyster accumulate glycogen (Martino & da Cruz, 2004), whereas other fish raw materials are virtually devoid of carbohydrates. Therefore, postmortem pH does not decrease to the same extent as pH in red meats and *Shewanella* species, which are quite sensitive to low pH can therefore grow and spoil chilled finfish similar to their involvement in spoilage of high-pH meat (Borch, KantMuermans, & Blixt, 1996). Crustacea have a high content of free amino acids, some of which (e.g., arginine and glycine) contribute to the characteristic crustacean flavor (Finne, 1992). Many cephalopods contain ammonia ($NH_4^+$) in quite high concentration

L. Gram (✉)
National Institute of Aquatic Resources, Technical University of Denmark, Soltofts Plads bldg 221, DK-2800 Kgs Lyngby, Denmark
e-mail: gram@aqua.dtu.dk

W.H. Sperber, M.P. Doyle (eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages*, Food Microbiology and Food Safety,
DOI 10.1007/978-1-4419-0826-1_4, 

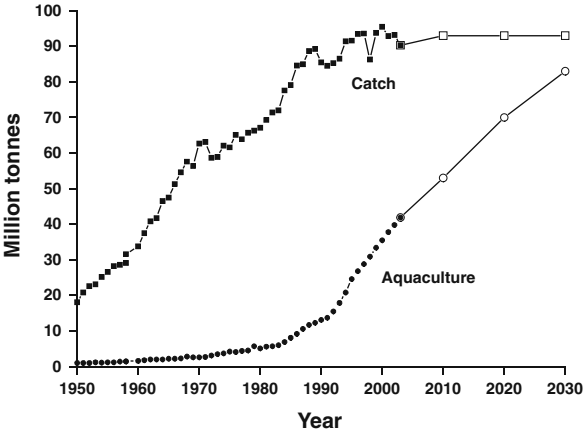


**Fig. 1** Global fish production from wild fish catches and from aquaculture (FAO, 2004)

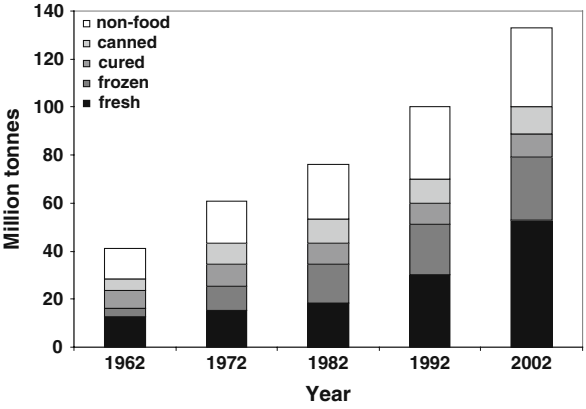


**Fig. 2** Utilization of fish catches (FAO, 2004)

(100–300 mM) (Seibel, Goffredi, Thuesen, Childress, & Robison, 2004). Seafood raw materials are, in general, very rich in nonprotein nitrogen (NPN) such as amino acids and trimethylamine oxide, and bacterial metabolism of these compounds is often the cause of spoilage.

## Spoilage Concepts

Spoilage of a food product is, in this chapter, a term used to describe that the product is no longer edible based on a sensory assessment. Safety issues are not included in the spoilage or shelf-life considerations. The sensory rejection may be caused by discoloration, physical changes, textural changes, slime or gas formation, or the development of off-flavors and off-odors. Spoilage may arise due to a number of chemical or microbiological changes. Lipid hydrolysis and oxidation are very

common causes of spoilage in many fatty fish species and rejection of small uneviscerated species such as anchovies may be caused by "belly burst" in which the enzymes and microorganisms of the digestive tract cause massive gas development (Careche, Garcia, & Borderias, 2002). Protein denaturation and development of "card box" flavor due to changes in the protein and lipid fraction are common causes of spoilage of frozen fish products. However, microbial growth and metabolism is the major cause of spoilage of fresh, lightly preserved, and semi-preserved fish products. Microbial spoilage involves growth of bacteria, yeast, or fungi to high numbers and products of their metabolism give rise to the sensory impressions perceived as spoilage. Visible slime may appear as a result of the formation of extracellular polysaccharides from carbohydrate substrates (Lyhs, Koort, Lundstrom, & Bjorkroth, 2004). Fungal growth on, for example, dried fish is also a very visible spoilage form (Chakrabarti & Varma, 2000) as is the red discoloration that often accompanies spoilage of heavily salted fish (Prasad & Panduranga Rao, 1994). Off-odors and off-flavors arise when low-molecular-weight compounds are degraded by microorganisms. Typical spoilage compounds of seafood products include ammonia from deamination of amino acids, sulfides formed from sulfur-containing amino acids (Herbert & Shewan, 1975), trimethylamine resulting from bacterial reduction of trimethylamine oxide, and esters that may arise from degradation of phospholipids (Table 1).

The microflora on newly caught or produced product is a function of the microorganisms present on the live animal and the microorganisms contaminating during processing. Each seafood processing operation has its own unique microflora reflecting the raw materials and the preservation parameters used (Bagge-Ravn, Yin, Hjelm, Christiansen, Johansen, & Gram, 2003). Only some of the microorganisms will be able to tolerate the product-specific conditions (temperature, packaging, pH, water activity) and proliferate during storage. Those successful will form the spoilage microflora (or microbiota), which simply are the microorganisms present on the product at the point of sensory rejection. Only some of the species present are able to produce the off-odors and off-flavors, which are typical of the spoiling product, and this spoilage potential is typically determined by inoculating pure cultures of bacteria in sterile food systems (Chinivasagam, Bremner, Wood, & Nottingham, 1998; Dalgaard, 1995b; Gram, Trolle, & Huss, 1987; Herbert, Hendrie, Gibson, & Shewan, 1971; Joffraud, Leroi, & Chevalier, 1998; Miller, Scanlan, Lee, & Libbey, 1973). Subsequently, it must be determined if the microorganisms with spoilage potential are capable of producing the amounts of compounds associated with the spoiling product under relevant conditions of storage. This assesses the so-called spoilage activity of the microorganisms. For instance, *Vibrio* spp., *Aeromonas* spp., *Shewanella* spp., and *Photobacterium phosphoreum* are capable of reducing trimethylamine oxide (TMAO) to trimethylamine (TMA). The latter, fishy smelling compound, is typical of spoiling gadoid fish species. Gadoid species are those fish belonging to the Gadidae family, which includes cod, haddock, whiting, and pollock.

*Shewanella* species can grow under aerobic storage to $10^8$–$10^9$ cfu/g and produce the amounts of TMA found in aerobically, iced-stored fish. When the fish is packed

**Table 1** Examples of substrates, spoilage products, and spoilage bacteria from different seafood products (modified from Gram, 2005)

| Sensory impression | Spoilage substrate | Spoilage product | Food product | Specific organism | Reference |
|---|---|---|---|---|---|
| Slime | Sugars | Extracellular polysaccharide (dextran) | Acetic acid herring preserve | *Leuconostoc gelidum*<br>*Leuconostoc gasicomitatum* | Lyhs et al. (2004) |
| Gas | Sugars, Protein | $CO_2$ | Preserved herring | *Lactobacillus alimentarius*<br>Yeast | Lyhs, Lahtinen, et al. (2001) |
| Fishy off-odor | Trimethylamine oxide | Trimethylamine | Several fish products | *Shewanella baltica*<br>*Photobacterium phosphoreum*<br>*Aeromonas* spp.<br>*Vibrio* spp.<br>Enterobacteriaceae | Dalgaard et al. (1993)<br>Gram et al. (1990)<br>Vogel et al. (2005) |
| Musty off-odor | | | Salted fish, fresh fish | *Pseudomonas*<br>*Psychrobacter* | Bjorkevoll, Olsen, and Skjerdal (2003)<br>Castell, Greenough, and Jenkin (1995) |
| Ammonia | Amino acids | Ammonia, $NH_3$ | Several fish products | Enterobacteriaceae, lactic acid bacteria<br>*P. phosphoreum* | Jorgensen, Huss, et al. (2000) |
| Sulfide | Cysteine | Hydrogen sulfide, $H_2S$ | Several fish products | *S. baltica*<br>Enterobacteriaceae<br>*Lactobacillus saké,*<br>*Lactobacillus curvatus* | Chai et al. (1968)<br>Hansen (1995)<br>Joffraud, Leroi, Roy, & Berdague (2001)<br>Kadota and Ishida (1972)<br>Segal and Starkey (1969) |
| Sulfhydryl off-odors | Methionine | Dimethyldisulfide, $(CH_3)_2S_2$ | Several fish products | *Pseudomonas*<br>Enterobacteriaceae | Chinivasagam, Bremner, et al. (1998)<br>Kadota and Ishida (1972)<br>Segal and Starkey (1969) |

in $CO_2$ atmosphere, large amounts of TMA are detected; however, counts of *Shewanella* remain below $10^6$ cfu/g. Although they have the spoilage potential, they do not possess sufficient spoilage activity. Instead, $CO_2$-tolerant *P. phosphoreum* grow and produce the TMA formed in packed fish (Dalgaard, 1995b; Dalgaard, Gram, & Huss, 1993).

In some products, the specific spoilage microorganisms are one single group or species, for example, *Shewanella baltica* in iced cod (Chai, Chen, Rosen, & Levin, 1968; Vogel, Venkateswaran, Satomi, & Gram, 2005), *P. phosphoreum* in $CO_2$-packed chilled fish (Dalgaard, Mejlholm, Christiansen, & Huss, 1997), or *Lactobacillus alimentarius* in some marinated herring (Lyhs, Korkeala, Vandamme, & Bjorkroth, 2001). The spoilage patterns of other products may be much more complex and spoilage is brought about by a combination of several microorganisms. Typically, these as single cultures do not give rise to the product spoilage off-odors but only when cocultured (Jorgensen, Huss, & Dalgaard, 2000; Mejlholm, Boknaes, & Dalgaard, 2005).

## *Fish Substrates of Spoilage*

Fish muscle is rich in nonprotein nitrogen and the amino acids, nucleotides, and trimethylamine oxide serve as microbial substrates or electron acceptors. The products of microbial metabolites result in the spoilage of the products (Table 1). Trimethylamine oxide is a small odorless compound which accumulates in finfish, elasmobranches, cephalopods, and some bivalves (Sadok, Uglow, & El-Abed, 2003; Seibel & Walsh, 2002). In finfish, it is mostly found in marine gadoid fish species but can also be formed in other species. It was believed for many years that the compound was not produced in freshwater fish species; however, it has been detected in both Nile perch and tilapia (Anthoni, Borresen, Christophersen, Gram, & Nielsen, 1990). The role of TMAO in fish is not known but it is generally believed that it acts as a compatible solute balancing the effects of salt levels in the marine environment, deep pressure, or high concentrations of urea found in elasmobranches (Seibel & Walsh, 2002; Yancey, Clark, Hand, Bowlus, & Somero, 1982), where it stabilizes protein folding (Yancey, 2005). Several bacterial species may use TMAO as an electron acceptor in an anaerobic respiration and the reduced compound, trimethylamine (TMA), is the most prominent compound giving rise to "fishy" odor. TMAO/TMA is a redox couple and its presence in fish ensures a positive redox potential of approximately 200 mV as opposed to red meat where the Eh is negative. The Eh decreases to negative values when TMAO is reduced to TMA (Huss & Larsen, 1980; Yancey, 2005).

The ability to use TMAO as an electron acceptor gives obligately respiratory microorganisms an advantage when oxygen becomes limited. Ringo, Stenberg, and Strom (1984) suggested that the complete Krebs' cycle was used when *Shewanella* respired using TMAO and that amino acids such as cysteine were used as substrates, resulting in the parallel formation of hydrogen sulfide ($H_2S$). More recent studies

have determined that the metabolism is slightly more complex and that TMAO reduction involves only part of the TCA cycle (Scott & Nealson, 1994).

Sulfurous odors arise from bacterial degradation of L-cysteine and L-methionine. The volatiles consist of an array of compounds including hydrogen sulfide, methyl mercaptan, and dimethyl sulfide (Herbert & Shewan, 1975). Some sulfurous off-odors give rise to more fruity or onion-like off-odors. The fruity off-odors also consist of esters and are typically formed from degradation of monoamine–monocarboxylic amino acids (Castell & Greenough, 1959) but also low-molecular-weight fatty acids can act as substrates for bacterial production of fruity off-odors (Reddy, Bills, & Lindsay, 1969).

Bacterial decarboxylation of amino acids gives rise to the formation of the so-called biogenic amines (Table 2), some of which are off-odorous. These compounds have mainly been of interest due to their role in food safety being the cause of histamine poisoning; however, they have also been used as indicators of spoilage in a number of products.

**Table 2** Amino acids and biogenic amines formed by bacterial degradation

| Amino acid | Biogenic amine | Reaction |
|---|---|---|
| Histidine | Histamine | Decarboxylase |
| Tyrosine | Tyramine | Decarboxylase |
| Lysine | Cadaverine | Decarboxylase |
| Arginine | Agmatine | Decarboxylase |
| L-Phenylalanine | β-Phenylethylamine | Decarboxylase |
| Ornithine | Putrescine | Decarboxylase |
| | Spermidine | Spermidine synthase |
| | Spermine | Spermine synthase |

Seafoods are products rich in protein and it is often stated that proteolytic bacteria are important for the spoilage process. However, the pool of free amino acids is more than sufficient to support bacterial growth and acts as substrates for the off-odors and off-flavors formed. In a series of classical experiments, Lerke and coworkers (Adams, Farber, & Lerke, 1964; Lerke, Adams, & Farber, 1963; Lerke, Adams, & Farber, 1965; Lerke, Farber, & Adams, 1967) inoculated spoilage bacteria into fish juice and separated it into a high-molecular-weight fraction (protein) and a low–molecular-weight fraction (amino acids and TMAO). In the LMW fraction, off-odors and spoilage compounds identical to the spoiling fish developed, whereas no off-odors were formed in the HMW fraction (Table 3).

The odor, flavor, and color of fish are influenced by its feed; however, little is known about the influence of fish feed on subsequent spoilage patterns. This is becoming an issue as an increasing amount of fish raw material is produced in aquaculture. Replacing 50% of fish meal in a typical trout diet with vegetable protein had no influence on subsequent growth of bacteria on iced fillets or on spoilage rates (Ozogul, Ahmad, Hole, Ozogul, & Deguara, 2006).

**Table 3** Spoilage of low-molecular-weight and high-molecular-weight fractions of fish muscle press juice inoculated with a fish spoilage bacterium (modified from Lerke et al., 1967)

| Sample | Day | Volatile reducing substances (microequivalents of reduction/5 ml) | Total volatile nitrogen (mg/100 ml) | Trimethylamine nitrogen (mg/100 ml) | Log (cfu/ml) |
|---|---|---|---|---|---|
| Whole | 0 | 7.5 | 0 | 0 | 3.3 |
| juice | 1 | 7.5 | 0 | 0 | 7.35 |
| | 2 | 36 | 5 | 3.3 | 8.5 |
| | 2.5 | – | 6 | 3.2 | 8.9 |
| Nonprotein | 0 | 5 | 0 | 0 | 3.3 |
| fraction | 1 | 4.5 | 0 | 0 | 6.8 |
| | 2 | 16 | 2.8 | 2.5 | 8.6 |
| | 2.5 | 36 | 5.5 | 4.7 | 8.6 |
| Protein | 0 | 5 | 0 | 0 | 3.3 |
| fraction | 1 | 5 | 0 | 0 | 6.3 |
| | 2 | 4 | 0 | 0 | 7.8 |
| | 2.5 | 4 | 0 | 0 | 8.1 |

## *Taxonomy of an Important Fish Spoilage Bacterium*

The understanding of the bacteriology of seafood spoilage was brought about by studies in a 20-year period from 1950 to 1970 by Castell (Castell & Anderson, 1948), by Lerke's group (Adams et al., 1964; Lerke et al., 1963, 1965, 1967), and by the microbiology team at Torry Research Station led by James Shewan (Castell & Anderson, 1948; Shaw & Shewan, 1968; Shewan, 1977). It was discovered, relatively early, that the "total bacterial count" was not a parameter that could indicate spoilage or remaining shelf life of fresh fish (Castell, Anderson, & Pivnick, 1948; Huss, Dalsgaard, Hansen, Ladefoged, Pedersen, & Zittan, 1974). However, the spoilage off-odors and off-flavors were produced by very specific bacteria, and the most noticeable were the fishy and sulfidy odors of spoiling gadoid fish species produced by Gram-negative, psychrotrophic bacteria capable of producing hydrogen sulfide and reducing trimethylamine oxide. The specific microorganisms associated with chill storage fish spoilage were originally grouped as *Achromobacter*, which was a compilation of Gram-negative, nonfermentative, rod-shaped bacteria, several of which today have been reclassified as *Acinetobacter*, *Moraxella, Psychrobacter*, and *Shewanella*. In 1941, Long and Hammer (Long & Hammer, 1941) reclassified the bacterium as *Pseudomonas* and due to its role in fish spoilage, the species became *Pseudomonas putrefaciens*. The research team at Torry Research Station classified pseudomonads into four groups (I, II, III, IV) (Shewan, Hobbs, & Hodgkiss, 1960) and the fish spoilage bacterium belonged to group IV. This bacterium was moved to the *Alteromonas* genera because of different GC contents between *Ps. putrefaciens* (43–53%) and the majority of pseudomonads (58–72%). Molecular approaches once again led to the bacterium's name being changed. Colwell and coworkers (Macdonell & Colwell, 1985) sequenced the 5S rRNA gene

of several marine bacteria and determined that the bacterium belonged to a completely new genus named *Shewanella.* Recently, results of 16S rRNA gene sequence analyses of genera from this group led to a proposal for a new family Shewanellaceae (Ivanova, Flavier, & Christen, 2004), which contains about 30 *Shewanella* spp. The number of new *Shewanella* species is constantly increasing. In 1998, Ziemke, Hofle, Lalucat, and Rossello-Mora (1998) characterized a large number of *Shewanella* isolates from the Baltic Sea using DNA–DNA hybridization, 16S rRNA gene sequencing, and phenotypic testing. They concluded that strains classified as *Shewanella putrefaciens* were a diverse group and belonged to two different species, one being *S. putrefaciens* and a new one being *S. baltica*. Recent studies (Vogel et al., 2005) have revealed that the $H_2S$-producing bacteria that develop during iced storage of cod and plaice are indeed *S. baltica* strains. Although it is not possible to know whether bacterial isolates from former studies are *S. baltica* or *S. putrefaciens* or even some of the new psychrotrophic *Shewanella* species such as *S. hafniensis* or *S. morhaue* (Satomi, Vogel, Gram, & Venkataraman, 2006; Shewan et al., 1960), one must assume that several of these probably today would be classified as *S. baltica*. Recent studies of low-temperature-stored garfish and tuna have also identified the $H_2S$-producing bacteria of the spoilage microflora as *S. baltica* (Dalgaard, Madsen, Samieian, & Emborg, 2006; Emborg, Laursen, & Dalgaard, 2005).

## Microbiology of Freshly Caught Fish

The muscles of healthy fish are sterile and microorganisms reside at the surfaces such as skin, gills, and gastrointestinal tract of finfish. The level of microorganisms vary depending on the area of catch; however, the skin typically contains $10^4$ cfu/cm$^2$, the gills $10^6$ cfu/g, and the digestive tract up to $10^8$ cfu/g (Austin, 2002). The level of microorganisms in the digestive tract may vary from $10^4$ to $10^9$ cfu/g (Spanggaard et al., 2000). Most of the microbiota are culturable under standard laboratory conditions; however, the digestive tract may contain high levels of anaerobic microorganisms (Huber, Spanggaard, Appel, Rossen, Nielsen, et al., 2004) requiring special culture conditions.

A wide array of different bacterial species can be found on fish; however, the dominant microbes are genera or species typical of the aquatic environment, including pseudomonads, coryneforms, and bacteria belonging to the *Acinetobacter/Moraxella* group (Table 4). In some studies of tropical fish species, Gram-positive bacteria dominate the microbiota; however, the bacterial flora mostly consists of Gram-negative species. The microbiota on the skin surface is often dominated by aerobic, nonfermentative microorganisms, whereas a higher proportion of fermentative bacteria are present on the gills and in the gastrointestinal tract (Spanggaard et al., 2001). The bacteria that subsequently become important in spoilage of fresh finfish are present only in small proportions. Vogel et al. (2005) determined that the number of $H_2S$-producing bacteria varied between $10^1$ and $10^3$ and constituted between 0.1 and 10% of the total bacterial count. After 3 weeks of storage, the counts had increased to $10^8$–$10^9$ and $H_2S$-producing bacteria constituted

**Table 4** Bacterial genera associated with raw finfish and crustaceans, and their percentage of the total microflora (modified from ICMSF, 2005)

| | Percentage Composition | | | |
|---|---|---|---|---|
| | Marine | | Freshwater | |
| Group or species | Temperate | Tropical | Temperate | Tropical |
| *Pseudomonas* | 3–32 | 2–22 | 0–26 | 0–6 |
| Vibrionaceae | 1–29 | 0–28 | 0–7 | 0–2 |
| *Acinetobacter–Moraxella* | 11–56 | 9–30 | 0–47 | 10–43 |
| *Flavobacterium–Cytophaga* | 2–22 | 4–25 | – | 0–11 |
| Other Gram-negative bacteria | 0–21 | 0–12 | 0–55 | 0–30 |
| Coryneforms | 3–81 | 0–43 | 0–15 | 0–5 |
| Gram-positive cocci | 1–23 | 3–51 | 0–45 | 30–50 |
| *Bacillus* | 0–4 | 0–2 | 0–5 | 0–40 |
| Others | 0–8 | 0–22 | 0–34 | – |

2–30% of the viable count. In the warm summer months, several of the $H_2S$-producing bacteria were mesophilic *Shewanella algae,* which is a human pathogen. These bacteria quickly disappeared upon iced storage and the psychrotrophic $H_2S$-producing bacteria subsequently dominated the spoilage bacterial community.

## Product Categories and Spoilage

The principal spoilage microorganisms and microbial spoilage processes are described in this chapter. The seafood products are grouped in categories having somewhat similar microbial ecology – and hence, similar spoilage processes. Table 5 provides examples of typical shelf lives of some seafood products.

### *Raw, Fresh Seafood*

#### Bivalve Mollusks

As mentioned, oysters have a relatively high content of glycogen and spoilage is characterized by the growth of lactic acid bacteria, the formation of lactic acid, and a concurrent decrease in pH. The pH of fresh oysters is approximately 6 and spoiled oysters have pH values of 4.9–5.3 (Aaraas et al., 2004; Cook, 1991). Other studies have noted an increase in bacteria such as *Ps. putrefaciens* (Brown & McMeekin, 1977) or *Pseudoalteromonas* (Romero, Gonzalez, & Espejo, 2002) during spoilage and this is more likely to cause development of amine compounds. It is often stated that bacteria will not grow when mollusks are stored live; however, Lorca, Pierson, Flick, and Hackney (2001) determined that counts of halotolerant bacteria (such as *Vibrio* spp.) increased from approximately $10^5$ to $10^8$ cfu/g over a 10-day storage period. The increase was seen at storage temperatures of 7, 13, and 21°C.

**Table 5** Typical shelf lives of some seafood products – shelf lives are a function of sensory acceptability and do not consider safety aspects

| Category | Subcategory | Product example | Storage conditions | Typical shelf life |
|---|---|---|---|---|
| Raw fresh seafood | Mollusks | Live oysters | Live, 10°C | 3–6 days |
| | Crustaceans | Shrimp | In ice (0°C) | 10–16 days |
| | Cephalopods | Squid | In ice (0°C) | 8–12 days |
| | Finfish | Cod | In ice (0°C) | 12–16 days |
| Packed fresh fish | | Cod | $CO_2$ packed, at 2°C | 11–12 days |
| Cured seafood | Salted, cold-smoked fish | Cold-smoked salmon | Vacuum packed, 5°C | 3–8 weeks |
| | Other lightly preserved products | Brined shrimp | Contain sorbate, benzoate, packed, 5°C | 1–2 months |
| | Semipreserved products | Marinated herring | Acetic acid brine, preservatives, 5°C | 3–6 months |
| | Heavily salted fish | Tropical salted fish | Aerobic | |
| Heated seafood products | Hot-smoked fish | Hot-smoked mackerel | Ambient temperature, aerobic, vacuum-packed, 5°C | 2–6 days<br>1–2 months |
| | Sous vide-cooked products | Cod | Heated, vacuum packed, 3°C | 1–2 weeks |
| | Fully canned products | Tuna | | 1–2 years |
| Miscellaneous | Frozen fish | Cod | –20°C | 1–12 months |
| | Surimi | White fish | 5°C | 5 days |
| | | | Double washed, –18°C | 12 months |

### Crustaceans

Fresh shrimp are often stored in ice for a few days to facilitate loosening of the shell, known as ripening (Hoegh, 1989). This process is autolytic and partly caused by the increase in pH which loosens the shrimp shell proteins that have a low p*I* (Hoegh, 1989). The content of ammonia increases steadily during iced storage to 1.5 g/kg after 7–8 days of storage. This is primarily a result of autolytic proteases and is accompanied by a concurrent increase in free amino acids (Hoegh, 1989).

Psychrotrophic bacteria increase during storage (Cann, 1973). The spoilage flora may contain a large proportion of *Moraxella* or *Acinetobacter* (Surendran, Mahadeva Iyer, & Gopakumar, 1985; Vanderzant, Cobb, Thompson, & Parker, 1973), but other studies have revealed that pseudomonads and alteromonads dominate (Shamshad, Kher, Riaz, Zuberi, & Qadri, 1990). *Pseudomonas fragi* and *Shewanella putrefaciens*have been identified as the primary spoilage agents of chill-stored shrimp, with *Ps. fragi* spoiling iced-stored shrimp and *S. putrefaciens* being the dominant microorganism in shrimp stored in ice slurry (Chinivasagam, Bremner, Thrower, & Nottingham, 1996). Naturally, spoiling prawns are characterized by amines, sulfides, and esters and both *Ps. fragi* and *S. putrefaciens* produced sulfides, whereas amines were mostly formed by *S. putrefaciens* and the spoilage esters mainly by *Ps. fragi* (Chinivasagam, Bremner, et al., 1998).

Indole has been suggested as a spoilage indicator for shrimp and prawn; however, this is not a universal compound in all species. Indole is produced by bacteria that degrade tryptophan. Levels may, in some species, increase to above 100 μg/100 g and some regulatory agencies use 250 μg/100 g as the spoilage limit. Indole production is mainly an issue of high-temperature storage and is likely caused by members of Enterobacteriaceae; however, indole is not formed in all species (Mendes, Goncalves, Pestana, & Pestana, 2005), not even at elevated temperatures in severely spoiled product (Table 6). Sensory rejection of crustacea may also be caused by the formation of black spots (melanosis). Melanin is formed from tyrosine and it has been suggested that this is a bacterial process as several spoilage pseudomonads can oxidize tyrosine (Chinivasagam, Bremner, & Reeves, 1998). This process is normally prevented or controlled by dipping the shrimp in sulfites or other reducing components.

### Cephalopods

The shelf life of iced-stored squid is slightly shorter than that of most finfish and the product is rejected in 8–12 days (Albanese, Cinquanta, Lanorte, & Di Matteo, 2005; Paarup et al., 2002; Vaz-Pires & Barbosa, 2004). Spoilage is characterized by ammoniacal off-odors and the rapid onset of ammonia production at relatively low cell densities suggested that spoilage is mainly autolytic and not caused by bacterial growth (Vaz-Pires & Barbosa, 2004). Paarup et al. (2002) determined that the spoilage microflora was dominated by *Pseudoalteromonas* that reached $10^7$ cfu/g. This bacterium may contribute to late ammonia formation. Trimethylamine also

**Table 6** Indole formation in shrimp/prawns (ICMSF, 2005)

| Species | Temperature (°C) | Time (days) | Indole (μg/100 g) | Reference |
|---|---|---|---|---|
| *Penaeus merguiensis* | 0–4 | 9–13 | 4 | Shamshad et al. (1990) |
| *Penaeus setiferus* or *Penaeus duorarum* | 0–4<br>12–22 | 8–13<br>1–2 | 10–15<br>>100 | Chang, Cheuk, Nickelson, Martin, and Finne (1983) |
| *Pandalus platycens* | 0 | 14–21 | 30–40 | Layrisse and Matches (1984) |
| *Pandalus jordani* | 0 | 10 | 65 | Matches (1982) |
| | 11 | 3 | 130 | |
| | 22 | 2 | 623 | |
| *Pandalus borealis* | 0 | 10 | 4 | Solberg and Nesbakken (1981) |
| | 22 | 1 | 1 | |

develops during iced storage (Paarup et al., 2002) but it is not known which microbes are responsible for its production.

## Finfish

Storage of finfish at ambient temperature leads to rapid growth of mesophilic Gram-negative bacteria belonging to Vibrionaceae or Enterobacteriaceae (Len, 1987; Liston, 1992). These bacteria reduce TMAO to TMA and produce several sulfides and shelf life is short, typically less than 24 h. Cooling, mostly in flaked or crushed ice, is the most common and most effective method for preservation of fresh finfish. The initial biochemical changes in the fish muscle are autolytic and related to the breakdown of ATP. These changes, however, do not have a major impact on sensory quality. Lipid hydrolysis and oxidation may proceed in fatty fish species and contribute to the development of unpleasant off-flavors and off-odors. However, the most offensive off-odors and flavors leading to spoilage at a low temperature are a consequence of bacterial action. As temperature decreases, the microbiota of the product changes and at 0–2°C, the spoilage microflora which reach $10^8$–$10^9$ cfu/g after 2–4 weeks is mostly dominated by pseudomonads and shewanellae (Fig. 3, Table 7) even though other psychrotrophic microbes sometimes also grow. Some studies have reported the growth of the Gram-positive bacterium *Brochothrix thermosphacta* in iced finfish (Grigorakis, Alexis, Gialamas, & Nikolopoulou, 2004; Lalitha et al., 2005) but their numbers are two orders of magnitude lower than numbers of *Shewanella* (Koutsoumanis & Nychas, 1999). *Photobacterium phosphoreum* may also grow during aerobic low-temperature storage of some fish species, and together with shewanellae and pseudomonads constitute the spoilage microflora (Dalgaard et al., 2006). The specific importance of each of these three groups in the actual spoilage process has not been described.

The dominance of pseudomonads and shewanellae has been seen in warm tropical freshwater fish and in fish caught in cold, marine waters (Gram, Wedell-Neergaard, & Huss, 1990; Koutsoumanis & Nychas, 1999). Growth of these psychrotrophic bacteria in marine fish is accompanied by the production of TMA

**Table 7** Changes in the composition of the microflora during storage of a marine (mackerel, *Rastrelliger faughni*Matsui) and a freshwater (*Tilapia aurea*) fish species in ice (Acuff, Izat, & Finne, 1984; Barile, Milla, Reilly, & Villadsen, 1985a, 1985b)

| Species/group | Percentage composition of the microflora at different times during iced storage | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mackerel | | | | | Tilapia | | | | |
| Storage time | 0 | 1 | 5 | 10 | 15 | 0 | 3 | 9 | 15 | 19 |
| cfu/g | $10^4$ | $10^5$ | $5 \times 10^5$ | $5 \times 10^7$ | $10^9$ | $2 \times 10^4$ | $2 \times 10^2$ | $10^3$ | $5 \times 10^6$ | $6 \times 10^7$ |
| *Pseudomonas* | 30 | 25 | 38 | 58 | 53 | 16 | 27 | 62 | 82 | 71 |
| *Shewanella* | | | 38 | 14 | 42 | | | | | |
| *Acinetobacter/Moraxella* | 10 | 8 | | | | 19 | 11 | 6 | 16 | 15 |
| *Flavobacterium* | | | | | | 13 | 1 | 7 | | |
| Vibrionaceae | | | | | | 16 | 1 | 6 | | |
| Enterobacteriaceae | 10 | | 6 | | | | 4 | | | |
| *Bacillus* | 40 | 42 | 18 | 14 | | 1 | 13 | | 1 | 6 |
| *Micrococcus* | | | | | | 26 | 22 | 3 | | 2 |
| Coryneform | 10 | 17 | | | | 4 | 22 | 16 | 1 | 6 |
| Unknown/other | | 8 | | 14 | 5 | 5 | | | | |

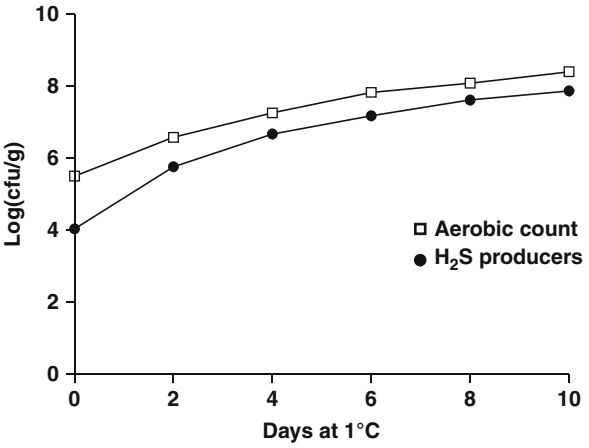


**Fig. 3** Changes in total bacterial count and in $H_2S$-producing bacteria during storage of haddock fillets at 1°C. At day 0 and 10, $H_2S$-producing bacteria account for 3 and 29% of the total bacterial count, respectively (Chai et al.,1968)

caused by *Shewanella* metabolism. *Pseudomonas* spp. dominate the spoilage of iced freshwater finfish (Chytiri, Chouliara, Savvaidis, & Kontominas, 2004; Gelman, Glatman, Drabkin, & Harpaz, 2001; Gram, 1989) and the spoilage off-odors are typically more fruity or onion-like. Remaining shelf life of iced gadoid species can be predicted based on numbers of $H_2S$-producing bacteria because their numbers correlate closely with sensory rejection (Fig. 4; Jorgensen, Gibson, & Huss, 1988).

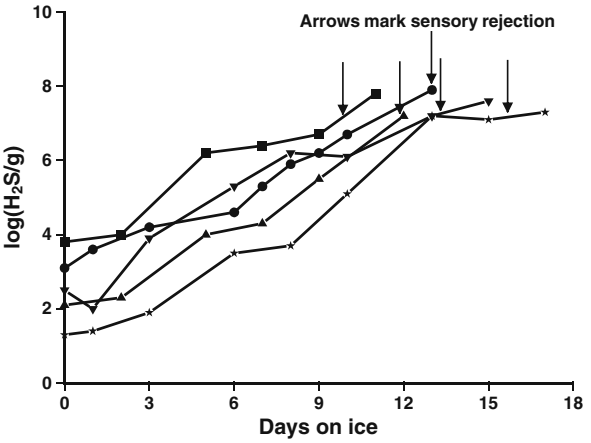


**Fig. 4** Growth of $H_2S$-producing bacteria in iced cod and the rejection based on sensory evaluation (Jorgensen et al., 1988)

Temperature is the most important factor influencing shelf life of fresh finfish. Shelf life of iced cod is approximately 14–16 days (Jorgensen et al., 1988), whereas increasing the temperature to 5°C shortens the shelf life to 6–7 days.

**Packed, Fresh Fish**

Spoilage of aerobic chill-stored fish is mainly caused by the growth of pseudomonads and shewanellae which are respiratory bacteria and it could be expected that removal of the oxygen-containing atmosphere either by vacuum packing or by $CO_2$ packaging would result in extension of shelf life. However, *Shewanella* species are capable of anaerobic respiration using TMAO as an electron acceptor (Jorgensen & Huss, 1989; Pitt & Hocking, 1999; Ringo et al., 1984; Scott & Nealson, 1994) and the $CO_2$-tolerant marine bacterium *P. phosphoreum* also grows well in vacuum-packed fish at low temperatures (Dalgaard et al., 1993). Therefore, vacuum packing does not result in a marked extension of the sensory shelf life of many marine fish species. Freshwater fish and some tropical species do not carry these two spoilage bacteria in high amounts and vacuum packing is likely to select for a microflora dominated by lactic acid bacteria (Hussain, Ehlermann, & Diehl, 1976; Pedersen & Snabe, 1995); however, little is known about the effect on shelf life. Concern has been raised that vacuum packing would increase the risk of botulism because *Clostridium botulinum* type E would be able to grow and produce toxin if temperatures were above 3°C. However, no case of botulism has ever been attributed to fresh, packed fish. First, no toxin was detected in a survey of 1,100 commercial packages of vacuum-packed fresh fish (Lilly & Kautter, 1990) and second, type E toxin is heat labile and would likely be inactivated in cooked product (Ohye & Scott, 1957).

One would assume that packaging in a $CO_2$-containing atmosphere would cause a dramatic extension of chilled shelf life because $CO_2$ has a marked inhibitory effect on respiratory bacteria such as shewanellae and pseudomonads. Red meat typically spoils due to the growth and metabolism of pseudomonads and packaging in $CO_2$ extends chilled shelf life from days to months, with the microflora becoming dominated by lactic acid bacteria (Borch et al., 1996). The shelf life of marine fish species packaged in a $CO_2$ atmosphere is only marginally extended as compared to nonpackaged product and in gadoid species, significant amounts of trimethylamine are produced (Dalgaard et al., 1993; Debevere & Boskou, 1996; Ruiz-Capillas, Saavedra, & Moral, 2003). This is due to the growth and metabolism of the $CO_2$-tolerant marine bacteria *P. phosphoreum* (Dalgaard et al., 1993; Debevere & Boskou, 1996; Emborg, Laursen, Rathjen, & Dalgaard, 2002) and remaining shelf life of $CO_2$-packaged cod can be modeled based on numbers of *P. phosphoreum* (Dalgaard, 1995a). *Photobacterium phosphoreum* grows to $10^7$–$10^8$ cfu/g during low-temperature storage of packed fish and the amounts of TMA produced far exceeds the amounts formed during spoilage of nonpacked fish. This is due to very high production of TMA per cell of *P. phosphoreum* (Dalgaard, 1995b).

$CO_2$ packaging of fish that do not contain *P. phosphoreum* extends shelf life to some degree. *Photobacterium phosphoreum* is sensitive to freezing and a freezing step before $CO_2$ packaging eliminates the bacteria and extends shelf life of the packaged product (Dalgaard, Munoz, & Mejlholm, 1998; Emborg et al., 2002). Also, the shelf life of freshwater fish or tropical water fish that do not naturally harbor *P. phosphoreum* may be extended by packaging either under vacuum (Merivirta,

Koort, Kivisaari, Korkeala, & Bjorkroth, 2005) or in a $CO_2$ atmosphere (Gimenez & Dalgaard, 2004; Reddy, Villanueva, & Kautter, 1995). The elimination of *P. phosphoreum* from packaged fish often leads to a dominance by lactic acid bacteria and *B. thermosphacta* which can reach $10^7$–$10^8$ cfu/g (Emborg et al., 2002; Lannelongue, Finne, Hanna, Nickelson, & Vanderzant, 1982), which is similar to patterns observed for stored, packaged red meats (Borch et al., 1996; Dainty & Mackey, 1992). A bacterium belonging to Enterobacteriaceae has also been detected as a major part of the spoilage microflora in $CO_2$-packaged tuna fish (Emborg et al., 2005). This bacterium and *P. phosphoreum* are both capable of forming large amounts of histamine at low temperatures (2°C) (Emborg et al., 2005, 2002). The production of biogenic amines is in several fish species believed to be a consequence of elevated temperatures that allow mesophilic *Morganella morganii* to grow and decarboxylate amino acids (Lehane & Olley, 2000). Amine production is of special interest in scombroid fish species which are rich in histidine, the precursor of histamine. *Photobacterium phosphoreum* may produce histamine (van Spreekens, 1987) at refrigeration temperatures but the finding of other *M. morganii*-like psychrotolerant histamine producers is novel. Histamine production in Sri Lankan tuna fish packed in $CO_2$ and $N_2$ and stored at 2°C began at day 3 and increased to more than 1,000 ppm on day 9 (Emborg et al., 2005). In garfish stored aerobically at 0°C, only low concentrations of histamine were detected and only after sensory rejection; however, at 5°C, more than 1,000 ppm were formed both in aerobically stored and $CO_2$-packaged garfish (Dalgaard et al., 2006). Histamine formation parallels TMA formation and, hence, is a similar spoilage indicator.

## *Cured Seafood*

Curing of fish refers to a wide variety of preservation principles combining salting, acidification, fermentation, and addition of preservatives such as sorbate, benzoate, lactate, and acetate. Although the proportion of the fish catch that is used for cured products is decreasing (Fig. 2), still at least 10 million tons are preserved in this manner. As the degree of preservation is "increased" compared to fresh fish, there is a change in the spoilage microflora toward fermentative Gram-negative bacteria, lactic acid bacteria, and yeast (Gram, 2005).

### Salted, Cold-Smoked Fish

Especially salmon, but also to some extent trout, cod, and halibut are processed as lightly salted, cold-smoked products. The salt concentration is typically between 3 and 6% (as water-phase salt) and the cold-smoking process never increases above 28–30°C. Fish proteins denature at higher temperatures and the fish would obtain a "cooked" appearance as in hot-smoked products. The process may also rely on a combination of drying and addition of liquid smoke (Siskos, Zotos, & Taylor, 2005). Cold-smoked products are typically packaged under vacuum and retail distributed at refrigeration temperature. Often the products are held under frozen storage before

display at retail. If products are stored aerobically, spoilage occurs more rapidly and visible colony growth of pseudomonads and yeast cells can be seen. Packaging of salmon and other fatty fish species also serves the purpose of eliminating oxidation of the lipid fraction as rancidity otherwise becomes a major off-odor.

The shelf life of vacuum-packaged, cold-smoked fish varies between species and factories and depends to some extent on the degree of drying, smoking, and the amount of salt added (Leroi & Joffraud, 2000). The sensory shelf life varies between 3 and 9 weeks (Jorgensen, Dalgaard, & Huss, 2000); however, the longer shelf lives may not be acceptable from a safety perspective as, for instance, *Listeria monocytogenes* and sometimes *C. botulinum* may be able to grow in this product (Gram, 2001a, 2001b). Recently, consumption of a packed cold-smoked salmon product 3 days after "use-by-date" was linked to a case of botulism (Dressler, 2005). The total bacterial count is reduced slightly by the cold-smoking process and is between $10^1$ and $10^4$ cfu/g in the freshly produced product (Dondero, Cisternas, Carvajal, & Simpson, 2004; Hansen, Drewes Rontved, & Huss, 1998; Leroi, Joffraud, Chevalier, & Cardinal, 1998).

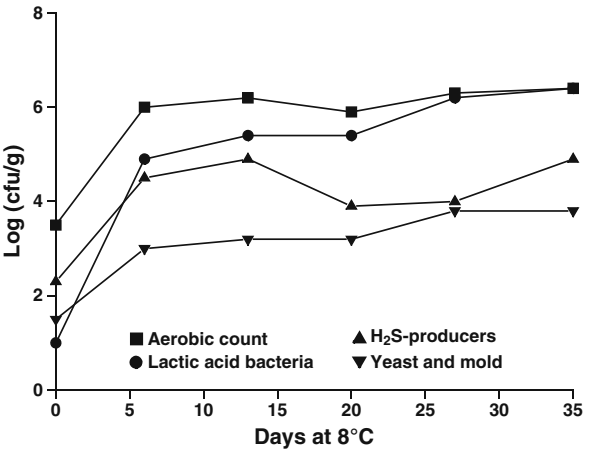


**Fig. 5** Changes in aerobic count, lactic acid bacteria, $H_2S$-producing bacteria, and yeasts and molds in cold-smoked salmon stored under vacuum at 8°C (Leroi et al., 1998)

The total bacterial count of vacuum-packaged, cold-smoked fish increases during refrigerated storage (4–5°) and the spoilage microflora is often dominated by lactic acid bacteria or a combination of lactic acid bacteria and fermentative Gram-negative bacteria (Enterobacteriaceae or *P. phosphoreum*) (Hansen et al., 1998; Leisner, Millan, Huss, & Larsen, 1994; Leroi et al., 1998; Lyhs, Bjorkroth, Hyytia, & Korkeala, 1998). Lactic acid bacteria counts typically increase to $10^6$–$10^8$ cfu/g during a few weeks of refrigerated storage (Fig. 5) (Jorgensen, Dalgaard, et al., 2000a; Leroi & Joffraud, 2000). The spoilage pattern of cold-smoked fish is complex and no single bacterial species has been identified as being responsible for the spoilage. Although autolytic changes may cause some textural changes, the actual spoilage is of bacterial origin (Hansen, Gill, Rontved, & Huss, 1996). Several types of lactic acid bacteria may become dominant in cold-smoked fish; some studies revealed that

the microflora is dominated by carnobacteria (Paludan-Muller, Dalgaard, Huss, & Gram, 1998) and in other trials different *Lactobacillus* species are dominant. Based on phenotypic characteristics, the lactobacilli have been identified as *Lb. curvatus, Lb. sakei*, and *Lb. plantarum,* with each of three processing plants having their own composition of the flora (Hansen & Huss, 1998). The odor profile of spoiled cold-smoked fish varies and a range of potentially odorous volatile compounds are produced during spoilage, including alcohols, aldehydes, esters ketones, and phenols (Jorgensen, Huss, & Dalgaard, 2001). Only some compounds were produced in quantities exceeding an odor threshold and trimethylamine and 3-methylbutanal were both believed to contribute to the spoilage odor profile (Jorgensen et al., 2001). Several of the bacteria that can be isolated from the spoilage microflora of cold-smoked fish produce spoilage off-odors either as pure cultures or as mixed cultures (Joffraud et al., 1998; Stohr, Joffraud, Cardinal, & Leroi, 2001). However, it has not been possible to correlate these odor profiles directly to the profile of the spoiling product.

Cold-smoked fish products are high-value delicatessen products which are traded globally, and an objective chemical "spoilage index" would be valuable in quality and shelf-life determinations (Leroi, Joffraud, Chevalier, & Cardinal, 2001). It has been demonstrated that the production of biogenic amines may be indicative of spoilage, although the amines themselves apparently do not contribute to the spoilage profile (Jorgensen, Huss, et al., 2000). Several different combinations of biogenic amines can be found at the point of spoilage (Table 8) (Jorgensen, Dalgaard, et al., 2000) and some are likely to be the result of metabolism by mixed bacterial communities. For instance, most spoilage bacteria produce only low concentrations (max 10 μg/10 g) of putrescine when grown as single cultures (Jorgensen, Huss, et al., 2000). The spoiling product is characterized by much higher putrescine concentrations and cocultures of lactic acid bacteria and Gram-negative bacteria give rise to the concentrations equivalent of the spoiling product. A similar metabiosis is seen in spoilage of vacuum-packed meat (Dainty, Edwards, Hibbard, & Ramantanis, 1986; Edwards, Dainty, & Hibbard, 1985). Putrescine is formed by ornithine decarboxylation which is common in several Gram-negative bacteria. The pool of ornithine may be replenished by lactic acid bacteria that use arginine deiminase to convert arginine to citrulline and ornithine carbamoyltransferase to convert citrulline to ornithine (Jorgensen, Huss, et al., 2000).

## Other Lightly Preserved Seafood Products

A number of other delicatessen seafood products are similar to the cold-smoked fish in preservation profile and, hence, in spoilage microbiology. For instance, cooked shrimp may be packaged in modified atmosphere and distributed at refrigeration temperature (Mejlholm et al., 2005) or brined before packaging (Dalgaard et al., 2003). These products contain 2–3% NaCl (water-phase salt) and are preserved with either lactic acid or a combination of citric, sorbic, and benzoic acid. The shelf life is 2–3 weeks and the spoilage microflora is dominated by lactic acid bacteria accompanied by *B. thermosphacta* that grow to $10^7$–$10^8$ cfu/g (Mejlholm et al., 2005).

**Table 8** Production of biogenic amines by pure and mixed cultures of bacteria isolated from spoiled cold-smoked salmon (Jorgensen, Dalgaard, et al., 2000; Jorgensen, Huss, et al., 2000)

| Species/group | No of strains or batches | Biogenic amines (μg/g) | | | | |
|---|---|---|---|---|---|---|
| | | Agmatine | Cadavarine | Histamine | Putrescine | Tyramine |
| *Photobacterium phosphoreum* | 3 | 200 | 400 | 180 | 9 | 90 |
| *Aeromonas* | 1 | < | 50 | < | < | < |
| *Serratia liquefaciens* | 2 | < | 400 | < | 9 | < |
| *Enterobacter* | 2 | 13 | 5 | < | 10 | < |
| *Hafnia* | 1 | < | 180 | < | 5 | < |
| *Lactobacillus curvatus* (I) | 5 | < | < | < | 6 | 0–200 |
| *Lactobacillus curvatus* (IV) | 2 | < | 5 | < | 6 | 0–100 |
| *Lactobacillus sakei* | 3 | < | < | < | 5 | < |
| *Carnobacterium divergens* | 2 | < | < | < | 4 | 95 |
| *S. liquefaciens* or *Hafnia* + *C. divergens* or *Lb. sakei* | | | | | 25–80 | |
| *C. divergens* + Gram-negative bacteria | | | | | | 80–130 |
| Spoiling product (I) | 6 | 90–270 | 150–350 | 100–240 | 3–35 | 80–140 |
| Spoiling product (II) | 3 | 2–30 | 100–135 | 3–50 | 8–40 | 130–180 |
| Spoiling product (III) | 2 | 2–25 | 180–300 | 10–15 | 190–380 | 225–335 |
| Spoiling product (IV) | 1 | 20 | 35 | 20 | 30 | 200 |

Single bacterial cultures did not produce the odor profile typical of the spoiling product, but a coculture of *Carnobacterium maltaromaticum* and *B. thermosphacta* produced the typical "wet dog" off-odors.

The Scandinavian speciality "gravad" fish is also a lightly preserved product similar to cold-smoked fish. Fillets are sprinkled with salt, sugar, herbs (typically dill), and spices and left under slight pressure for 1–2 days at refrigeration temperature. The product is stored aerobically or vacuum packaged (Leisner et al., 1994; Lyhs, Bjorkroth, & Korkeala, 1999; Lyhs, Lahtinen, et al., 2001). The aerobic count increases in vacuum-packed product to approximately $10^8$ cfu/g over 2–3 weeks and the microflora is dominated by lactic acid bacteria and also $H_2S$-producing bacteria, which may increase to $10^6$ cfu/g (Lyhs, Lahtinen, et al., 2001). The dominant lactic acid bacteria are *Lactobacillus sakei*, *Lb. curvatus*, and *Carnobacterium piscicola* (now *C. maltaromaticum*) (Lyhs, Korkeala, & Bjorkroth, 2002).

Barrel-salted lumpfish roe (as described below) is used in a desalted type of product in which the salt concentration is adjusted to 4% and pH is reduced with lactic acid to approximately 5.5 (Basby, Jeppesen, & Huss, 1998a). This product is packed in airtight containers and distributed at refrigeration temperature. Spoilage which manifests itself with off-odors characterized as sulfidy, sour, or rotten is caused by bacterial growth (Basby, Jeppesen, & Huss, 1998b). Lactic acid bacteria increased to $10^7$–$10^8$ cfu/g and Enterobacteriaceae were detected at levels between $10^5$ and $10^6$ cfu/g. When assessing spoilage potential, only *M. morganii* strains produced off-odors in a heat-treated roe (Basby, Jeppesen, & Huss, 1998c). Although not tested, it is likely that spoilage in this product is brought about by an interaction between the lactobacilli and the Gram-negative bacteria present.

### Semipreserved Seafood Products

Several fish products are preserved by NaCl, acid, and preservatives like benzoic acid, sorbic acid, lactate, acetate, and/or nitrate. Some of the products are based on raw fish such as Scandinavian marinated herrings, the German rollmops, or the anchovies of Southern Europe. Other types of products such as brined shrimp are based on cooked raw materials.

The raw materials for marinated herring are either barrel-salted herring stored for 6–12 months or fillets that are acid-brined for 2–3 weeks. Subsequently, the fish is drained and covered in a brine (or marinade) with salt, acetic acid, sugar, and preservatives. The products are left to ripen and stored at 5–10°C. Often these products are microbiologically stable; however, when spoilage occurs, it is typically due to the growth of acetic acid-tolerant lactic acid bacteria (Lyhs et al., 2004; Lyhs, Korkeala, et al., 2001) or yeasts (Somners, 1975). Spoilage can be characterized by gas formation produced by heterofermentative lactobacilli such as *Lb. alimentarius* (Lyhs, Korkeala, et al., 2001). Spoilage may also be manifest by slime or ropiness which is caused by the growth of *Leuconostoc* species (Lyhs et al., 2004) or halophilic Gram-negative rod-shaped bacteria (Magnusson & Moeller, 1985). pH is an important parameter in preventing the growth of spoilage lactobacilli and yeasts (Fig. 6).

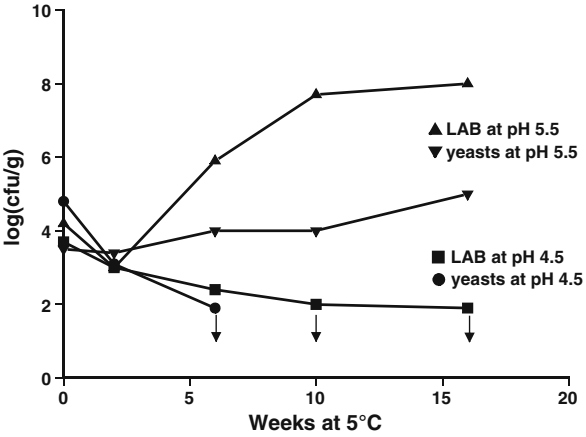


**Fig. 6** Growth of lactic acid bacteria and yeasts in brine of marinated herring is dependent on pH (Jessen, 1987)

Ripened anchovies are prepared by salting partially gutted (or nongutted) fresh anchovies. By tradition, the product is packaged in cans but these are not heat sterilized and must be held at refrigeration temperature. Microbial counts decrease during the ripening process to less than $10^4$ cfu/g (Pons-Sanchez-Cascado, Veciana-Nogues, & Vidal-Carou, 2003). Biogenic amines increase during the ripening process but do not reach hazardous levels under controlled salt and storage conditions (Pons-Sanchez-Cascado et al., 2003). Total volatile bases increase during ripening (Pons-Sanchez-Cascado, Veciana-Nogues, Bover-Cid, Marine-Font, & Vidal-Carou, 2005) but this is believed, as the herring ripen, to be caused by enzymes from the gut and is not a consequence of bacterial action. The microflora rapidly becomes dominated by halophilic bacteria but their counts remain relatively low ($10^4$–$10^5$ cfu/g) (Perrez Villarreal & Pozo, 1992).

Similar to the herring and anchovy products, some types of fish roe are also barrel salted (at 15–25% NaCl) for months before further processing to caviar products. Caviar is produced from a number of fish species of which the most famous is sturgeon, but also a number of other species such as cod and lumpfish are used (Bledsoe, Bledsoe, & Rasco, 2003). Some of these products retain high levels of salt during subsequent marketing but others are desalted before marketing. Yet others are processed directly to lower levels of salt, i. e., 5–8% NaCl (water phase salt) (Bledsoe et al., 2003). Very little is known about spoilage of these products and microbiological studies have mainly addressed food safety issues related to potential for growth of *C. botulinum* and *L. monocytogenes*.

## Heavily Salted Fish

Some fish, including gadoid species, are sometimes preserved exclusively by heavy salting. This process eliminates all of the spoilage bacteria described in the products above and the product is shelf stable at ambient temperature for a long time.

**Table 9** Fungal spoilage of heavily salted fish (data from Pitt & Hocking, 1999)

| Geographical region | Genera of filamentous fungi | Species of filamentous fungi |
|---|---|---|
| Temperate climate | *Wallemia* | *W. sebi* |
| Subtropical climate | *Hortaea* | *H. werneckii* |
| Tropical | *Aspergillus* | *A. flavus, A. niger* |
| | | *A. clavatus, A. penicilliodes, A. wentii* |
| | | *A. fumigatus*,*A. restrictus* |
| | *Penicillium* | *P. chrysogenum, P. citrinum* |
| | | *P. thomi, P. chalybeum* |
| | *Polypaecilum* | *P. pisce* |
| | *Eurotium* | *E. rubrum, E. amstelodami, E. repens* |
| | *Basipetospora* | *B. halophila* |
| | *Cladosporium* | *C. cladosporioides* |
| | *Scopulariopsis* | *S. brevicaulis* |

However, the use of poor-quality salt can lead to a very characteristic type of spoilage called pinking. This is due to the growth of red-pigmented halophilic bacteria (Lamprecht, 1988; Prasad & Panduranga Rao, 1994; Prasad & Seenayya, 2000) that are strongly proteolytic causing a softening of the muscle and rotten off-odors. Pink is a traditional term for visible growth of extremely halophilic Gram-negative bacteria, such as *Halobacterium salinarium*, that belong to the family Halobacteriaceae. Most are nonmotile and obligate aerobes. Also, especially in tropical countries, this type of product may spoil due to fungal growth visible on the salted fillets or fish (Santoso, Gandjar, Sari, & Sembiring, 1999). Several different filamentous fungi have been isolated from spoiled, salted fish (Pitt & Hocking, 1999). Fungal spoilage of salted fish from temperate or subtropical climates differs from that of tropical salted fish with respect to genera and species (Pitt & Hocking, 1999) (Table 9). The spoilage described as "dun" is the visible appearance of brown colonies (1–2 mm in diameter). This is a caused by the growth of the fungus *Wallemia sebi*, an obligate aerobe.

## *Heated Seafood Products*

Seafood raw materials are heated or cooked and result in a number of products such as hot-smoked fish, pasteurized crab meat, sous vide-cooked fillets, and fully canned products. Typically, the temperatures used are sufficient to result in substantial reduction of any microorganism present and it is either postprocess contamination that introduces spoilage microorganisms or spores surviving heat treatment that give rise to vegetative cells with spoilage potential.

### Hot-Smoked Fish

The hot-smoking process is very similar to the cold smoking described above in that the fish are typically salted to 3–6% NaCl (water phase salt). However, the smoking process typically takes place at higher temperatures (50–80°C) which results in "cooking" of the fish flesh. Several species, such as herring and mackerel, are hot smoked but recently salmon has also become popular as a hot-smoked product. Hot smoking is also a common preservation technology in many developing countries. The hot-smoking process of mackerel can involve a drying step (30°C) and heating step at 50 and 80°C. The entire process usually requires 3 h. Larger and heavier fish require longer smoking (cooking) time.

Lightly salted, hot-smoked fish can be stored aerobically and bacterial growth limits shelf life of the product (Karnop, 1980). Also, fungal growth is quite common and is a shelf-life-limiting factor (Efiuvwevwere & Ajiboye, 1996). The fungi isolated are typically identified as *Aspergillus* or *Penicillium* species (Lilabati, Vishwanath, & Shymkesho Singh, 1999). If the product is vacuum packaged immediately after smoking under hygienic conditions, there is virtually no change in microbial levels during subsequent storage. The product becomes dry and somewhat tasteless, but microbial spoilage is not apparent.

### Sous Vide-Cooked Fish

The "sous vide" (under vacuum) technology involves heating of the product typically at less-than-boiling temperature (65–75°C) followed by storage of the product at refrigeration temperature. The technique has been developed to improve sensory quality of several types of cooked food. Due to risk from *C. botulinum*, e.g., survival of spores and subsequent growth and toxin production, it has been recommended that sous vide fish products be heated to at least 90°C for 10 min. To control *L. monocytogenes*, heating to an internal temperature of 70°C for 2 min must be done (ACMSF, 1992). Several types of bacteria grow during subsequent chill storage and length of lag phase and extent of growth depend on the severity of the preceding heat treatment; however, sensory rejection does not appear to be a consequence of bacterial metabolism (Gonzalez-Fandos, Villarino-Rodriguez, Garcia-Linares, Garcia-Arias, & Garcia-Fernandez, 2005) and shelf lives range from 3 to 5 weeks (Gonzalez-Fandos et al., 2005; Nyati, 2000). Sporadically, Ben Embarek (1994) found that sous vide-cooked cod developed very strong putrid off-odors after storage at 5°C. Spore-forming, Gram-positive bacteria were isolated from these samples; however, their identity (*Bacillus* or *Clostridium*) was never determined (Ben Embarek, 1994).

Crab products are important especially to the US market. Crabs are harvested around the world. Some species are dissected before cooking, whereas others are cooked as whole animals. Subsequently the meat is removed from the shell (Cockey & Chai, 1991). Although cooking leaves the flesh sterile, subsequent picking and handling recontaminates the product. Spoilage of canned crab has, as with spoilage of sous vide cod, been caused by sporeformers (Cockey & Chai, 1991).

### Fully Canned Products

Some species of fish are often processed and distributed as fully canned products. This is typical of tuna, mackerel, and salmon. These products are shelf stable if correctly processed and microbiological spoilage is not an issue. The use of raw materials of poor quality may result in the presence of biogenic amines in the canned products. The amines are heat stable and will not be inactivated even during canning (Luten et al., 1992)

## *Miscellaneous*

### Frozen Fish

Almost 20% of the global fish production is preserved by freezing (Fig. 2) and storage at −18 to −20°C. Growth of bacteria is stopped at these temperatures and sensory rejection is caused by nonmicrobial changes in the protein and lipid fractions of the fish flesh. Spoilage bacteria may grow in the raw fish if stored above 0°C before freezing and this may be reflected in the quality of the frozen product.

Filamentous fungi may grow in these products if stored at elevated freezing temperatures (−10 to −5°C). Many bacteria may, to some extent, survive frozen storage and if the product is thawed, grow and cause a normal type of spoilage. As mentioned above, *P. phosphoreum* is very sensitive to freezing and a freezing step before thawing and storage in $CO_2$ atmosphere may increase the shelf life of $CO_2$-packaged fish at refrigeration temperature quite substantially (Boknes, Osterberg, Nielsen, & Dalgaard, 2000; Dalgaard et al., 2006; Emborg et al., 2002).

### Surimi

Surimi is a product prepared from either deboned meat or fillets. It consists mainly of muscle protein fibers that have been washed several times. Surimi is also prepared by mechanical deboning but is reduced essentially to muscle protein fibers by repeated washing. Some studies have reported that the microflora on surimi is similar to the microflora of fresh fish and consists of *Moraxella*, pseudomonads, and *Corynebacterium* (Himelbloom, Brown, & Lee, 1991), with counts of approximately $10^6$ cfu/g (Matches, Raghubber, Yoon, & Martin, 1987). Bacteria grow very well in surimi and surimi-based products; however, these are mostly stored frozen, hence bacterial growth is not a problem (Elliott, 1987).

### Dried Fish

The water activity of fully dried or salted and dried fish is so low that no bacteria can grow. However, fungal growth is a major problem, specifically in developing countries and spoilage is caused by *Aspergillus* and *Penicillium* species (Chakrabarti & Varma, 2000), but a range of other fungi may also be isolated from these products (Santoso et al., 1999). *Aspergillus niger, Aspergillus flavus*, and *Penicillium*spp. are

among the dominant fungi in salted, dried fish (Chakrabarti & Varma, 2000) and *A. niger* also appear to dominate in dried fish (Atapattu & Samarajeewa, 1990). Not only does visible fungal growth spoil the product per se, but filamentous fungi may also produce mycotoxins, hence constituting a health risk. Aflatoxin has been detected in smoked freshwater fish from Africa (Jonsyn & Lahai, 1992).

## References


Aaraas, R., Hernar, I. J., Vorre, A., Bergslien, H., Lunestad, B. T., Skeie, S., et al. (2004). Sensory, histological and bacteriological changes in flat oysters, *Ostrea edulis* L., during different storage conditions. *Journal of Food Science, 69*, S205–S210.

ACMSF (1992). Report on vacuum packaging and associated processes. *Advisory committee on microbiological safety of foods*. London, UK: HMSO.

Acuff, G., Izat, A. L., & Finne, G. (1984). Microbial flora of pond-reared tilapia (*Tilapia aurea*) held on ice. *Journal of Food Protection, 47*, 778–780.

Adams, R., Farber, L., & Lerke, P. (1964). Bacteriology of spoilage of fish muscle 2. Incidence of spoilers during spoilage. *Applied Microbiology, 12*, 277–279.

Albanese, D., Cinquanta, L., Lanorte, M. T., & Di Matteo, M. (2005). Squid (*Sepia officinalis*) stored in active packaging: Some chemical and microbiological changes. *Italian Journal of Food Science, 17*, 325–332.

Anthoni, U., Borresen, T., Christophersen, C., Gram, L., & Nielsen, P. H. (1990). Is trimethylamine oxide a reliable indicator for the marine origin of fish? *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 97*, 569–571.

Atapattu, R., & Samarajeewa, U. (1990). Fungi associated with dried fish in Sri Lanka. *Mycopathologia, 111*, 55–59.

Austin, B. (2002). The bacterial microflora of fish. *The Scientific World Journal, 2*, 558–572.

Bagge-Ravn, D., Yin, N., Hjelm, M., Christiansen, J. N., Johansen, C., & Gram, L. (2003). The microbial ecology of processing equipment in different fish industries – analysis of the microflora during processing and following cleaning and disinfection. *International Journal of Food Microbiology, 87*, 239–250.

Barile, L. E., Milla, A. D., Reilly, A., & Villadsen, A. (1985a). Spoilage patterns of mackerel (*Rastrelliger faughni* Matsui). I. Delays in icing. *ASEAN Food Journal, 1*, 70–77.

Barile, L. E., Milla, A. D., Reilly, A., & Villadsen, A. (1985b). Spoilage patterns of mackerel (*Rastrelliger faughni* Matsui). II. Mesophilic and psychrophilic spoilage. *ASEAN Food Journal, 1*, 121–126.

Basby, M., Jeppesen, V. F., & Huss, H. H. (1998a). Chemical composition of fresh and salted lumpfish (*Cyclopterus lumpus*) roe. *Journal of Aquatic Food Product Technology, 7*, 7–21.

Basby, M., Jeppesen, V. F., & Huss, H. H. (1998b). Spoilage of lightly salted lumpfish (*Cyclopterus lumpus*) roe at 5°C. *Journal of Aquatic Food Product Technology, 7*, 23–34.

Basby, M., Jeppesen, V. F., & Huss, H. H. (1998c). Characterization of the microflora of lightly salted lumpfish (*Cyclopterus lumpus*) roe stored at 5°C. *Journal of Aquatic Food Product Technology, 7*, 35–51.

Ben Embarek, P. K. (1994). Microbial safety and spoilage of sous vide fish products. Kgs. Lyngby, Denmark: (Ph.D. Thesis) Technological Laboratory and the Royal Veterinary and Agricultural University.

Bjorkevoll, I., Olsen, R. L., & Skjerdal, O. T. (2003). Origin and spoilage potential of the microbiota dominating genus *Psychrobacter* in sterile rehydrated salt-cured and dried salt-cured cod (*Gadus morhua*). *International Journal of Food Microbiology, 84*, 175–187.

Bledsoe, G. E., Bledsoe, C. D., & Rasco, B. (2003). Caviars and fish roe products. *Critical Reviews in Food Science and Nutrition, 43*, 317–356.

Boknes, N., Osterberg, C., Nielsen, J., & Dalgaard, P. (2000). Influence of freshness and frozen storage temperature on quality of thawed cod fillets stored in modified atmosphere packaging. *Food Science and Technology, 33*, 248.

Borch, E., KantMuermans, M. L., & Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology, 33*, 103–120.

Brown, R. K., & McMeekin, T. A. (1977). Microbiological aspects of oyster production in Southern Tasmania. *Food Technology in Australia, 29*, 103–106.

Cann, D. C. (1973). Bacteriological aspects of tropical shrimp. In R. Kreuzer (Ed.), *Fishery products* (pp. 338–344). Surrey, UK: Fishing News (Books) Ltd.

Careche, M., Garcia, R., & Borderias, J. (2002). Anchovy shelf life as affected by different chilling methods during distribution. *Journal of Food Protection, 65*, 353–361.

Castell, C., & Anderson, G. W. (1948). Bacteria associated with the spoilage of cod fillets. *Journal of Fisheries Research Board Canada, 7*, 370–377.

Castell, C., Anderson, G. W., & Pivnick, H. (1948). Relation of bacterial counts to quality of cod fillets. *Journal of Fisheries Research Board Canada, 7*, 378–388.

Castell, C., & Greenough, M. F. (1959). The action of *Pseudomonas* on fish muscle 4. Relation between substrate composition and the development of odours by *Pseudomonas fragi*. *Journal of Fisheries Research Board Canada, 16*, 21–31.

Castell, C., Greenough, M. F., & Jenkin, N. L. (1995). The action of *Pseudomonas* on fish muscle: 2. Musty and potato-like odours. *Journal of Fisheries Research Board Canada, 14*, 775–782.

Chai, T., Chen, C., Rosen, A., & Levin, R. E. (1968). Detection and incidence of specific species of spoilage bacteria on fish. II. Relative incidence of *Pseudomonas putrefaciens* and fluorescent pseudomonads on haddock fillets. *Applied Microbiology, 16*, 1738–1741.

Chakrabarti, R., & Varma, P. R. G. (2000). The sensitivity of halotolerant *Aspergillus flavus, Aspergillus niger* and *Penicillium* sp. to propionate, sorbate and benzoate. *Journal of Food Science and Technology, India, 37*, 72–74.

Chang, O., Cheuk, W. L., Nickelson, R., Martin, R., & Finne, G. (1983). Indole in shrimp: Effect of fresh storage temperature, freezing and boiling. *Journal of Food Science, 48*, 813–816.

Chinivasagam, H. N., Bremner, H. A., & Reeves, R. (1998). Can spoilage bacteria cause blackspot (melanosis) in stored prawns? *Letters in Applied Microbiology, 27*, 5–8.

Chinivasagam, H. N., Bremner, H. A., Thrower, S. J., & Nottingham, S. M. (1996). Spoilage pattern of five species of Australian prawns: Deterioration is influenced by environment of capture and mode of storage. *Journal of Aquatic Food Product Technology, 5*, 25–50.

Chinivasagam, H. N., Bremner, H. A., Wood, A. F., & Nottingham, S. M. (1998). Volatile components associated with bacterial spoilage of tropical prawns. *International Journal of Food Microbiology, 42*, 45–55.

Chytiri, S., Chouliara, I., Savvaidis, I. N., & Kontominas, M. G. (2004). Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology, 21*, 157–165.

Cockey, R. R., & Chai, T. (1991). Microbiology of crustaceae processing: Crabs. In D. Ward & C. R. Hackney (Eds.), *Microbiology of marine food products* (pp. 41–63). New York: Van Nostrand Reinhold.

Cook, D. (1991). Microbiology of bivalve molluscan shellfish. In D. Ward & C. R. Hackney (Eds.), *Microbiology of marine food products* (pp. 19–39). New York: Van Nostrand Reinhold.

Dainty, R. H., Edwards, R. A., Hibbard, C. M., & Ramantanis, S. V. (1986). Bacterial sources of putrescine and cadaverine in chill stored vacuum-packaged beef. *Journal of Applied Bacteriology, 61*, 117–123.

Dainty, R. H., & Mackey, B. M. (1992). The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *Journal of Applied Bacteriology, Symposium Supplement 73*, S103–S114.

Dalgaard, P. (1995a). Modelling of microbial activity and prediction of shelf life for packed fresh fish. *International Journal of Food Microbiology, 26*, 305–317.

Dalgaard, P. (1995b). Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology, 26*, 319–333.

Dalgaard, P., Gram, L., & Huss, H. H. (1993). Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology, 19*, 283–294.

Dalgaard, P., Madsen, H. L., Samieian, N., & Emborg, J. (2006). Biogenic amine formation and microbial spoilage in chilled garfish (*Belone belone belone*) – effect of modified atmosphere packaging and previous frozen storage. *Journal of Applied Microbiology, 101*, 80–95.

Dalgaard, P., Mejlholm, O., Christiansen, T. J., & Huss, H. H. (1997). Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. *Letters in Applied Microbiology, 24*, 373–378.

Dalgaard, P., Munoz, L. G., & Mejlholm, O. (1998). Specific inhibition of *Photobacterium phosphoreum* extends the shelf life of modified-atmosphere-packed cod fillets. *Journal of Food Protection, 61*, 1191–1194.

Dalgaard, P., Vancanneyt, M., Euras Vilalta, N., Swings, J., Fruekilde, P., & Leisner, J. J. (2003). Identification of lactic acid bacteria from spoilage associations of cooked and brined shrimps stored under modified atmosphere between 0°C and 25°C. *Journal of Applied Microbiology, 94*, 80–89.

Debevere, J., & Boskou, G. (1996). Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. *International Journal of Food Microbiology, 31*, 221–229.

Dondero, M., Cisternas, F., Carvajal, L., & Simpson, R. (2004). Changes in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature. *Food Chemistry, 87*, 543–550.

Dressler, D. (2005). Botulism caused by consumption of smoked salmon. *Nervenarzt, 76*, 763–766.

Edwards, R. A., Dainty, R. H., & Hibbard, C. M. (1985). Putrescine and cadaverine formation in vacuum packed beef. *Journal of Applied Bacteriology, 58*, 13–19.

Efiuvwevwere, B. J. O., & Ajiboye, M. O. (1996). Control of microbiological quality and shelf-life of catfish (*Clarias gariepinus*) by chemical preservatives and smoking. *Journal of Applied Bacteriology, 80*, 465–470.

Elliott, E. L. (1987). Microbiological quality of Alaska pollock surimi. In D. E. Kramer & J. Liston (Eds.), *Seafood quality determination* (pp. 269–281). Amsterdam: Elsevier

Emborg, J., Laursen, B. G., & Dalgaard, P. (2005). Significant histamine formation in tuna (*Thunnus albacares*) at 2°C – effect of vacuum- and modified atmosphere-packaging on psychrotolerant bacteria. *International Journal of Food Microbiology, 101*, 263–279.

Emborg, J., Laursen, B. G., Rathjen, T., & Dalgaard, P. (2002). Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2°C. *Journal of Applied Microbiology, 92*, 790–799.

FAO (2004). *The state of world fisheries and aquaculture*. Rome, Italy: Food and Agricultural Organization.

Finne, G. (1992). Non-protein nitrogen compounds in fish and shellfish. In G. J. Flick & R. E. Martin (Eds.), *Advances in Seafood Biochemistry, Composition and Quality* (pp. 393–401). Lancaster: Technomic

Gelman, A., Glatman, L., Drabkin, V., & Harpaz, S. (2001). Effects of storage temperature and preservative treatment on shelf life of the pond-raised freshwater fish, silver perch (*Bidyanus bidyanus*). *Journal of Food Protection, 64*, 1584–1591.

Gimenez, B., & Dalgaard, P. (2004). Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage micro-organisms in cold-smoked salmon. *Journal of Applied Microbiology, 96*, 96–109.

Gonzalez-Fandos, E., Villarino-Rodriguez, A., Garcia-Linares, M. C., Garcia-Arias, M. T., & Garcia-Fernandez, M. C. (2005). Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. *Food Control, 16*, 77–85.

Gram, L. (2001a). Potential hazards in cold-smoked fish: *Clostridium botulinum* type E. *Journal of Food Science, 66*, S-1082–S-1087.

Gram, L. (2005). Microbial food spoilage. In Y. H. Hui (Ed.), *Handbook in food science, technology and engineering* (pp. 51-1–51-16). Boca Raton, FL, USA: Taylor and Francis, CRC Press

Gram, L. (2001b). Potential hazards in cold-smoked fish: *Listeria monocytogenes*. *Journal of Food Science, 66*, S-1072-S-1081.

Gram, L. (1989). *Identification, characterization, and inhibition of bacteria isolated from tropical fish*. Kgs. Lyngby, Denmark: Technological Laboratory and the Royal Veterinary and Agricultural University.

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

Gram, L., Wedell-Neergaard, C., & Huss, H. H. (1990). The bacteriology of fresh and spoiling Lake Victorian Nile perch (*Lates niloticus*). *International Journal of Food Microbiology, 10*, 303–316.

Grigorakis, K., Alexis, M., Gialamas, I., & Nikolopoulou, D. (2004). Sensory, microbiological and chemical spoilage of cultured common sea bass (*Dicentrarchus labrax*) stored in ice: A seasonal differentiation. *European Food Research and Technology, 219*, 584–587.

Hansen, L. T. (1995). *Quality of chilled vacuum-packed cold-smoked salmon*. Lyngby, Denmark: Danish Institute for Fisheries Research, The Royal Veterinary and Agricultural University.

Hansen, L. T., Drewes Rontved, S., & Huss, H. H. (1998). Microbiological quality and shelf life of cold-smoked salmon from three different processing plants. *Food Microbiology, 15*, 137–150.

Hansen, L. T., Gill, T., Rontved, S. D., & Huss, H. H. (1996). Importance of autolysis and microbiological activity on quality of cold-smoked salmon. *Food Research International, 29*, 181–188.

Hansen, L. T., & Huss, H. H. (1998). Comparison of the microflora isolated from spoiled cold-smoked salmon from three smokehouses. *Food Research International, 31*, 703–711.

Herbert, R. A., Hendrie, M. S., Gibson, D. M., & Shewan, J. M. (1971). Bacteria active in the spoilage of certain sea foods. *Journal of Applied Bacteriology, 34*, 41–50.

Herbert, R. A., & Shewan, J. M. (1975). Precursors of the volatile sulphides in spoiling North Sea cod (*Gadus morhua*). *Journal of the Science of Food and Agriculture, 26*, 1195–1202.

Himelbloom, B. H., Brown, E. K., & Lee, J. S. (1991). Microorganisms isolated from surimi processing operations. *Journal of Food Science, 56*, 299–301.

Hoegh, L. (1989). *Quality index for shrimp [in Danish]*. Kgs. Lyngby: (Ph.D. Thesis) Technological Laboratory and Technical University of Denmark.

Huber, I., Spanggaard, B., Appel, K. F., Rossen, L., Nielsen, T., & Gram, L. (2004). Phylogenetic analysis and in situ identification of the intestinal microbial community of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Applied Microbiology, 96*, 117–132.

Huss, H. H., Dalsgaard, D., Hansen, L., Ladefoged, H., Pedersen, A., & Zittan, L. (1974). The influence of hygiene in catch handling on the storage life of iced cod and plaice. *Journal of Food Technology, 9*, 213–221.

Huss, H. H., & Larsen, A., (1980). Changes in the oxidation-reduction potential (Eh) of smoked and salted fish during storage. *Lebensmittel-Wissenschaft und -Technologie, 13*, 40–43.

Hussain, A. M., Ehlermann, D., & Diehl, J. F. (1976). Effect of radurization on microbial flora of vacuum-packaged trout (*Salmo gairdneri*). *Archiv fur Lebensmittelhygiene, 27*, 223–225.

ICMSF (2005). Fish and fish products. In ICMSF (Eds.), *Microorganisms in foods 6. Microbial ecology of food commodities* (pp. 174–249). New York: Kluwer Academic and Plenum Publishers

Ivanova, E. P., Flavier, S., & Christen, R. (2004). Phylogenetic relationships among marine *Alteromonas*-like proteobacteria: Emended description of the family Alteromonadaceae and proposal of Pseudoalteromonadaceae fam. nov., Colwelliaceae fam. nov., Shewanellaceae fam. nov., Montellaceae fam. nov., Ferrimonadaceae fam. nov., Idiomarinaceae fam. nov and Psychromonadaceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology, 54*, 1773–1788.

Jessen, B. (1987). Semi-preserved herring: Final products [in Danish]. *Fisker-Bladet* 1–4.

Joffraud, J. J., Leroi, F., & Chevalier, F. (1998). Development of a sterile cold-smoked fish model. *Journal of Applied Microbiology, 85*, 991–998.

Joffraud, J. J., Leroi, F., Roy, C., & Berdague, J. L. (2001). Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. *International Journal of Food Microbiology, 66*, 175–184.

Jonsyn, F. E., & Lahai, G. P. (1992). Mycotoxic flora and mycotoxins in smoke-dried fish from Sierra Leone. *Nahrung, 36*, 485–489.

Jorgensen, B. R., Gibson, D. M., & Huss, H. H. (1988). Microbiological quality and shelf life prediction of chilled fish. *International Journal of Food Microbiology, 6*, 295–307.

Jorgensen, B. R., & Huss, H. H. (1989). Growth and activity of *Shewanella putrefaciens* isolated from spoiling fish. *International Journal of Food Microbiology, 9*, 51–62.

Jorgensen, L. V., Dalgaard, P., & Huss, H. H. (2000). Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *Journal of Agricultural and Food Chemistry, 48*, 2448–2453.

Jorgensen, L. V., Huss, H. H., & Dalgaard, P. (2001). Significance of volatile compounds produced by spoilage bacteria in vacuum-packed cold-smoked salmon (*Salmo salar*) analyzed by GC-MS and multivariate regression. *Journal of Agricultural and Food Chemistry, 49*, 2376–2381.

Jorgensen, L. V., Huss, H. H., & Dalgaard, P. (2000). The effect of biogenic amine production by single bacterial cultures and metabiosis on cold-smoked salmon. *Journal of Applied Microbiology, 89*, 920–934.

Kadota, H., & Ishida, Y. (1972). Production of volatile sulfur-compounds by microorganisms. *Annual Review of Microbiology, 26*, 127.

Karnop, G. (1980) [Quality and storage quality of hot-smoked fish products. III. Microbiology and effects of intensity of smoking on the keeping quality of halibut, buckling and eels.]. *Deutsche Lebensmittel-Rundschau, 76*, 125–134.

Koutsoumanis, K., & Nychas, G. J. E. (1999). Chemical and sensory changes associated with microbial flora of Mediterranean boque (*Boops boops*) stored aerobically at 0, 3, 7 and 10°C. *Applied and Environmental Microbiology, 65*, 698–706.

Lalitha, K. V., Sonaji, E. R., Manju, S., Jose, L., Gopal, T. K. S., & Ravisankar, C. N. (2005). Microbiological and biochemical changes in pearl spot (*Etroplus suratensis* Bloch) stored under modified atmospheres. *Journal of Applied Microbiology, 99*, 1222–1228.

Lamprecht, A. (1988). The heat resistance of red halophiles in salt. *Food Review, 15*, 27.

Lannelongue, M., Finne, G., Hanna, M. O., Nickelson, R. I., & Vanderzant, C. (1982). Microbiological and chemical changes during storage of swordfish (*Xiphias gladius*) steaks in retail packages containing $CO_2$-enriched atmospheres. *Journal of Food Protection, 45*, 1197–1203.

Layrisse, M. E., & Matches, J. R. (1984). Microbiological and chemical changes of spotted shrimp (*Pandalus platyceros*) stored under modified atmospheres. *Journal of Food Protection, 47*, 453–457.

Lehane, L., & Olley, J. (2000). Histamine fish poisoning revisited. *International Journal of Food Microbiology, 58*, 1–37.

Leisner, J. J., Millan, J. C., Huss, H. H., & Larsen, L. M. (1994). Production of histamine and tyramine by lactic acid bacteria isolated from vacuum-packed sugar-salted fish. *Journal of Applied Bacteriology, 76*, 417–423.

Len, P. P. (1987). Mesophilic spoilage of marine fish – bay trout (*Arripis trutta*), bream (*Acanthopagrus butcheri*) and mullet (*Aldrichetta forsteri*). *Food Technology in Australia, 39*, 277–282.

Lerke, P., Adams, R., & Farber, L. (1963). Bacteriology of spoilage of fish muscle .1. Sterile press juice as a suitable experimental medium. *Applied Microbiology, 11*, 458–462.

Lerke, P., Adams, R., & Farber, L. (1965). Bacteriology of spoilage of fish muscle 3. Characterization of spoilers. *Applied Microbiology, 13*, 625.

Lerke, P., Farber, L., & Adams, R. (1967). Bacteriology of spoilage of fish muscle 4. Role of protein. *Applied Microbiology, 15*, 770.

Leroi, F., & Joffraud, J. J. (2000). Salt and smoke simultaneously affect chemical and sensory quality of cold-smoked salmon during 5°C storage predicted using factorial design. *Journal of Food Protection, 63*, 1222–1227.

Leroi, F., Joffraud, J. J., Chevalier, F., & Cardinal, M. (2001). Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. *Journal of Applied Microbiology, 90*, 578–587.

Leroi, F., Joffraud, J. J., Chevalier, F., & Cardinal, M. (1998). Study of the microbial ecology of cold-smoked salmon during storage at 8°C. *International Journal of Food Microbiology, 39*, 111–121.

Lilabati, H., Vishwanath, W., & Shymkesho Singh, M. (1999). Changes in bacterial and fungal quality during storage of smoked *Esomus danricus* of Manipur. *Fishery Technology, 36*, 36–39.

Lilly, T., & Kautter, D. A. (1990). Outgrowth of naturally-occurring *Clostridium botulinum* in vacuum-packaged fresh fish. *Journal of the Association of Official Analytical Chemists, 73*, 211–212.

Liston, J. (1992). Bacterial spoilage of seafood. In H. H. Huss, M. Jakobsen, & J. Liston (Eds.), *Quality assurance in the fish industry* (pp. 93–105). Amsterdam: Elsevier Science Publishers

Long, H. F., & Hammer, B. W. (1941). Classification of organisms important in dairy products. III. *Pseudomonas putrefaciens*. *Iowa Agricultural Experimental Station Research Bulletin, 285*, 176–195.

Lorca, T. A., Pierson, M. D., Flick, G. J., & Hackney, C. R. (2001). Levels of *Vibrio vulnificus* and organoleptic quality of raw shellstock oysters (*Crassostrea virginica*) maintained at different storage temperatures. *Journal of Food Protection, 64*, 1716–1721.

Luten, J. B., Bouquet, W., Seuren, L. A. J., Burggraaf, M. M., Riekwell-Booy, G., Durand, P., et al. (1992). Biogenic amines in fishery products: Standardization methods within EC. In H. H. Huss, M. Jakobsen, & J. Liston (Eds.), *Quality assurance in the fish industry* (pp. 427–439). Amsterdam: Elsevier.

Lyhs, U., Bjorkroth, J., Hyytia, E., & Korkeala, H. (1998). The spoilage of flora of vacuum-packaged, sodium nitrite or potassium nitrate treated, cold-smoked rainbow trout stored at 4°C or 8°C. *International Journal of Food Microbiology, 45*, 135–142.

Lyhs, U., Bjorkroth, J., & Korkeala, H. (1999). Characterisation of lactic acid bacteria from spoiled vacuum-packaged, cold-smoked rainbow trout using ribotyping. *International Journal of Food Microbiology, 52*, 77–84.

Lyhs, U., Koort, J. M. K., Lundstrom, H. S., & Bjorkroth, K. J. (2004). *Leuconostoc gelidum* and *Leuconostoc gasicomitatum* strains dominated the lactic acid bacterium population associated with strong slime formation in an acetic-acid herring preserve. *International Journal of Food Microbiology, 90*, 207–218.

Lyhs, U., Korkeala, H., & Bjorkroth, J. (2002). Identification of lactic acid bacteria from spoiled, vacuum-packaged "gravad" rainbow trout using ribotyping. *International Journal of Food Microbiology, 72*, 147–153.

Lyhs, U., Korkeala, H., Vandamme, P., & Bjorkroth, J. (2001). *Lactobacillus alimentarius*: A specific spoilage organism in marinated herring. *International Journal of Food Microbiology, 64*, 355–360.

Lyhs, U., Lahtinen, J., Fredriksson-Ahomaa, M., Hyytia-Trees, E., Elfing, K., & Korkeala, H. (2001). Microbiological quality and shelf-life of vacuum-packaged "gravad" rainbow trout stored at 3 and 8°C. *International Journal of Food Microbiology, 70*, 221–230.

Macdonell, M. T., & Colwell, R. R. (1985). Phylogeny of the Vibrionaceae, and recommendation for 2 new genera, *Listonella* and *Shewanella*. *Systematic and Applied Microbiology, 6*, 171–182.

Magnusson, H., & Moeller, A. (1985). Ropiness in the brine of sugar-salted herring. *International Journal of Food Microbiology, 1*, 253–261.

Martino, R. C., & da Cruz, G. M. (2004). Proximate composition and fatty acid content of the mangrove oyster *Crassostrea rhizophorae* along the year seasons. *Brazilian Archives of Biology and Technology, 47*, 955–960.

Matches, J. R. (1982). Effects of temperature on the decomposition of Pacific coast shrimp (*Pandalus jordani*). *Journal of Food Science, 47*, 1044–1047.

Matches, J. R., Raghubber, E., Yoon, I. H., & Martin, R. E. (1987). Microbiology of surimi-based products. In D. E. Kramer & J. Liston (Eds.), *Seafood quality determination* (pp. 373–387). Amsterdam: Elsevier.

Mejlholm, O., Boknaes, N., & Dalgaard, P. (2005). Shelf life and safety aspects of chilled cooked and peeled shrimps (*Pandalus borealis*) in modified atmosphere packaging. *Journal of Applied Microbiology, 99*, 66–76.

Mendes, R., Goncalves, A., Pestana, J., & Pestana, C. (2005). Indole production and deepwater pink shrimp (*Parapenaeus longirostris*) decomposition. *European Food Research and Technology, 221*, 320–328.

Merivirta, L. O., Koort, J. M. K., Kivisaari, M., Korkeala, H., & Bjorkroth, K. J. (2005). Developing microbial spoilage population in vacuum-packaged charcoal-broiled European river lamprey (*Lampetra fluviatilis*). *International Journal of Food Microbiology, 101*, 145–152.

Miller, A. I., Scanlan, R. A., Lee, J. S., & Libbey, L. M. (1973). Identification of the volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas fragi*. *Applied Microbiology, 25*, 952–955.

Nyati, H. (2000). An evaluation of the effect of storage and processing temperatures on the microbiological status of sous vide extended shelf-life products. *Food Control, 11*, 471–476.

Ohye, D. F., & Scott, W. J. (1957). Studies on the physiology of *Cl. botulinum* type E. *Australian Journal of Biological Science, 10*, 85–94.

Ozogul, Y., Ahmad, J. I., Hole, M., Ozogul, F., & Deguara, S. (2006). The effects of partial replacement of fish meal by vegetable protein sources in the diet of rainbow trout (*Oncorhynchus mykiss*) on post mortem spoilage of fillets. *Food Chemistry, 96*, 549–561.

Paarup, T., Sanchez, J. A., Moral, A., Christensen, H., Bisgaard, M., & Gram, L. (2002). Sensory, chemical and bacteriological changes during storage of iced squid (*Todaropsis eblanae*). *Journal of Applied Microbiology, 92*, 941–950.

Paludan-Muller, C., Dalgaard, P., Huss, H. H., & Gram, L. (1998). Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5°C. *International Journal of Food Microbiology, 39*, 155–166.

Pedersen, L., & Snabe, L. (1995). Isolation of bacteriocin producing lactic acid bacteria from chilled vacuum packed temperate and tropical fish products. Royal Veterinary and Agricultural University. Danish Institute for Fisheries Research.

Perrez Villarreal, B., & Pozo, R. (1992). Ripening of the salted anchovy (*Engraulis encrasicolus*): Study of the sensory, biochemical and microbiological aspects. In H. H. Huss, M. Jakobsen, & J. Liston (Eds.), *Quality assurance in the fish industry* (pp. 157–167). Amsterdam: Elsevier.

Pitt, J. I., & Hocking, A. D. (1999). *Fungi and food spoilage*. Gaithersburg, MD: Aspen Publishers.

Pons-Sanchez-Cascado, S., Veciana-Nogues, M. T., Bover-Cid, S., Marine-Font, A., & Vidal-Carou, M. C. (2005). Volatile and biogenic amines, microbiological counts, and bacterial amino acid decarboxylase activity throughout the salt-ripening process of anchovies (*Engraulis encrasicolus*). *Journal of Food Protection, 68*, 1683–1689.

Pons-Sanchez-Cascado, S., Veciana-Nogues, M. T., & Vidal-Carou, M. C. (2003). Effect of delayed gutting on biogenic amine contents during ripening of European anchovies. *European Food Research and Technology, 216*, 489–493.

Prasad, M. M., & Panduranga Rao, C. C. (1994). Storage studies on commercial salt cured dry fish with special reference to red discolouration. *Fishery Technology, 31*, 163–166.

Prasad, M. M., & Seenayya, G. (2000). Effect of spices on the growth of red halophilic cocci isolated from salt cured fish and solar salt. *Food Research International, 33*, 793–798.

Reddy, M. C., Bills, D. D., & Lindsay, R. C. (1969). Ester production by *Pseudomonas fragi* 2. Factors influencing ester levels in milk cultures. *Applied Microbiology, 17*, 779–782.

Reddy, N. R., Villanueva, M., & Kautter, D. A. (1995). Shelf life of modified-atmosphere-packaged fresh tilapia fillets stored under refrigeration and temperature-abuse conditions. *Journal of Food Protection, 58*, 908–914.

Ringo, E., Stenberg, E., & Strom, A. R. (1984). Amino acid and lactate catabolism in trimethylamine oxide respiration of *Alteromonas putrefaciens* NCMB 1735. *Applied and Environmental Microbiology, 47*, 1084–1089.

Romero, J., Gonzalez, N., & Espejo, R. T. (2002). Marine *Pseudoalteromonas* sp. composes most of the bacterial population developed in oysters (*Tiostrea chilensis*) spoiled during storage. *Journal of Food Science, 67*, 2300–2303.

Ruiz-Capillas, C., Saavedra, A., & Moral, A. (2003). Hake slices stored in retail packages under modified atmospheres with $CO_2$ and $O_2$ enriched gas mixes. *European Food Research and Technology, 218*, 7–12.

Sadok, S., Uglow, R. F., & El-Abed, A. (2003). Nitrogenous compound changes in live, stored clam, *Tapes decussatus*: The effects of temperature and emersion. *Journal of Aquatic Food Product Technology, 12*, 113–128.

Santoso, I., Gandjar, I., Sari, R. D., & Sembiring, N. D. (1999). Xerophilic moulds isolated from salted and unsalted dried fish from traditional markets in Jakarta. *Indonesian Food and Nutrition Progress, 6*, 55–58.

Satomi, M., Vogel, B. F., Gram, L., & Venkataraman, R. (2006). Description of two *Shewanella* species, *Shewanella hafniensis* sp. nov., and *Shewanella morhaue* sp. nov., isolated from the marine fish of the Baltic Sea, Denmark. *International Journal of Systematic and Evolutionary Microbiology, 56*, 243–249.

Scott, J. H., & Nealson, K. H. (1994). A biochemical study of the intermediary carbon metabolism of *Shewanella putrefaciens*. *Journal of Bacteriology, 176*, 3408–3411.

Segal, W., & Starkey, R. L. (1969). Microbial decomposition of methionine and identity of resulting sulfur products. *Journal of Bacteriology, 98*, 908–913.

Seibel, B. A., Goffredi, S. K., Thuesen, E. V., Childress, J. J., & Robison, B. H. (2004). Ammonium content and buoyancy in midwater cephalopods. *Journal of Experimental Marine Biology and Ecology, 313*, 375–387.

Seibel, B. A., & Walsh, P. J. (2002). Trimethylamine oxide accumulation in marine animals: Relationship to acylglycerol storage. *Journal of Experimental Biology, 205*, 297–306.

Shamshad, S. I., Kher, U. N., Riaz, M., Zuberi, R., & Qadri, R. B. (1990). Shelf life of shrimp (*Penaeus merguiensis*) stored at different temperatures. *Journal of Food Science, 55*, 1201–1205.

Shaw, B. G., & Shewan, J. M. (1968). Psychrophilic spoilage bacteria of fish. *Journal of Applied Bacteriology, 31*, 89–&.

Shewan, J. M. (1977). The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. *Proceedings of the conference on handling, processing and marketing of tropical fish* (pp. 51–66). London: Tropical Products Institute.

Shewan, J. M., Hobbs, G., & Hodgkiss, W. (1960). A determinative scheme for the identification of certain general of gram-negative bacteria with special reference to the *Pseudomonadaceae*. *Journal of Applied Bacteriology, 23*, 379–390.

Siskos, I., Zotos, A., & Taylor, K. D. A. (2005). The effect of drying, pressure and processing time on the quality of liquid-smoked trout (*Salmo gairdnerii*) fillets. *Journal of the Science of Food and Agriculture, 85*, 2054–2060.

Solberg, T., & Nesbakken, T. (1981) Quality changes in iced shrimps (*Pandalus borealis*). III. Indole and pH in shrimps caught in the Barents Sea compared with shrimps caught in the Far East. *Nordisk Veterinaermedicin, 33*, 446–453.

Somners, J. M. (1975). Herring marinades. *Food-Progress, 2*, 2–4.

Spanggaard, B., Huber, I., Nielsen, J., Nielsen, T., Appel, K. F., & Gram, L. (2000). The microflora of rainbow trout intestine: A comparison of traditional and molecular identification. *Aquaculture, 182*, 1–15.

Spanggaard, B., Huber, I., Nielsen, J., Sick, E. B., Pipper, C. B., Martinussen, T., et al. (2001). The probiotic potential against vibriosis of the indigenous microflora of rainbow trout. *Environmental Microbiology, 3*, 755–765.

Stohr, V., Joffraud, J. J., Cardinal, M., & Leroi, F. (2001). Spoilage potential and sensory profile associated with bacteria isolated from cold-smoked salmon. *Food Research International, 34*, 797–806.

Surendran, P. K., Mahadeva Iyer, K., & Gopakumar, K. (1985). Succession of bacterial genera during iced storage of three species of tropical prawns, *Penaeus indicus, Metapenaeus dobsoni* and *M. affinis*. *Fishery Technology, 22*, 117–120.

van Spreekens, K. J. A. (1987). Histamine production by the psychrophilic flora. In D. E. Kramer & J. Liston (Eds.), *Seafood quality determination* (pp. 309–318). Amsterdam: Elsevier Science Publishers B.V.

Vanderzant, C., Cobb, B. F., Thompson, C. J., & Parker, J. C. (1973). Microbial flora, characteristics, and shelf life of four species of pond-reared shrimp. *Journal of Milk and Food Technology, 36*, 443–446.

Vaz-Pires, P., & Barbosa, A. (2004). Sensory, microbiological, physical and nutritional properties of iced whole common octopus (*Octopus vulgaris*). *Lebensmittel-Wissenschaft und - Technologie, 37*, 105–114.

Vogel, B. F., Venkateswaran, K., Satomi, M., & Gram, L. (2005). Identification of *Shewanella baltica* as the most important $H_2S$-producing species during iced storage of Danish marine fish. *Applied and Environmental Microbiology, 71*, 6689–6697.

Yancey, P. H. (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *Journal of Experimental Biology, 208*, 2819–2830.

Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., & Somero, G. N. (1982). Living with water-stress – evolution of osmolyte systems. *Science, 217*, 1214–1222.

Ziemke, F., Hofle, M. G., Lalucat, J., & Rossello-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *International Journal of Systematic Bacteriology, 48*, 179–186.