



## Evaluation of Seafood Safety Health Hazards for Traditional Fish Products: Preventive Measures and Monitoring Issues

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### Abstract

Traditional fish products (TFPs) are usually produced by applying old preserving methods such as salting, fermenting, drying and smoking. These products also greatly varies amongst the countries as well as within the same country by using many different applications such as differences in additives, percentage of salt or vinegar and maturing temperatures. Moreover, modifications in these techniques are also known due to food safety issues and changes in customer preference of new generation. Although such processing/preserving methods have been known as old techniques for many years, they have still wide acceptance around the world because of their specific taste and aroma.

Due to their specific characteristics for varying many types, they have both advantages and disadvantages relating to seafood health risks that makes them difficult to identify, establish effective preventive and/or monitoring procedures. In this paper, the most common seafood health hazards were evaluated under five main traditional fish processing methods. The preventive measures were discussed along with effective monitoring in Hazard Analysis Critical Control Points (HACCP) system application for specific products by reviewing current literature and regulations under this subject. Seafood safety hazards were evaluated under two sections as 'raw material receiving and storage stage before processing including other ingredients and packaging materials', and 'processing and storage stage'.

Although it is easy to prevent certain health hazards at the receiving stage, some of them have to be monitored from harvesting along with processing until consumption. The most common seafood health hazards, which threatens TFPs, were mainly found as histamine, parasites, *Listeria monocytogenes* and *Clostridium botulinum*, and they were discussed under each process type. In the scope of this study, preventive measures and further studies related to major health hazards are suggested under each specific TFPs.

**Keywords:** traditional fish, seafood safety hazards, HACCP, parasites, histamine, *L. monocytogenes*, *C. botulinum*.

### Geleneksel Balık Ürünlerinde Su Ürünleri Gıda Güvenliği Tehditlerinin Değerlendirilmesi: Önleyici Tedbirler ve İzleme Prosedürü

#### Özet

Geleneksel balık ürünleri genelde tuzlama, kurutma ve tütsüleme gibi muhafazaya dayalı eski işleme yöntemleriyle üretilirler. Bu ürünler, hem ülkeler arasında ve hem de aynı ülke içinde işleme teknolojisinde kullanılan farklı katkıları, farklı oranlarda tuz veya sirke uygulaması ve farklı sıcaklıklarda olgunlaştırma gibi uygulamalar nedeniyle geniş bir varyasyona sahiptirler. Aynı zamanda, bu tür ürünlerin işleme tekniklerinde gıda güvenliği ve yeni nesil tüketici taleplerindeki değişiklikler gibi nedenlerle modifikasyonlar da bilinmektedir. Bu ürünler eski muhafaza/işleme teknikleri ile üretilmesine rağmen günümüzde spesifik tat ve aromaları nedeniyle tüm dünyada hala çok kabul görmektedirler.

Çeşitli farklılıklara sahip pek çok geleneksel balık ürünleri her birinin spesifik karakterleri nedeniyle gıda güvenliği riski bakımından hem avantaj ve hem de dezavantajlara sahiptirler. Bu özellikler onların su ürünlerini tehdit eden tehlikelerin tanımlanması ve etkili koruma ve/veya izleme yöntemlerinin oluşturulmasını zorlaştırır. Bu makalede, en yaygın su ürünleri tehlikeleri beş farklı işleme yöntemi altında incelenip değerlendirilmiştir. Önleyici tedbirler, spesifik ürünler bazında Tehlike Analizi Kritik Kontrol Noktaları (HACCP) sisteminin uygulanması ile etkili izleme, ve bu alandaki literatür ve yasalar altında ele alınarak tartışılmıştır. Su ürünleri gıda emniyeti ile ilgili tehlikeler, 'diğer katkı ve paketleme materyalleri dahil ham maddenin kabulü ve işlem öncesi depolanması' ile 'işleme ve depolama' başlıkları altında iki aşamada incelenmiştir.

Kabul aşamasında belirli gıda tehlikelerini önlemek kolay olmasına karşın, bazı tehlikelerin hammaddenin hasat edilmesinden tüketime kadar izlenmesi gerekmektedir. Geleneksel balık ürünlerini tehdit eden en yaygın su ürünleri tehlikeleri, histamin, parazitler, *Listeria monocytogenes* ve *Clostridium botulinum* olarak belirlenmiştir. Bu tehlikeler her bir işleme yöntemi başlığı altında tartışılmıştır. Bu makalenin amacı doğrultusunda, su ürünleri gıda emniyetini tehdit eden başlıca tehlikelerle ilgili önleme yöntemleri incelenmiş ve bu konu ile ilgili geleceğe yönelik önerilen çalışmalara her bir geleneksel balık ürünü başlığı altında yer verilmiştir.

**Anahtar Kelimeler:** geleneksel balık, su ürünleri gıda tehlikeleri, HACCP, parazitler, histamin, *L. monocytogenes*, *C. botulinum*.

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## 1. Introduction

Traditional seafoods were originally developed to preserve fisheries products for a long storage life by either lowering water activity ( $a_w$ ) and/or changing pH of the products. In addition, preservation was also carried out by applying antibacterial activity of salt and/or smoke components or other preservative compounds to increase shelf-life and improve safety of such products. Although new technologies such as canning, high pressure processing (HPP) and modified or controlled atmospheric packaging (MAP, CAP) methods have been developed to improve safety of seafood products, traditional preserving methods of fish products have still wide acceptance around the world due to their accustomed taste and aroma.

Despite the preserving aim of such traditional methods, these products are still under risk of several hazards due to following reasons: (i) these products have long maturation time, (ii) they are generally consumed without further cooking, (iii) changes in the original methodologies over the years such as decreasing salt content. Small and medium sized (SMEs) fish processing companies are usually suffering from preparing efficient Hazard Analysis Critical Control Points (HACCP) plan to prevent such hazards (Köse *et al.*, 2010).

Traditionally processed fish products (TFPs) are reported to carry high potential risk for human health for halophilic pathogenic bacteria, histamine and parasites (Taylor, 1986; Essuman, 1992; Lehane and Olley, 2000; FDA, 2001; Kirschbaum *et al.*, 2000; Karaçam *et al.*, 2002; Kuda *et al.*, 2002; Mah *et al.*, 2002; Murrell, 2002; Huss *et al.*, 2003; Tsai *et al.*, 2006; Huss *et al.*, 2003; EU, 2004; Hansen, 2008). Due to legal criteria set for potential hazards, domestic and international marketing of these products is becoming difficult and limited.

This paper aims to discuss seafood safety issues for TFPs by using previous publications as well as regulations of the European Union (EU) and the USA (FDA). It also targets to evaluate these hazards under different types of TFPs for preventive measures and monitoring.

Seafood health hazards have been outlined in several guides in the literature (FDA, 2001; Huss *et al.*, 2003) and can be classified as (i) biological hazards (biogenic amines - in some literature is classified under chemical hazards or biotoxins, parasites, pathogenic bacteria, viruses, biotoxins and allergens), (ii) chemical hazards (chlorophenicol and other antibiotic residues for farmed fish, fish originated from contaminated waters such as heavy metals, dioxins, chemical contaminants originated from processing areas, chemicals formed by fish processing such as nitrosamines and polycyclic aromatic hydrocarbons (PAH)), and (iii) physical hazards such as bones, plastic, glass and metals. TFPs usually carry all these health risks although some of them are specific to other seafoods such as shellfish (e.g., paralytic shellfish poisoning) (FDA, 2001; Huss *et al.*, 2003; Stolyhwo and Sikorski, 2005; Yurchenko and Molder, 2006; Karl, 2008; Al Bulushi *et al.*, 2009).

## 2. Evaluation of Seafood Health Hazards at Incoming Material Receiving Stage

Some of above-mentioned hazards, such as biotoxins, viruses and chemical contaminants can easily be controlled at raw material receiving and storage step and/or during processing by applying good hygienic and manufacturing practices (GHP, GMP). In controlling such hazards at raw material receiving and storage stage, quality control/safety personnel (QC) for the processing unit or other personnel responsible for receiving should ask for 'Certificate of origin' for the raw material at receiving step. Beginning from 2010, a new EU regulation is going to be in application called as 'catch certificate' (Council Regulation EC, 2008; URL-1; EC, 2010). Such personnel should check if the raw material (fish) originates from a safe area (farm or catching area) or not. Similar attitude must be applied for the ingredients other than fish and as well as packaging materials. If the incoming material comes from unreliable source, then the personnel should 'Reject' it. Although checking the necessary certificates for incoming materials in terms of food safety is sufficient for preventive measures, it is also advisable to send occasional samples to an accredited laboratory for verification procedures which is the part of HACCP plan.

Poisonous fish species such as *Tetraodontidae*, *Molidae*, *Diodontidae* and *Canthigasteridae* are usually forbidden from the market and some species of *Gempylidae* family are only allowed in certain conditions (in wrapped/packaged form and must be

appropriately labelled) (EU, 2005). Therefore, it is unlikely that such poisonous fish will be landed on the market for processing. However, processors should indicate this at their HACCP plan.

There are certain types of hazards like histamine, parasites and some pathogenic bacteria that are not easy to control at incoming material stage or during GHP and GMP applications. Therefore, the preventive measures and careful monitoring have to be done starting from incoming material stage until consumption. Some information related to these hazards and monitoring practices at raw material stage are given below.

## 2.1. Histamine and Other Biogenic Amines

### **Formation of Biogenic Amines and Involved Products:**

Biogenic amines (BAs) are mainly formed in foods by microbial decarboxylation of amino acids and transamination of aldehyde and ketones. Certain biogenic amines such as histamine, cadaverine, putrescine and tyramine are of importance due to the risk of food intoxication and also they serve as chemical indicators of fish spoilage (Lehane and Olley, 2000; Kim *et al.*, 2009). Histamine is one of the main concerns in fisheries products formed by microbial decarboxylation of histidine as a result of time/temperature abuse in certain fish species. Histamine poisoning is often referred to as 'scombrototoxin poisoning' because of the frequent association of the illness with the consumption of spoiled scombroid fish such as tuna, bonito and mackerel. However, non-scombroid fish such as herring, anchovies and mahi-mahi have also been implicated in outbreaks (Lehane and Olley, 2000; Huss *et al.*, 2003).

### **Toxicity Levels:**

In most cases, histamine levels in illness-causing fish have been above 200 ppm, often above 500 ppm. However, there is some evidence that other biogenic amines such as putrescine and cadaverine may also play a role in this type of poisoning (FDA, 2001; Huss *et al.*, 2003; Lehane and Olley, 2000). A hazardous level of histamine for human health has been suggested as 500 mg/kg although low levels as 50 mg/kg (50 ppm) have been reported in histamine poisoning (FDA, 2001; Huss *et al.*, 2003). Shalaby (1996) suggested the following guideline levels for histamine content of fish as regards to health hazard as; (i) <5 mg/100 g (safe for consumption), (ii) 5-20 mg/100 g (possibly toxic), (iii) 20-100 mg/100 g (probably toxic), (iv) >100 mg/100 g (toxic and unsafe for human consumption).

### **The type of Fish Species Involved:**

Free histidine is generally found in large amounts in the muscle of fatty, red-meat, active and migratory species compared to that in the white meat

of slower species. Therefore, formation of histamine primarily relates to marine fish species and is not a potential hazard when freshwater fishes are used as raw material (Huss *et al.*, 2003). Table 1 demonstrates the list of fish species presenting a potential health hazard for histamine poisoning (FDA, 2001; Dalgaard *et al.*, 2008; Tsai *et al.*, 2006; Chang *et al.*, 2008; Rabie *et al.*, 2009).

### **The type of Bacteria Involved:**

Certain species of *Enterobacteriaceae*, *Clostridium* and *Lactobacillus* produce the enzyme histidine decarboxylase during growth (Frank, 1985; Lehane and Olley, 2000). Enteric bacteria have been found to be the most important histamine forming bacteria (HFB) in fish. *Morganella morganii*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Hafnia alvei* are known to originate from fish implicated incidents of histamine poisoning (Frank, 1985; Lehane and Olley, 2000; Huss *et al.*, 2003).

### **The risk of Histamine Poisoning for Traditional Fish Products:**

Some of the HFB are reported to be halotolerant (salt-tolerant) or halophilic (salt-loving). This causes some salted and smoked fish products produced from histamine forming species to continue to be suspected for histamine development. Furthermore, a number of HFB are facultative anaerobes that can grow in reduced oxygen environments. The investigations also proved that such bacteria can still be isolated from salted fish products that contain high salt level and long storage time (Köse *et al.*, 2007a). Although histamine poisoning cases or outbreaks have been reported worldwide for TFPs (Lehane and Olley, 2000; Kanki *et al.*, 2004; Tsai *et al.*, 2007), many incidents have been claimed to be left unreported (Tsai *et al.*, 2005; Mah and Hwang, 2009a; Mah *et al.*, 2009; Rabie *et al.*, 2009). Development of biogenic amines in TFPs occurs at maturation stage during salting or fermenting, poor handling of the raw materials and improper storage conditions.

### **Preventive Measures:**

Preventive measures for histamine formation are mainly based on preventing or delaying the growth of HFB and also slowing down the activity of enzymes which are produced by the related bacteria. Therefore, time/temperature control is mainly used for critical limit for monitoring histamine formation at raw material stage. HFB are capable of growing and producing histamine over a wide temperature range. Growth is more rapid, however, at high-abuse temperatures (e.g. 70°F [21.1°C]) than at moderate abuse temperatures (e.g. 45°F [7.2°C]). Growth is particularly rapid at temperatures near 90°F (32.2°C). Histamine is formed more commonly as a result of high temperature spoilage than that of long term, relatively low temperature spoilage. Nonetheless, there are a number of opportunities for histamine to

**Table 1.** Most common species identified in terms of health risk of histamine poisoning (FDA, 2001; Dalgaard *et al.*, 2008, Tsai *et al.*, 2006; Chang *et al.*, 2008; Rabie *et al.*, 2009).

Common English names	Species Latin names
Anchovy	<i>Anchoa spp.</i> , <i>Anchoviella spp.</i> , <i>Cetengraulis mysticetus</i> , <i>Engraulis spp.</i> , <i>Stolephorus spp.</i>
Escolar Or Oil Fish	<i>Lepidocybium flavobrunneum</i> , <i>Ruvettus pretiosus</i>
Gem Fish	<i>Lepidocybium flavobrunneum</i>
Bluefish	<i>Pomatomus saltatrix</i>
Bonito	<i>Cybiosarda elegans</i> , <i>Gymnosarda unicolor</i> , <i>Orcynopsis unicolor</i> , <i>Sarda spp.</i>
Bouri	<i>Mugil cephalus*</i>
Garfish	<i>Belone belone</i>
Herring	<i>Alosa spp.</i> , <i>Alosa pseudoharengus</i> , <i>Etrumeus teres</i> , <i>Harengula thrissina</i> , <i>Ilisha spp.</i> , <i>Opisthopterus tardoore</i> , <i>Pellona ditchela</i> , <i>Clupea spp.</i> (Sild and roe), <i>Opisthonema spp.</i> (Thread).
Jack	<i>Caranx spp.</i> , <i>Oligoplites saurus</i> , <i>Selene spp.</i> , <i>Seriola rivoliana</i> , <i>Urapsis secunda</i> , <i>Caranx crysos</i> (Blue Runner), <i>Alectis indica</i> (Crevalle), <i>Elagatis bipinnulata</i> (Rainbow Runner), <i>Nematistius pectoralis</i> (Roosterfish).
Jobfish	<i>Aphareus spp.</i> , <i>Aprion virescens</i> , <i>Pristipomoides spp.</i>
Kahawai	<i>Arripis spp.</i>
Mackerel	<i>Gasterochisma melampus</i> , <i>Grammatorcynus spp.</i> , <i>Rastrelliger kanagurta</i> , <i>Scomber scombrus</i> and <i>Scomber spp.</i> (Chub mackerel), <i>Trachurus spp.</i> (Jack Mackerel), <i>Scomberomorus spp.</i> (Spanish Mackerel)
Mahi-Mahi	<i>Coryphaena spp.</i>
Marlin	<i>Makaira spp.</i> , <i>Tetrapturus spp.</i>
Pilchard or Sardine	<i>Sardina pilchardus</i> , <i>Sardinops spp.</i> , <i>Harengula spp.</i> , <i>Sardinella spp.</i>
Sailfish	<i>Istiophorus albicans</i> , <i>Istiophorus platypterus</i>
Salmon	<i>Onchorhynchus keta</i> , <i>O. kisutch</i> , <i>O. gorbuscha</i> , <i>O. nerka</i> and others
Saury	<i>Cololabis saira</i> , <i>Scomberesox saurus</i>
Shad, Gizzard	<i>Dorosoma spp.</i> , <i>Nematalosa vlaminghi</i>
Snapper	<i>Pristipomoides spp.</i>
Sprat or bristling	<i>Sprattus spp.</i>
Swordfish	<i>Xiphias gladius</i>
Trevally	<i>Caranx sexfasciatus</i>
Tuna	<i>Allothunnus fallai</i> , <i>Auxis spp.</i> , <i>Euthynnus spp.</i> , <i>Katsuwonus pelamis</i> , <i>Thunnus tonggol</i> , <i>T. alalunga</i> , <i>T. albacares</i> , <i>T. atlanticus</i> , <i>T. maccoyii</i> , <i>T. obesus</i> , <i>T. thynnus</i> .
Wahoo	<i>Acanthocybium solandri</i>
Yellowtail or Amberjack	<i>Seriola lalandei</i>

\*Reported to present histamine health risk for fermented fish.

be formed under more moderate abuse temperature conditions (FDA, 2001). Moreover, recent findings indicated that histamine food poisoning can also be caused by psychrotolerant bacteria (*Morganella psychrotolerans* and *Photobacterium phosphoreum*) due to their ability of producing toxic concentrations of histamine at temperatures as low as 2°C (Emborg *et al.*, 2005). Dalgaard *et al.* (2008) pointed out that both bacteria can produce histamine in toxic levels at 0-5°C. Therefore, histamine formation during extended storage of fish at low temperature must not be disregarded.

There are several ways of controlling histamine formation in fish products. FDA (2001) indicated that freezing may inactivate the enzyme-forming bacteria. However, once the enzyme histidine decarboxylase has been formed, it can continue to produce histamine in the fish even if the bacteria are not active. The enzyme can be active at or near refrigeration temperatures. The enzyme is likely to remain stable while in the frozen state and may be reactivated very rapidly after thawing. Both the enzyme and the bacteria can be inactivated by cooking. However, once histamine is formed, it cannot be eliminated by

heat or freezing. After cooking, recontamination of the fish with the HFB is necessary for additional histamine to form (Köse, 1993; FDA, 2001; Huss *et al.*, 2003). For these reasons, histamine development more likely occurs in raw, unfrozen fish. Therefore, it is important to control histamine formation before processing, i.e. at raw material stage.

HFB naturally exist on the gills and in the gut of live, salt water fish. Although evisceration and removal of the gills in a sanitary manner may, however, reduce, however, under unsanitary conditions, these steps may accelerate the process of histamine development in the edible portions of the fish by spreading the bacteria to the flesh of the fish (FDA, 2001).

Rapid chilling of fish immediately after death is the most important element in any strategy for preventing the formation of histamine, especially for fish that are exposed to warmer waters or air. The time required to lower the internal temperature of fish after capture will be dependent upon a number of factors (FDA, 2001), including;

(i) *The harvest method:* Delays in removing fish from a long line may significantly limit the amount of

time left for chilling and may allow some fish to heat up after death. The quantity of fish landed in a purse seine or on a long line may exceed a vessel's ability to rapidly chill the product.

(ii) *The size of the fish:* Bigger fish will be chilled down slower than small sized fish.

(iii) *The chilling method:* As a consequence of reduced contact area and heat transfer, ice alone takes longer to chill fish than ice slurry or re-circulated refrigerated sea water or brine does. The quantity of ice or ice slurry and the capacity of refrigerated sea water or brine systems must be suitable for the quantity of catch.

### **Regulations for Histamine Presence in Fish Products:**

Usually, there are two main regulations stated by FDA and the EU, and the other countries apply either these regulations or modified versions. Because histamine is generally not uniformly distributed in a decomposed fish, a guidance level of 50 ppm has been set by FDA (FDA, 2001). If 50 ppm is found in one section, it means that it is possible for other sections to exceed 500 ppm. The European Union Directive (EC, 2007: Directive No: 1441/2007) requires that nine samples must be taken from each batch of fish species, particularly of the following families: *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryfenidae* (*Coryphaenidae*), *Pomatomidae*, *Scombrosidae*. These samples must fulfil the following requirements; the mean value must not exceed 10 mg/100g (100 ppm), two samples may have a value of more than 10 mg/100 g (100 ppm) but less than 20 mg/100g (200 ppm) and no sample may have a value exceeding 20 mg/100g (200 ppm).

On the other hand, the EU gives higher levels for 'fishery products which have undergone enzyme maturation treatment in brine and manufactured from fish species associated with a high amount of histidine' (EC, 2007, the EU regulation for microbial criteria) such as; two samples may have a value of more than 20 mg/100g (100 ppm) but less than 40 mg/100g (400 ppm) and no sample may have a value exceeding 40 mg/100g (400 ppm).

### **Monitoring Histamine Formation:**

Although FDA (2001) gave a valuable guide on how long fish should be kept at/or exposed to certain temperatures for a safe shelf life in relation to histamine formation, such guidance is more suitable or much easier to be used for fresh/frozen fish marketing for developed countries where seafood safety regulations are effectively in use. There are several factors that can affect such guidance to be used effectively around the world, especially in undeveloped countries and some developing countries where seafood regulations are lacking in effectiveness. Moreover, environmental conditions may differ around the world at different regions. Therefore, processors for TFPs are advised to follow

up careful monitoring procedures to allow safe shelf-life in terms of histamine specifically at raw material stage since histamine formation may continue during processing for certain types of products.

For controlling histamine and possibly other amines, the following guidance is suggested for raw material stage.

To monitor total exposure time of implicated fish to elevated and/or cold storage temperatures before processing: Such activity can be carried out depending on processors' choices, if they can trace fish harvesters (fishermen or farmers) through their recordings or not. If the answer is 'yes', then they can set up their critical limit as decided time/temperature according to experimental histamine test results for such delivery conditions as well as the type of processing to be applied. If the answer is 'no', then processors should apply several critical limits to monitor histamine formation. These are; (i) checking the temperature of fish and water at arrival (ii) checking sensory quality of fish (set up the freshest quality parameters as given in several guidance by Huss (1988), Botta (1995)), (iii) testing histamine levels at the arrival for suspected lot using rapid test kits and set up critical histamine levels according to the type of processing method to be applied, and (iv) if fish arrived as frozen, then the processors can perform histamine testing for a specified critical limit according to type of processing method, then to continue monitoring temperature during storage and thawing.

FDA (2001) pointed out that although sensory evaluation is generally used to screen fish for spoilage odours, such examination alone is ineffective control for histamine. Moreover, toxic histamine levels can also be observed in fish despite their acceptable sensory quality (Köse *et al.*, 1997). Federal Register (1995) reported that the best quality fish has histamine values less than 10 ppm, while histamine values between 10-30 ppm are accepted as middle quality and 30-50 ppm histamine value is critical since it is close to the level of FDA regulation (50 ppm). Therefore, histamine testing is an effective way of monitoring its formation at raw material stage before processing. However, approved histamine testing procedures are time-consuming and require technical skills that are mostly lacking at processing companies. Practical test kits are suggested to be used during monitoring histamine hazard in the HACCP plan starting from raw material receiving. Some test kits have been reported to work well for testing histamine in fish and several types of TFPs (Köse *et al.*, 2007b; Köse *et al.*, 2009a).

## **2.2. Nitrosamines**

Nitrosamines are reported to cause cancer and are often associated with TFPs such as smoked, fermented, salted and salt-dried products. Although various causes have been indicated with the formation

of nitrosamines in foods, the mechanisms of nitrosamine formation in fish products and factors influencing their formation have not been clearly elucidated. Nitrosamines are generally formed through reactions between secondary and tertiary amines and nitrite under certain conditions (Al Bulushi *et al.*, 2009). The presence of secondary amines such as dimethylamine (DMA) and tertiary amines such as TMA has been found to be implicated in nitrosamine formation. In fish products such as salted, pickled, smoked, fermented and canned fish, the presence of nitrosodimethylamine (NDMA) which is formed from DMA and nitrite, has been widely reported (Mitacek *et al.*, 1999). Primary amines such as putrescine and cadaverine have been suggested to cyclize during heating to secondary amines such as pyrrolidine and piperidine, which react with nitrite to form carcinogenic nitrosamines (Al Bulushi *et al.*, 2009). The International Agency for Research on Cancer (IARC, 1978) classified a number of NAs with respect to the cancer risk for humans. The IARC considers NDMA and N-nitrosodiethylamine (NDEA) into the group of probably carcinogenic to human, and N-nitrosodibutylamine (NDBA), N-nitrosopiperidine (NPIP) and N-nitrosopyrrolidine (NPYR) into the group of possibly carcinogenic to human (Yurchenko and Mölder, 2006). The USA has set 10 µg/kg NPYR as the maximum allowable limit in bacon (Domanska-Blicharz *et al.*, 2005; Al Bulushi *et al.*, 2009) while not many countries including the EU regulations set regulations for nitrosamines originated from food. Estonian government has maximum permitted concentration of the sum of two NAs (NDMA and NDEA) in fresh and smoked fish, which is 3 µg/kg (Yurchenko and Mölder, 2006). The EU regulations (EU, 1993) relate to the release of the N-Nitrosamines and N-nitrosatable substances from elastomer or rubber teats and soothers.

Al Bulushi *et al.* (2009) reviewed the nitrosamine levels in traditional products originated from various countries (China, Estonia, Poland, Thailand and South Korea) and reported that NDMA accounted for 86% of the total N-nitrosamines in salted fish in China. Yurchenko and Mölder (2006) found varying nitrosamine levels in Estonian fish samples including TFPs. They reported that the sum of the average of 5 important NAs in cold-smoked, hot-smoked, fried, pickled, salted and salted/dried fish samples was 1.92, 4.36, 8.29, 5.37, 3.16 and 3.81 µg/kg, respectively. In fresh fish, the levels were below detection limit. The authors indicated that their results were lower than the findings of other researchers obtained in fisheries products of Russia, Japan and China but in agreement with the results reported for France, Denmark and Sweden. Other studies also proved that while TFPs from the EU origin show lower levels of nitrosamines, the levels were found to be higher for such products from other countries (S. Korea, China, Thailand) (Al Bulushi *et al.*, 2009).

There are many factors that influence nitrosamine formation or degradation in food (Zou *et al.*, 1994; Mitacek *et al.*, 1999; Rywotycki, 2007; Yurchenko and Mölder, 2006; Al Bulushi *et al.*, 2009). These are (i) level of nitrite and nitrate in food or water that is used to process food, (ii) type of bacteria present in the product or contaminated via water (because some of them may accelerate nitrosating reaction and increase nitrosamine amount, e.g. bacteria can convert nitrate to nitrite), (iii) the purity of salt (sodium chloride has been shown to have inhibitory action on the formation of nitrosamines, while impure salt, particularly with high nitrite content can accelerate its formation), (iv) the pH of the product (low pH enhances nitrosamine formation in fish products and the optimum pH for the formation of the highest levels of nitrosamines has been found to be 3.8), (v) species of the fish, (vi) temperature (although in vitro formation of NPYR and NPIP has been found to occur at high temperature, such as 160°C for 2h, the reaction between nitrite and putrescine was found to occur at low temperature such as 22°C over 6 days), (vii) fish quality prior to processing (some fish can contain high amount of BAs that can affect nitrosamine formation, and (viii) type of processing methods (e.g., the USA allows addition of sodium nitrite in smoked fish to prevent *C. botulinum* but the EU does not allow it).

All these factors are studied in vitro: however, little is known about the effect of these factors in vivo (Al Bulushi *et al.*, 2009). Thus, one expects that formation of nitrosamines in fish products produced from fish with a high content of BAs may be significant. Although NDMA and NPIP increase significantly in meat products treated with nitrites during storage at 4–8°C for 72 h (Domanska and Kowalski, 2002), little is known about the impact of storage conditions on the formation of these compounds in fish products (Al Bulushi *et al.*, 2009). Ahn *et al.* (2003) reported that gamma irradiation has a possibility to reduce N-nitrosamines in salted and fermented anchovy sauce. Considering the possible effects on nitrosamine formation in fish products, it is advisable to use good quality fish and water, limited amount of nitrite, pure salt and apply GHP.

### 2.3. Pathogenic Bacteria

Fish can contain pathogenic bacteria, such as *Clostridium botulinum* (usually type E which is marine origin), *Staphylococcus aureus* and *Vibrio parahaemolyticus*. The pathogens grow well in temperature abused raw fish include: *Vibrio vulnificus*, *V. parahaemolyticus*, *V. cholerae*, and *Listeria monocytogenes*. Some other pathogens can contaminate through mishandling of fish. Such contamination can be eliminated by GHP and GMP starting from harvesting to consumer. It is not easy to eliminate such hazards at raw material stage;

however, increase in their numbers can be slowed down at this step. FDA gives some guidance on growth limiting factors at Table 2 (FDA, 2001). Since some of the pathogenic bacteria are poor competitors in the presence of other bacteria especially spoilage bacteria, their growth is not expected as fast as after or during processing when such competitors are decreased in number. Some pathogens such as *L. monocytogenes* and *C. botulinum* type E can grow well at low temperatures. Therefore, *C. botulinum* presents health risk at raw material stage when bulk storage without gutting takes place.

#### Monitoring Pathogenic Bacteria in TFPs at Raw Material Stage:

The guidance is as follows according to choices of processors;

- Frozen storage at -18°C or colder can stop bacterial growth and may slightly decrease in number. Temperature is the critical limit and used for monitoring.

- If cold storage between 0-4°C is applied, growth of some pathogenic bacteria can be stopped but some still can grow slowly. Time and temperature are used for critical limit to monitor both receiving and storing stages of fish. Similar conditions used to monitor histamine can also be applied to monitor bacterial growth.

- Other choices are also available, such as washing fish with sterile water, using ozonated or salt water.

- Gutting is advised to prevent *C. botulinum* in bulk storage of fish at elevated temperatures.

Huss *et al.* (2003) suggested that control of *C. botulinum* in fishery products can be achieved by inactivation of spores or by inhibition of growth. They listed the guidelines as; (i) storage at all times at

<3.3°C, (ii) storage at 5-10°C for a shelf life of <5 days, (iii) heat treatment of 90°C for 10 min combined with chill storage (<10°C), (iv) adjustment of pH 5.0 throughout the food combined with chilled storage (<10°C), and (v) salt-on-water concentration (3.5% or  $a_w$ : 0.97 throughout the food combined with chill storage (<10°C). This requirement fills in our suggestions that fish must be stored either as frozen or chilled. It is indicated that evisceration of fish before processing is necessary (FDA, 2001). Because spores are known to be present in the viscera of fish, any product that will be preserved by salting, drying, pickling, or fermentation must be eviscerated prior to processing. Without evisceration, toxin formation is possible during the process even with strict control of temperature. Evisceration must be thorough and performed to minimize contamination of the fish flesh. Even if a portion of the viscera or its contents is left behind, the risk of toxin formation remains. Small fish, less than 5 inches (12.7 cm) in length (e.g. anchovies and sprats), that are processed in a manner that prevents toxin formation, and that reach a water phase salt (WPS) content of 10% in refrigerated products, or having a  $a_w$  of below 0.85 (Note: this value is based on the minimum  $a_w$  for growth of *S. aureus*) or a pH of 4.6 or less, in shelf-stable products are exempt from the evisceration requirement. WPS is the amount of salt in the product relative to the product moisture and is found using the following calculation (Losikoff, 2008).

$$\%WPS = \% \text{ salt} \times 100 / \% \text{ salt} + \% \text{ moisture}$$

#### 2.4. Parasites

FDA (2001) reported that parasites (in the larval stage) consumed in uncooked, or undercooked, unfrozen seafood can present a human health hazard.

**Table 2.** FDA guidance for limiting conditions for pathogen growth

Bacteria species	Min. $a_w$ using salt	Min pH	Max pH	WPS %	Min temp. °C	Max. temp. °C	Oxygen requirement
<i>Bacillus cereus</i>	0.92	4.3	9.3	10	4	55	Aerobe
<i>Campylobacter jejuni</i>	0.987	4.9	9.5	1.5	30	45	Microaerophilic
<i>Clostridium botulinum</i> , type A, and proteolytic B& F	0.935	4.6	9	10	10	48	Anaerobe
<i>C. botulinum</i> , type E, and nonproteolytic B & F	0.97	5	9	5	3.3	45	Anaerobe
<i>C. perfringens</i>	0.93	5	9	7	10	52	Anaerobe
Pathogenic strains of <i>Escherichia coli</i>	0.95	4	9	6.5	6.5	49.4	Facultative anaerobe
<i>Listeria monocytogenes</i>	0.92	4.4	9.4	10	-0.4	45	Facultative anaerobe
<i>Salmonella</i> spp.	0.94	3.7	9.5	8	5.2	46.2	Facultative anaerobe
<i>Shigella</i> spp.	0.96	4.8	9.3	5.2	6.1	47.1	Facultative anaerobe
<i>Staphylococcus aureus</i> growth	0.83	4	10	20	7	50	Facultative anaerobe
<i>S. aureus</i> – toxin	0.85	4	9.8	10	10	48	
<i>Vibrio cholerae</i>	0.97	5	10	6	10	43	Facultative anaerobe
<i>V. parahaemolyticus</i>	0.94	4.8	11	10	5	45.3	Facultative anaerobe
<i>V. vulnificus</i>	0.96	5	10	5	8	43	Facultative anaerobe
<i>Yersinia enterocolitica</i>	0.945	4.2	10	7	-1.3	42	Facultative anaerobe

Among parasites, the nematodes or roundworms (*Anisakis* spp., *Pseudoterranova* spp., *Eustrongylides* spp. and *Gnathostoma* spp.), cestodes or tapeworms (*Diphyllobothrium* spp.) and trematodes or flukes (*Clonorchis sinensis*, *Opisthorchis* spp., *Heterophyes* spp., *Metagonimus* spp., *Nanophyetes salminicola* and *Paragonimus* spp.) are of the most concern in seafood (Rim, 1998; FDA, 2001; Murrell, 2002; Huss *et al.*, 2003). Some TFPs such as fermented and marinated do not include cooking step to kill these parasites. These products are also mainly consumed without cooking. Therefore, they are very likely to cause disease and must be regarded as a significant hazard. FDA (2001) reported several TFPs implicated in human infection, which are ceviche (fish and spices marinated in lime juice), lomi lomi (salmon marinated in lemon juice, onion and tomato), poisson cru (fish marinated in citrus juice, onion, tomato and coconut milk), green herring (lightly brined herring) and cold-smoked fish. Such hazard is more likely to threaten processing companies which are close to harvest areas where raw material is delivered as fresh and subjected to processing without freezing.

Huss *et al.* (2003) reported that more than 50 species of helminth parasites from fish and shellfish are known to cause diseases in man. They also indicated that although the most are rare and involve only slight to moderate injury but some pose serious potential health risks. *Anisakis simplex* which is usually associated with herring, cod, mackerel and salmon is considered to be the most pathogenic, while *P. decipiens*, which is associated with cod, halibut and flatfish family or Pacific red snapper is frequently reported to cause symptoms (Murrell, 2002). More than 95% of cases manifest as acute gastric anisakidosis, in which severe epigastric pain is experienced shortly after ingestion of fish carrying parasite larvae incorporated in the fish muscle (Murrell, 2002). Huss *et al.* (2003) reported that 65-100% of wild salmon samples originated from Washington, Atlantic and Japan, 86-88% of herring samples from Mediterranean sea and Pacific Ocean, and 84% of cod samples obtained from Pacific Ocean contained *A. simplex*. Therefore, monitoring parasites, specifically nematodes either at raw material stage or during processing is necessary. While *Anisakidae* family is often involved with marine seafood, *Gnathostoma* (Nematode), Trematodes and Cestodes are usually involved with freshwater seafood. *Clonorchiasis sinensis*, which is a trematode, is widespread throughout Asia and the former Soviet Union. *O. felineus* is generally found in Eastern Europe, Poland, Germany, and Siberia (Murrell, 2002). Orlandi *et al.* (2002) have reviewed various parasites and their involvement with fish.

#### **Preventive Measure and Monitoring Parasites at Raw Material Step:**

Parasites are considered as health hazards for seafood products at raw material stage if fish is (i)

obtained from faeces-contaminated waters, (ii) not previously frozen, (iii) not going to be cooked and (iv) not going to be subjected to salting using efficient salt content to kill parasites. Although visual inspection (candling and physical removal) and trimming away the belly flap are suggested to prevent this hazard, it is also known that such activities are not a complete control (FDA, 2001). According to the EU (EC, 2004), food business operators must ensure that fishery products have been subjected to a visual examination for the purpose of detecting visible parasites before being placed on the market. EC also requires that fishery products must be frozen at a temperature of not more than  $-20^{\circ}\text{C}$  in all parts of the product for not less than 24 h; this treatment must be applied to the raw product or the finished product if:

(a) fisheries products has to be consumed raw or almost raw,

(b) the following fish species,

(i) herring,

(ii) mackerel,

(iii) sprat, and

(iv) (wild) Atlantic and Pacific salmon

have to undergo a cold smoking process in which the internal temperature of the fishery product is not more than  $60^{\circ}\text{C}$ ,

(c) the processing is insufficient to destroy nematode larvae in the marinated and/or salted fishery products.

FDA guideline suggests different approach to freezing along with visual inspection, brining, pickling and trimming. FDA (2001) recommends following guideline for freezing and frozen storage; (i) critical limit for freezing and storing must be at  $-20^{\circ}\text{C}$  or below for 7 days (total time), (ii) or freezing at  $-35^{\circ}\text{C}$  or below until solid and storing at  $-35^{\circ}\text{C}$  or below for 15 h, (iii) or freezing at  $-35^{\circ}\text{C}$  or below until solid and storing at  $-20^{\circ}\text{C}$  or below for 24 h. FDA (2001) also pointed out that these conditions may not be suitable for freezing particularly large fish (e.g. thicker than 6 inches). Therefore, longer frozen storage is more advisable.

Murrell, (2002) reported that 32 days is required to inactivate *O. felineus* metacercariae in fish at  $-28^{\circ}\text{C}$ . Therefore, for freshwater fish species longer freezing and frozen storage time is necessary for an efficient HACCP plan. Murrell (2002) also suggested several control measures for preventing parasite infection originated from freshwater. These are (i) environmental control of surface water where fish are caught, (ii) hygienic aquaculture, and the control or elimination of the first intermediate hosts (snails).

FDA (2001) indicated that the effectiveness of freezing to kill parasites depends on several factors, including temperature of the freezing process, length of time needed to freeze the fish tissue, length of time that fish is held frozen, fat content of the fish, and type of parasite present. Among those, the temperature of the freezing process, length of time



that fish is held frozen, and the type of parasite appear to be the most important factors. For example, tape worms are more susceptible to freezing than are roundworms. Flukes appear to be more resistant than roundworms. Table 3 also represents the effect of freezing variables in different parasites. The guidance of the EU or FDA sometimes can mislead processors since there are several factors affecting parasite inhibition as also pointed out by FDA (2001). Therefore, if frozen storage is going to be used as preventive measure, critical limit for monitoring should be frozen storage at -20°C or below for 10 days or longer depending on the fish whether it is freshwater or salt water fish and the risk of parasite infections. If frozen storage is not going to be applied, then the preventive measures must be considered during processing. Among those heating is another way of preventing parasites. FDA (2001) indicated that heating raw fish sufficiently to kill bacterial pathogens is also sufficient to kill parasites. However, such case usually represents inactivation of nematodes.

High hydrostatic pressure (HHP) is a recently proposed method to control decontamination of foods. Studies by Slifko *et al.* (2000a cited in Orlandi *et al.*, 2002; Slifko *et al.*, 2000b) demonstrated that more than 99% of *Cryptosporidium parvum* oocysts were inactivated following >60 sec HHP treatment. The use of HHP on fish for inactivation of helminths is currently investigated. While some of the control measures discussed exhibit efficacy against certain parasites, none of these procedures has been demonstrated to be as uniformly reliable as heat. Furthermore, such application is not suitable for small sized fish processors (SMEs) due to high cost.

Among the number of preventive measures, ozone is also suggested as a possible disinfectant for some parasites. Although successful results observed by some researchers on treatment of *Cryptosporidium parvum* in ozone (Peeters *et al.*, 1989; Gyürék *et al.*, 1999; Owens *et al.*, 2000; Orlandi *et al.*, 2002; Ortega, 2006), inactivation is dependent on several parameters including temperature, medium pH, and the amount of extracellular organic matter residing around the parasite; therefore, ozone is not found

suitable (Orlandi *et al.*, 2002).

Orlandi *et al.* (2002) suggested that irradiation serves as another possible measure for parasite control. However, its economic feasibility has to be determined. Besides, most consumers are reluctant to use irradiated food (Murrell, 2002). On the other hand, brining and pickling may reduce the parasite hazard in a fish, but they do not eliminate or minimize it to an acceptable level. Nematode larvae have been shown to survive for 28 days in the 80° salinometer brine (21% salt by weight) (FDA, 2001). This area is further discussed under section relating to brining.

Fish that contain parasites in their flesh may also contain parasites within their egg skeins, but generally not within the eggs themselves. For this reason, eggs that have been removed from the skein and rinsed are not likely to contain parasites. Trimming away the belly flaps of fish or candling and physically removing parasites are effective methods for reducing the number of parasites. However, they do not completely eliminate or minimize the hazard to an acceptable level (FDA, 2001). The only reference procedure for detection is visual inspection (EC, 2005). There is lack of fast and reliable techniques for identification of parasites in fish products.

Audicana and Kennedy (2008) pointed out that unfortunately, even dead *A. simplex* can cause allergic reactions to humans due to allergens left in the food. Therefore, the current preventive or control measures for a complete safety from *A. simplex* are not efficient. Several studies have concentrated on *A. simplex* antigen characterization and innate as well as adaptive immune response to this parasite over the last 20 years (Audicana and Kennedy, 2008). Aquaculture could have advantages over extractive fishing in supplying fish guaranteed to be free from *A. simplex* and related parasites. However, allergen traceability in feed is crucial; therefore, studies on the detection of this allergen in fish and fisheries products are future concern. Since it is difficult to trace allergen of *A. simplex*, current regulations have no limitations on such allergen although strict rules exist on live *A. simplex*. Recent regulations can only inform allergic people for such antigen by labelling.

**Table 3.** Conditions to kill some parasites using freezing and heating (Derived from Murrell, 2002; Huss *et al.*, 2003).

Preservative parameter	Parasite	Process variable	Time	Reference	
Freezing	Nematodes	- 20°C	24 hours	Huss <i>et al.</i> , 2003	
	Trematodes	<i>Clonorchis</i> & <i>Opisthorchis</i> metacercariae <i>O. felineus</i> metacercariae	-10°C	5 days	Murrell, 2002
			-28°C	32 days	Murrell, 2002
			-35°C	14 hours	Murrell, 2002
			-40°C	7 hours	Murrell, 2002
Heating	Nematodes	55-63°C	1 min	Huss <i>et al.</i> , 2003	
	Trematodes	<i>O. viverrini</i> free metacercariae	50°C	5 hours	Murrell, 2002
			70°C	30 min	Murrell, 2002
			80°C	5 min	Murrell, 2002

### 3. Evaluation of Health Hazard at Processing, Storage and Distribution Stages

Since each process type applies different preserving conditions, such as addition of salt decreases  $a_w$ , marination and fermentation decreases pH preventing certain pathogens, and heating kills parasites, the evaluation of such hazards is carried out under each type for TFPs. Prevention of physical and some chemical hazards can be controlled similarly in each type of processing methods as commonly known; therefore, they are not covered under this section either. In this section, hazards that vary according to processing methods in terms of health risk, preventive measures and monitoring procedures are evaluated.

#### 3.1. Salting

There are different types of salting methods. Some of them are combined with other traditional methods such as marinating, drying, smoking and fermenting. In this section, evaluation on and monitoring of hazards are discussed under two subsections as dry salting, and brining and others (such as injecting fish with salt etc.). The salt penetration will depend on fat content, temperature, amount of salt, salt composition, brine concentration, etc. (Mol and Özden, 2004; Codex Alimentarius, 2009). Therefore, risk and monitoring procedures for different health hazards depend on these conditions.

##### 3.1.1. Dry Salting

#### *Histamine and Other Biogenic Amines and Pathogenic Bacteria:*

Although some histamine-forming bacteria are reported to be halotolerant or halophilic, efficient, dry salting processes for fish products are unlikely to allow such bacteria to grow due to low  $a_w$  values. Köse *et al.* (2007a) and Üzen (2008) demonstrated that  $a_w$  of dry salted anchovies both at cold storage and ambient temperature were under 0.77. Most analysed dry salted commercial and home-made salted products also showed low  $a_w$  around 0.75 and salt content over 15% WPS (Köse *et al.*, 2007a; Köse *et al.*, 2009a; Köse, 2009). Such conditions are effective ways of preventing histamine formation and pathogenic bacteria at both room and cold temperatures during maturation and subsequent storage conditions. Some pathogenic bacteria and HFB can still be isolated from both salted and brined fish products (Köse *et al.*, 2008a; Üzen, 2008). Therefore, care should be taken after desalting. Veciana-Nogues *et al.* (1997) pointed out that biogenic amine formation can sharply increase if salted products would be desalted and packed in oil as applied in Spain. Practically, most producers including SMEs use enough salt to prevent above mentioned hazards. However, sometimes they use

larger piece of fish for salting which prevents salt uptake as fast as required. It should be noted that, fish must be eviscerated before salting due to possible risk for *C. botulinum* as mentioned in raw material section.

**Parasites:** Although research studies indicated that parasites can be eliminated at high salt concentrations for a short period of time, some of them can also be killed at low salt content. Murrell (2002) reported that dry salting is lethal for anisakids within ten minutes of direct contact. Therefore, marine fishes are usually safe for parasites. However, the appropriate inactivation technologies in freshwater fish are not well established. Some studies are to investigate the effect of salt in brined solution on *O. viverrini* and *Opisthorchis metacercariae* in fish. The varying concentrations from 0.9% to 30% were used and 10 days were enough to inactivate parasites in the lowest salt content. Conditions to kill or inactive some parasites in fish using salting are shown in Table 4 that can be used for monitoring purposes.

##### 3.1.2. Brining and Other Salting Methods

#### *Histamine and Other Biogenic Amines (BAs):*

Maturing brined fish products at room temperature present health risk for histamine and/or other BAs such as cadaverine, putrescine and tyramine if brining is carried out at 25% and lower salt concentrations (Karaçam *et al.*, 2002; Köse *et al.*, 2007a; Köse *et al.*, 2008a; Köse *et al.*, 2009a). Therefore, unless heavily salted brine (30% and over) is used, maturing and storage of the brined fish should be carried out at cold storage conditions until it reaches to consumer (prior to eating). Cold storage and brine (salt) together are very effective for controlling histamine formation. However, such prevention is only effective until certain period of time. Usually, products are safe within the sensory shelf-life especially for small sized fish such as anchovies (Köse *et al.*, 2008a; Köse *et al.*, 2009a; Köse, 2009).

Although cold storage conditions may be effective in preventing histamine formation within the sensory shelf-life in fish brined using <10% salt, some other BAs may still present health risk for such products (Köse *et al.*, 2008a; Köse *et al.*, 2009a; Köse, 2009). Therefore, testing histamine and other BAs to define a safe maturing period as well as allowing marketing shelf-life is advised. Time/temperature as well as salt content is the main parameters to be monitored during HACCP system. Using practical test kits for histamine measurements is also advisable. Some test kits are found to be reliable for testing histamine in salted products as well as other TFPs during EU funded project called TRUEFOOD (Köse *et al.*, 2007b; Köse *et al.*, 2008b; Köse *et al.*, 2009c). In the same project, it is also reported that TVB-N values reach unacceptable levels

**Table 4.** Conditions to kill or inactive some parasites in fish using salting

	Parasites	NaCl (WPS%)	Time to inactivation	Reference
Nematodes		4-5	>17 weeks	Karl <i>et al.</i> , 1995
		6-7	10-12 weeks	Huss <i>et al.</i> , 2003
		8-9	5-6 weeks	Huss <i>et al.</i> , 2003
		12-13	35 days (5 weeks)	Karl, 2008
		15	28 days (4 weeks)	Karl, 2008
		20	21 days	Karl, 2008
		21	28 days	Huss <i>et al.</i> , 2003
		22•	10 days	Murrell, 2002
	Dry salting		10 minutes direct contact*	Murrell, 2002
Trematodes	<i>C. sinensis</i>	30 (wt based)	8 days	Huss <i>et al.</i> , 2003
	<i>O. viverrini</i> (free metacercariae)	0.9%♣	10 days	Murrell, 2002
		10%♣	3.6 hours	Murrell, 2002
		20%♣	12 hours	Murrell, 2002
		30%♣	1 hour	Murrell, 2002
	<i>Opisthorchis</i> metacercariae in fish	13.6%♣	24 hours	Murrell, 2002

♣Percentage salinity in fermented fish.\*Anisakids, •Saturated NaCl.

for brined anchovies at the same time or before histamine levels reach to 50 ppm. Therefore, TVB-N analysis along with sensory analysis can also be used for monitoring histamine in brined products if such levels are previously confirmed for the particular brined fish products.

It is known that producers commonly add other ingredients such as lemon, herbs or antimicrobial agents either to alter product taste/aroma or to delay spoilage. However, slowing down the spoilage is not usually to decrease histamine formation since enzymatic actions (histidine decarboxylase) may still continue being active in the product. Moreover, decreasing pH either by adding lemon or due to lactic acid bacteria can enhance histamine formation. Although some of these types of brined products mixed with other ingredients should be classified under either marinated or fermented depending on the treatment, producers are usually not aware of such classification. Some producers also apply different processes to fish before brining such as boiling, smoking (lemon sauce herring), or dry salting (as in the case of processing Lakerda which is a Turkish and Greek origin product). In such cases, such additional preserving steps can help prevent histamine formation. For example, cooking can inactivate both HFB as well as decarboxylase enzymes.

#### **Pathogenic Bacteria:**

According to FDA (2001) guide and Huss *et al.* (2003), brined products containing >10% WPS are usually safe for most pathogens except *S. aureus* at both ambient and cold storage because of the level of  $a_w$ . It is difficult to control growth of *S. aureus* at temperatures over 7°C, especially at ambient temperatures if the salt content is lower than 20% WPS since such bacteria is reported to be active up to this level. However, toxin formation is limited to 10 % WPS. It is advisable to store/mature such products

at cold storage to prevent pathogen risk as well as histamine formation. Although low  $a_w$  is another possibility of preventing pathogen growth, it is less likely to drop  $a_w$  to the low levels for brined products. *L. monocytogenes* and *C. botulinum* (type E and non-proteolytic type B and F) are other pathogens that present health hazard even at cold storage conditions if the salt content is lower than 10 %WPS, especially under reduced oxygen packing (e.g., vacuum packing). To prevent health hazard for such low salt level, similar procedures suggested for monitoring pathogens mentioned in the sections for cold smoked or fermented products can be applied. Testing *S. aureus* is also advisable to be included in HACCP plan for verification purposes. Codex Alimentarius (2009) advised that salting process, including the temperature, should be sufficiently controlled to prevent development of *C. botulinum*, or the fish should be eviscerated prior to brining. GHP and GMP are also necessary prerequisite programs for preventing such hazards. As mentioned earlier, the processing of brined products may further contain some variations in different countries and even within the same country as reported by OECD (2008). These are applications of additives, antimicrobial agents or other additives that can prevent the growth of target bacteria. Therefore, it is advisable to carry out a safe shelf-life test for certain pathogens, particularly for *L. monocytogenes*.

#### **Parasites:**

Monitoring parasites using salt content is necessary if the products are not previously frozen or if they are not going to be cooked/or freezed during or after processing. Preventive measures that can be applied according to Table 4 as the critical parameters are salt percentage and time. Products containing salt content over 20% WPS are likely to be regarded as safe for parasites unlike lower concentrations, which

require more time to be kept in such salt concentrations before consumption. Therefore, storage at refrigerated conditions is advisable so that products can be stored for longer periods before marketing.

### 3.2. Fermented Fish Products (FFPs)

There are numerous types of fermented fish products (FFPs) processed in the world (Essuman, 1992; Saisithi, 1994; Hall, 2002; Köse *et al.*, 2007a; OECD, 2008). Most common ones are obtained from SE Asia (Saisithi, 1994). Essuman (1992) defined fermented fish as any fishery product which has undergone degradative changes through enzymatic or microbiological activity either in the presence or absence of salt. Traditionally, the term "fermented fish" covers both enzyme-hydrolysed and microbially fermented fish products; however, a clear distinction has not been made between these products (Huss *et al.*, 2003). Most FFPs involve salting and occasionally smoking, marinating and drying. Saisithi (1994) has revised FFPs under three categories. These are;

(i) products usually prepared from whole fish with the addition of salt to reduce  $a_w$  to prevent microbial spoilage (e.g., fish paste and fish sauce). The enzymes for the fermentation process come partly from the fish digestive system and partly from the bacteria that are naturally present in the fish and salt.

(ii) products prepared with different forms of fish (e.g. whole or sliced) with the addition of carbohydrates as substrate and salt. Fermenting microorganisms are known as naturally occurring microorganisms.

(iii) products similar to 2<sup>nd</sup> type except that starter cultures are added for fermentation.

#### **Histamine and Other Biogenic Amines (BAs):**

Østergaard *et al.* (1998) pointed out that the fermentation process for fish may fulfill the conditions required for abundant formation of BAs, such as availability of free amino acids, presence of decarboxylase-positive microorganisms, and conditions allowing bacterial growth, which are decarboxylase synthesis and decarboxylase activity. Although few food poisoning outbreaks have been attributed to FFPs, high levels of histamine and other BAs have been detected by several researchers suggesting that such cases may have not been reported. Other possible reason may be that some of these products, especially fish sauce, are consumed in small amounts as an ingredient with/or side dish along with other dishes that is likely to reduce the risk. Therefore, the EU allows higher histamine levels as 200 ppm (2 samples/9 as 400 ppm) in FFPs compared to other fish products. However, FDA does not make any exception for such products and permitted level for histamine is even lower than the

EU-permitted amount for normal fisheries products. Karnop (1988) demonstrated that *Pediococcus halophilus*, is often isolated and present in FFPs, is able to produce histamine during long storage at ambient temperatures of 20-25°C (cited in Essuman, 1992). Tapingkae *et al.* (2009a) pointed out the difficulty of preventing histamine in Thai fish sauce. It was demonstrated in a recent study that despite high levels of putrescine were obtained from one sample stored at ambient temperature, lower levels of histamine were detected in European FFPs (Köse *et al.*, 2008a). Due to the EU restrictions, such products are usually produced and stored at cold storage conditions in the EU countries. On the other hand several studies indicated high histamine levels in different FFPs originated from other regions, especially S. East Asia (Essuman, 1992; Mah *et al.*, 2002; Tsai *et al.*, 2006; Mah *et al.*, 2009). Therefore, processing methods of FFPs and their country of origin have a great effect on the safety of such products in relation to BAs. Mah *et al.* (2002) demonstrated that the levels of 7 BAs including histamine in 8 *Jeotkal* samples were in the range of 0–70 mg/kg and these levels changed little for a period of 20 days at 4, 10 and 15°C. This result indicates the effect of low temperature storage on preventing BAs in such products. However, they observed that levels of cadaverine, histamine and spermidine were significantly high in *Myeolchi-jeot* which is another type of fermented fish and increased considerably during storage for longer than 10 days at the same temperatures after processing. The reason may be attributed to their methodology and poor handling during processing and raw material step. Mah *et al.* (2009) described that *Jeotkal* contains large amounts of precursor amino acids. Rabie *et al.* (2009) also reported high histamine as well as other BA levels in salted-fermented fish (*Feseekh*) and they demonstrated that amine levels increased during ripening period of the product.

Tsai *et al.* (2006) reported high levels of histamine and BAs in 27 FFPs (fish sauce, fish paste and shrimp waste) from Taiwan. The average amount of 8 different BAs was less than 90 ppm but average histamine content was 394 ppm in fish sauce, 263 ppm in fish paste. They observed that most of the samples had histamine levels greater than the levels in FDA guideline, while 7 of them contained >500 ppm of histamine. These results are also well above the EU permitted levels of 200 ppm for fermented fish indicating the safety concern for FFPs.

The main reasons of having difficulty in preventing histamine in FFPs are its fermenting temperature, which is carried out at ambient conditions and processing techniques involving bacterial enzymes originated from fish. Due to difficulty in preventing histamine formation in most SE Asian FFPs, especially in fish sauce, several studies are concentrated on decreasing histamine

levels using inhibition effect of additives or using other degradative compounds such as enzymes. Mah and Hwang (2009b) studied the inhibition effect of *Staphylococcus xylosus* on BAs including histamine in salted and fermented anchovy (*Myeolchi-jeot*). They found that *S. xylosus* possessed capability to degrade histamine and tyramine as 38 and 4.4%, respectively within 25h in a phosphate buffer. They observed a reduction in overall production of BAs as 16.0% in real samples. The same researchers carried out a recent study on the same type of product on the inhibitory effects of garlic and other spices (Mah *et al.*, 2009). They found that overall production of BAs was reduced by up to 8.7%, compared to control samples. Another study of same authors (Mah and Hwang, 2009a) on a similar subject was more successful in reducing histamine in similar products. They observed that addition of glycine in culture reduced putrescine, cadaverine, histamine, tyramine and spermidine by 32.6%, 78.4%, 93.2%, 100.0% and 100.0%, respectively. In a similar study, during the ripening of *Myeolchi-jeot*, it was observed that overall BAs were reduced by down to 63.0% and 73.4%, in samples prepared with 0% and 20% NaCl, respectively. Tapingkae *et al.* (2009a) have reported a histamine dehydrogenase enzyme purified from *Natrinema gari* BCC 24369. The authors performed a study to investigate the effect of this enzyme on degrading histamine in Thai fish sauce (Tapingkae *et al.*, 2009b) and observed a degrading activity of 70%.

The antimicrobial effects of clove, cinnamon, cardamom, turmeric and pepper at a level of 3% on histamine production and histidine decarboxylase activity of *Morganella morgani* (a main HFB in fish) in mackerel have been investigated (Nilsson and Gram, 2002). Clove and cinnamon showed a significant inhibitory effect on histamine, putrescine and tyramine.

Cold storage is recommended for monitoring histamine formation for FFPs. However, as explained before, there are numerous types of FFPs around the world. Some of them require ambient temperature for maturation according to their procedure. Therefore, it is rather a complex issue to monitor the formation of BAs in such products. The recommended monitoring approach should include prior histamine testing to identify critical limit for time/temperature and salt content that can suit for different companies according to their processing methods. After that, histamine testing is vital to be included in their HACCP plan along with other parameters. Köse *et al.* (2009c) investigated the suitability of some current commercial histamine test kits for TFPs of the EU origin and some were found as effective in testing histamine in such products.

#### **Pathogenic Bacteria:**

TFPs are usually under risk for pathogenic halophilic bacteria. Huss *et al.* (2003) reported that natural presence of pathogenic bacteria from the

aquatic and general environment is not considered as a significant hazard in FFPs due to their low numbers. Most important limiting factors are salt content,  $a_w$  and pH of the products. However, conditions for growth of some pathogens (*C. botulinum* type A and B, *L. monocytogenes*, *Vibrio* sp.) are good until the pH decreases to near 4.5 which takes about 1-2 days at 30°C in a natural fermentation. Rapid and adequate acidification is the preventive measure for this significant hazard. All types of *C. botulinum* are inhibited by salt content of 10-12% and a pH below 4.5. *C. botulinum* types E, F and non-proteolytic type B are able to grow between 8°C and 10°C but are inhibited below 4°C (Essuman, 1992). Meyers *et al.* (2007) reported the case of foodborne botulism from home-prepared fermented tofu in California in 2006. On the other hand, Essuman (1992) reported that toxins of micro-organisms such as *C. botulinum* are inactivated by the proteolytic enzymes in fish fermentation process.

Contamination of FFPs with pathogenic bacteria from the animal/human reservoir and with pathogenic virus is a potential hazards which can be controlled by GHP. It is well known that lactic acid bacteria (LAB) used in fermentation have inhibitory effect on pathogenic bacteria due to their production of bacteriocins, probiotic and other inhibitory effects such as production of acid that reduces pH of the product and hydrogen peroxide and carbondioxide. This subject is well reviewed by Hall (2002). Among bacteriocins; nisin, pediocin and reuterin are reported to be active against *B. cereus*, *C. botulinum*, *S. aureus*, *L. monocytogenes*, *S. typhimurium* and *Shigella* spp. (Hall, 2002). Østergaard *et al.* (1998) showed that many strains of LAB isolated from Thai FFPs (plaa som, som fak and hoi dorgn) had an inhibitory effect on *L. monocytogenes*, *Vibrio cholera* and *V. parahemolyticus* as well as some spoilage bacteria such as *Aeromonas* spp. Most isolated strains were *Lactobacillus* spp., and grew well at ambient temperatures (25–37°C) and tolerated up to 6.5% NaCl. Petäjä *et al.* (2000) demonstrated that fermentation with LAB can produce storable and microbiologically safe cold-smoked fish products. Past studies may indicate effective inhibitory effects on certain pathogens. Hall (2002) indicated that use of LAB fermentation in combination with other preservative techniques such as modified atmosphere packaging (MAP) and vacuum packing can contribute to the control of microbial safety and quality.

Huss *et al.* (2003) suggested that for complete safety, temperatures during fermentation should be kept at <10°C until final pH has been reached. There is a possible risk of *C. botulinum* type E of aquatic origin if large fish (over 12 cm) are salted ungutted. Therefore, gutting and cutting fish into smaller pieces are advisable for such fish. Efficient salting techniques that provide uniform application in short time can also contribute benefits in preventing pathogenic bacterial growth and toxin formation.

**Parasites:**

Parasites are one of the other main health risks for FFPs since they are usually consumed without cooking. If freezing and cooking are not going to be used for such products, salting seems to be the unique alternative to prevent parasites in fermented products. Similar monitoring given for brined fish products can also be applied for these products. If such monitoring is not suitable, labelling guidance should be given either for freezing or cooking before consumption. Huss *et al.* (2003) reported that *Opisthorchis* metacercariae in fermented fish that contains 13.6 % salt was inhibited in 24 h, while *O. viverrini* metacercariae in fermented fish containing 20% (wt based) salt was eliminated in 5 h.

**3.3. Marinating**

Marination process involves addition of acid (acetic acid, usually vinegar) and salt to the product. There are two main types of marinating, (i) cold or salt marinating and (ii) warm marinades prepared from pre-fried, pre-cooked or pre-smoked fish. Cold marinades are extensively used since they keep well and it is easier to prepare them (Zaitsev *et al.*, 2007). It has been reported that acetic acid or acidity in general stimulates the bacteriostatic and bacteriocidal action of salt, while salt stimulates the action of acetic acid. Marinated fish are kept in solutions containing 6-18% salt and 0.3–2% acetic acid. In Western Europe, marinades are more acidic, with concentrations up to 6% for the initial solution (Zaitsev *et al.*, 2004). Özden and Varlık (2004) reported that the activity of spoilage bacteria is inhibited at pH 4-4.5. This pH is also used for safety regulations for such products since it also prevents most pathogenic bacteria growth or toxin formation. Zaitsev *et al.* (2004) suggested that marinades are best matured at around 0°C. They also estimated maturation time between 10 to 30 days depending on salt and vinegar concentrations, and on the ripeness of the fish before marinating. Marinades can be prepared from a variety of fish species, with different sizes and shapes such as filleted, headed and gutted. Cold marinades may be prepared from both fresh and salted fish. Fresh fish is usually considered to give pleasanter taste, but the better opportunities offered today for processing light salted intermediate products have led to the bulk of marinades to be prepared from lightly cured fish (Zaitsev *et al.*, 2004). Marinated products are considered as lightly preserved fish products (Özkan and Varlık, 2004) with a short shelf life and must be kept in cold storage during processing until consumption.

**Histamine and Other Biogenic Amines (BAs):**

In the context of the TRUEFOOD Project studies, Köse *et al.* (2007a) and Köse *et al.* (2009a) reported that histamine levels were low in different commercial marinated products that are kept at cold

storage conditions (0-4°C). Olgunoğlu (2007) also supported these results with his study, which were carried out on marinated anchovy stored at cold temperature. Zaitsev *et al.* (2004) reported that to obtain a good quality product, lightly salted raw material should be used if marinades are prepared from pre-salted raw material. In these cases, histamine formation might occur if long maturation and/or storage at elevated temperatures are applied. Gökoğlu (2003) demonstrated that histamine and some other BAs in two different marinated sardine reached to unsafe levels within a day during maturation at ambient temperature (25±2°C). Several factors are reported contributing histamine development in marinated fish products (Gökoğlu, 2003). They may be summarized as follows: (i) amino acid decarboxylase activity is higher in an acidic environment (the optimum pH being between 4.0 and 5.5) and bacteria are more strongly encouraged to produce decarboxylases, as a part of their defense mechanisms against the acidity. (ii) acidic conditions of marinades make the tissue cathepsins more active resulting in the degradation of some muscle proteins into peptides and amino acids which are the precursors of amine formation in the presence of amino acid decarboxylase-positive microorganisms. Therefore, cold storage below 2°C is advised and commonly used for seafood processing companies.

**Pathogens:**

As mentioned before, low pH (<4.5) and high salt content (>10% WPS) can prevent most pathogens such as *C. botulinum*. On the other hand, *V. parahaemolyticus*, *L. monocytogenes* can grow at minimum pH of 4.4 and at maximum salt content of 10% WPS. Minimum toxin formation pH for *S. aureus* is 4 and salt content is 10%. Although *S. aureus* can be prevented at low temperatures <10°C, it is difficult to prevent *L. monocytogenes* due to its ability to grow at cold storage conditions (0-2°C). Therefore, salt content of such products is vital to monitor and it is advisable to use salt content of 12% as critical limit. However, such amount of salt may not be suitable for some customers around the world. Therefore, several additives are added to marinated products, some of which are added for preservation purposes, such as sodium benzoate, sorbate, nitrate and some of which are used for taste, flavor and preservation purposes. These are herbs, sugar, olives, ginger and vegetable leaves. Although numerous researchers proved the effect of several preservatives in preventing risk of pathogenic bacteria (Nilsson and Gram, 2002; Lai and Roy, 2004; Lin *et al.*, 2004; Zaika, 2007), except few, their amount and application procedures are not standardised for all types of marinated or other lightly preserved fish products. Therefore, preventive measures of such preservatives should be used with caution in the HACCP plan.



FDA guidelines suggest (FDA, 2001) that controlling *C. botulinum* for refrigerated pickled fish in ROP pack can be carried out by following applications (i) adding sufficient salt to produce a 5% WPS, (ii) adding sufficient acid to reduce the acidity (pH) to 5.0 or below, (iii) reducing the amount of moisture that is available for growth  $a_w$  to below 0.97 (e.g., by adding salt or other substances that "bind" the available water), or (iv) making a combination of salt, pH, and/or  $a_w$  adjustments that, when combined, prevent the growth of *C. botulinum* type E and nonproteolytic types B and F. FDA (2001) also pointed out that these control measures are not sufficient to prevent toxin formation by *C. botulinum* type A and proteolytic types B and F. Therefore, strict refrigeration control (i.e. at or below 40°F [4.4°C]) during storage and distribution must be maintained to prevent growth and toxin formation of relating types of *C. botulinum*. Huss *et al.* (2003) grouped marinated fish products under either lightly preserved fish or semi-preserved fish according to salt percentage or pH level of the products. They pointed out that pathogens originated from general environment are usually present in low numbers in such products, and with efficient GHP and storing fish products under 10°C can prevent such pathogens. However, growth of *L. monocytogenes* in lightly preserved fish (WPS <6% and pH >5.0) cannot be prevented with the applied salt and acidity conditions. Therefore, alternative solution is to reduce shelf life of the products to a period of no growth of *L. monocytogenes*. The length of this period needs to be established by experimentation.

#### Parasites:

Parasites are the most difficult hazards to monitor in marinated fish products since acidic pH conditions are not usually effective in preventing parasites, especially anisakid nematodes (Murrell, 2002). Although, some studies indicated inactivation of some parasites in acid conditions as given in Table 5, acid prevention was not found effective for nematodes. Murrell (2002) also reported that as concentration of salt in the product dropped, the time required to kill the nematodes increased. Under 15% salt and 7% acid conditions, 97% of worms were killed after 30 days while it took 70 days for 6% salt and 4% acid conditions. Since these products have

low salt content and short shelf life, parasite risk is high for products if they are not previously cooked or frozen. Therefore, freezing raw material seems to be the best way to prevent parasite risk in marinades since these products are usually consumed without cooking.

#### 3.4. Smoking

Two methods of smoking have to be distinguished, which are cold and hot smoking. There are also variations within these types (Zaitsev *et al.*, 2004) as well as other types such as electrostatic smoking where fish is firstly dried, smoked and then cooked. Stolyhwo and Sikorski (2005) divided smoking into three categories according to temperature of the smoke applied. They reported that the temperature of the smoke is in the range of 12–25°C during cold-smoking and 25–45°C in warm-smoking. In hot-smoking, the process may be carried out in different stages, during which the temperature of the smoke ranges from about 40–100°C and that in the centre of the product, temperature may reach up to 85°C. Various pre-treatments prior to smoking such as salting and drying and/or after treatments e.g., cooking, marinating are applied in the industry. A number of authors have been reviewed both for home-made and industrial fish smoking methods with a wide range of products (Horner, 1997; Erkan, 2004; Zaitsev *et al.*, 2004). Other than its acquired taste and flavor, smoking has known to have great preservative effect. Horner (1997) pointed out that the combined preservative effect of smoking arises from 4 main factors. These can be summarized as;

- Surface drying providing physical barrier to bacterial pathogens and preventing aerobic microbial proliferation,
- Salting decreases water activity and has inhibition effect on pathogenic bacteria (although a certain salt content is required),
- Deposition of phenolic antioxidant substances delaying autoxidation (and rancidity),
- Deposition of antimicrobial substances such as phenols, formaldehyde and nitrites.

Heat can also be added as a preservative factor, especially for hot-smoked fish products in killing some pathogens, HFB and parasites. Additionally, packaging methods such as MAP and vacuum packaging, and storage conditions such as freezing can also help as preventing food safety hazards in smoked products. However, vacuum packing can also create bacterial health hazard if not applied appropriately. Various salt contents were suggested for salting fish prior to smoking. Horner (1997) reported that 2-3% salt content in the fish is the maximum salt content required if the product has to be eaten as a main dish rather than a condiment. Zaitsev *et al.* (2004) suggested salt content of 1.8-2 % although some products may contain higher salt contents for such products.

**Table 5.** Inactivation of free *O. viverrini* metacercariae in pickled fish products (Murrell, 2002).

Acid Type	Acid concentration	Inactivation time
Commercial vinegar	4%	1 h
Acetic acid	4%	1.5 h
Lactic acid	4%	1.5 h
Citric acid	4%	1 h

### **Histamine and other biogenic amines (BAs):**

There have been several reports on high histamine levels in smoked fish products (SFPs) which exceeds either the FDA or EU-permitted levels (Lehane and Olley, 2000; Rauscher-Gabernig *et al.*, 2009). However, Lehane and Olley (2000) indicated that hot smoking 'practically sterilizes' the product and denatures enzymes, imparting some degree of preservation, but does not destroy histamine already formed. Therefore, the presence of histamine in hot SFPs is usually associated with poor handling of raw material when time/temperature abuse usually occurs. On the other hand, such products are susceptible to histamine production if recontamination occurs due to poor handling during packaging and storing. Emborg *et al.* (2005) reported high histamine concentration of >7000 mg in vacuum packed tuna which caused histamine fish poisoning and they linked high concentration with the growth of psychrotolerant *Morganella morganii*-like bacteria or with *Photobacterium phosphoreum* in vacuum packed fish. A number of the HFB are facultative anaerobes that can grow in reduced oxygen environments (Tsai *et al.*, 2007; Mah and Hwang, 2009a). Since such bacteria can also grow at refrigerated temperatures, vacuum packing of both cold and hot SFPs are at risk for histamine forming if GMP/GHP are not applied. Preventive measures for controlling pathogens in SFPs are also usually efficient to prevent histamine formation in such products. For verification purposes, histamine testing prior to processing and after processing at defined times is advised. Jahncke (2008) advised the most effective methods of preventing biogenic amines formation as handling and processing under sanitary conditions, rapid cooling of the fish, and continued refrigeration from harvest through consumption.

### **Pathogens:**

Despite the antimicrobial effect of smoking, *C. botulinum* and *L. monocytogenes* are the main pathogenic bacteria associated with SFPs, particularly for cold smoked and reduced oxygen packed products (ROP) i.e. vacuum and MAP products (FDA, 2001; Huss *et al.*, 2003; Losikoff, 2008; Janchke, 2008). Rarely, other pathogenic bacteria such as *S. aureus* are also involved in smoked fish (Huss *et al.*, 2003; Himelbloom, 2008). Anaerobic conditions can also occur in air packed products when fillets overlap each other or aerobic spoilage bacteria consume oxygen present in the pack. Although *C. botulinum* type A, B and F (proteolytic types) are reported to grow at salt concentration up to 10% salt (WPS) and minimum temperature is 10°C, the type E, and nonproteolytic B and F are able to grow at 5% salt (WPS) and at a minimum temperature of 3.3°C (FDA guide, 2001). Losikof (2008) has reviewed the botulism cases for SFPs. She indicated that although there is low incidence of disease, the mortality rate is high if the

symptoms are not treated immediately. Janchke (2008) has reviewed processing parameters to control *C. botulinum* in particularly smoked fish and identified 4 main control measures to control *C. botulinum* growth and toxin production in ROP products. These are (1) a minimum concentration of 3.5% WPS in the thickest part of the fish, or a combination of at least 3% WPS and a nitrite level of 100-200 ppm is necessary (2) packages containing refrigerated cold SFPs should be labelled as 'keep refrigerated at 40°F (4.4°C) or below'; (3) packages containing smoked frozen fish should be labelled as 'keep frozen until thawed at refrigeration temperatures and shall not be refrozen' and (4) products should not be packaged in ROP by the retailer. The US require that vacuum packed cold smoked fish must contain 3.5% NaCl (WPS) or 3% if combined with 100-200 ppm nitrite (only for sablefish, salmon, shad, chub, and tuna). For air packaged fish, not less than 2.5% NaCl (WPS) in the loin muscle is required (FDA, 2001; Huss *et al.*, 2003; Janchke, 2008). However, the EU does not allow nitrite in the food products (Jahncke, 2008).

FDA (2001) has outlined detailed guidelines for monitoring *C. botulinum* along with other pathogens in cold and hot SFPs. According to the guideline, for both cold-smoked and hot-SFPs, the temperature of heating/smoking is critical. The heating/smoking step for hot-smoked fish must be sufficient to damage spores and make them more susceptible to salt inhibition. The smoking step for cold-smoked fish must not be so severe that it kills the natural spoilage bacteria. These bacteria are necessary so that the product will spoil before toxin production occurs. It is likely that they will also produce acid, which will further inhibit *C. botulinum* growth and toxin formation. Sikorski *et al.* (1998) pointed out that GMP for hot smoking of fish required a heating regime sufficient to keep the center of the thickest part of the fish, which was at 82°C for 30 min, when the water phase of the muscle contains 3.5% NaCl. At 5% NaCl, the temperature may be reduced to 65.5°C, while at 3.5% NaCl<sup>+</sup> 200-300 ppm NaNO<sub>2</sub>, temperature may be reduced to 71°C. FDA (2001) also pointed out the importance of WPS% level as a critical parameter for the safety of the product. Salt and nitrite (if permitted) are the principal inhibitors for *C. botulinum* type E and nonproteolytic types B and F toxin formation in these products. the EU considers smoked fish as ready to eat (RTE) food commodity and requires that WPS% for MAP must be equal to or greater than 3.0% (EC, 2005).

*B. cereus* can grow and form toxin at salt concentrations of 18%. Therefore, in these products, storage temperature of finished product must be controlled (FDA, 2001). It was reported that *V. parahaemolyticus* could multiply to significant numbers in cold-smoked fish. Thus, the hazard associated with food poisoning via smoked fish



should be considered in areas where the fish may recontaminated with this organism (Sikorski *et al.*, 1998).

Himmelbloom *et al.* (2008) demonstrated that pellicle formation and inactivation of *L. monocytogenes* generally present health risks for SFPs and isolation of *Listeria* in such products is widely reported around the world (Huss *et al.*, 2003; Nakamura *et al.*, 2004; Vaz-Velho *et al.*, 2006; Himmelbloom *et al.*, 2008; Jahncke, 2008). Himmelbloom *et al.* (2008) pointed out that prevalence of *L. monocytogenes* is valid more often and at higher proportions for cold-smoked fish samples than for hot-smoked fish. The reason was given as the raw nature of cold-smoked fish, which retains viable bacterial cells that have entered the process at susceptible steps. The mild temperatures (<30°C) and low salt concentrations (<5% WPS) used in the cold-smoked processing of fish are also not sufficient to inactivate *L. monocytogenes*. The growth of the organism may occur in the product during storage and cold smoked fish does not undergo cooking before consumption (Vaz-Velho *et al.*, 2006). Sikorski *et al.* (1998) pointed out that in a smoked product, *Listeria* may stem from the original bacterial population, which survive after the treatment applied during smoking. It may also be introduced as the result of post processing contamination, especially during slicing of the product. Wiedmann and Gall (2008) reported that *L. monocytogenes* is often isolated in seafood smoking plants, and this pathogen may be introduced into processing plants through a variety of routes, including raw materials, employees, and equipment. Since *L. monocytogenes* tend to form a biofilm, which enhances its survival, it is difficult to eliminate this hazard with general cleaning and common sanitizing procedures. They also pointed out that implementing an effective *Listeria* control program is a long-term commitment and suggested at least five key elements necessary for an effective control program for RTE seafood products like smoked fish. These are (i) *Listeria* specific GMP and sanitation procedures (ii) employee training (iii) environmental microbiological monitoring and testing

(iv) raw material controls, and (v) controls to minimize growth in the finished products.

Since heat applied during cold smoking procedures is not sufficient to kill *Listeria*, the preventive measures must be applied starting from raw material through all steps of processing. Although temperature used for hot smoking is sufficient to kill *Listeria*, such products are susceptible to recontamination after smoking. Therefore, control efforts should focus on preventing contamination after smoking (Wiedmann and Gall, 2008). Due to the conflict between the EU requirement and FDA concerning preventive measures for *C. botulinum* in terms of %WPS requirement (since the EU customers prefer less salty products), Codex Alimentarius suggested a different approach preventing *C. botulinum* in SFPs (Table 6).

Although there are several successful studies in the literature concerning the prevention of this pathogen in smoked products, where bioprotective cultures ozone, irradiation, HHP are used (Nilsson *et al.*, 1999; Dufes *et al.*, 1999; Vaz-Velho *et al.*, 2006), such preventive measures should be applied in caution since the efficiency of such applications are affected by several factors such as intrinsic food characteristics. Although the US requires zero tolerance for *L. monocytogenes* in SFPs. EU allows <100 cfu/25 g for RTE although it allows zero for infants or immune-compromised persons. *Salmonella* presence is not acceptable for regulatory bodies of most countries including US and EU countries (FDA, 2001; EU, 2005).

#### Parasites:

If hot smoking is used, temperature is usually effective to prevent parasites. Care should be taken to ensure that the product reaches an appropriate temperature for a sufficient period (Murrell, 2002). For cold SFPs, fish must be either frozen before or after processing (as packed). Using salt as preventive measure for cold smoked products can be difficult if the salt content and time are not adequate for a safe shelf-life. EU and US require zero tolerance for alive parasites in smoked products. EU also requires that

**Table 6.** Recommendations of Codex Alimentarius to prevent *C. botulinum* and several other pathogens in smoked fish products (Modified from Hansen, 2008).

Storage temperature	Packaging type	WPS (%)	Comments
0°C to 3°C	Any	No minimal limitation	Temperature monitoring on each package
3°C< to 5°C	Aerobic packaging	No minimal limitation	Storage temperature is for general pathogen control and quality. Sensory signs indicate spoilage
Frozen (≤ -18°C)	ROP or VAC/MAP	No minimal limitation	<i>C. botulinum</i> cannot form-need label information when thawed
5°C≤	ROP or VAC/MAP	3.0 to 3.5% selected by country where product is consumed	Applied WPS will delay/prevent toxin formation
5°C< to 10°C	ROP or VAC/MAP	5%	Non-proteolytic <i>C. botulinum</i> is controlled

cold SFPs must be held frozen at  $-20^{\circ}\text{C}$  for not less than 24 h to control alive parasites (EC, 2004; Hansen, 2008).

#### **Polycyclic Aromatic Hydrocarbons (PAH):**

PAH contents in fisheries products which are contaminated from natural environment are usually low since fish have the ability to oxidize and further metabolize PAHs to water-soluble compounds that are excreted by the living organism. On the other hand, the wood smoke used in smoking of fish may contain, a large variety of PAHs, including the most carcinogenic ones depending predominantly on the temperature of generation. Among PAHs, Benzo(a)pyrene (BaP which is 252 Da) is known to be very mutagenic and carcinogenic and; therefore, has been accepted as a marker of carcinogenic PAHs in wood smoke and smoked products as well as environmental samples (Yean *et al.*, 1998; Stolyhwo and Sikorski, 2005). Stolyhwo and Sikorski (2005) well reviewed PAH issues in SFPs and pointed out that smoking under mild conditions, in modern smokehouses supplied with filtered smoke from external generators, does not lead to significant contamination of the products with carcinogenic PAHs. The contents of BaP in the meat of hot-smoked fish are, on average, not higher than the limit set by different national and European regulations. However, heavily smoked products from traditional kilns, particularly the outer parts of such commodities, may contain up to about 50  $\mu\text{g}$  BaP/kg wet weight.

To prevent the health risk relating to PAH, it is advised to use traditional kilns. It has been reported that BaP is not formed if the temperature of wood pyrolysis in a two-stage smoke generator is below  $425^{\circ}\text{C}$  and if oxidation of the volatile products of pyrolysis is below  $375^{\circ}\text{C}$ . Therefore, by lowering the temperature of the smouldering pile of wood shavings or sawdust to  $300\text{--}400^{\circ}\text{C}$  and using filters, the content of PAH in the smoke can be decreased about 10-fold (Stolyhwo and Sikorski (2005). Therefore, it is also wise to use lower smoke generating temperatures to decrease the health risk. the EU required limit of PAH for smoked products is 5 mg/kg (Hansen, 2008). It has been reported that finished products can be contaminated with PAHs via other ingredients such as vegetable oil (Stolyhwo and Sikorski (2005). Therefore, such ingredients should also be monitored for a reliable source if they are used in the process of SFPs.

### **3.5. Drying**

Drying after salting is one of the oldest ways of preserving fish, which is simple, traditional, does not require complicated equipment, and gives a product with a long storage life. Both mechanical and natural drying methods are utilized (Zaitsev *et al.*, 2004). Although most dried products include dehydrated fish which needs to be soaked and cooked before use

(Zaitsev *et al.*, 2004), some products can be eaten without further cooking as described by Özden (2004). Various countries may differ in the species of fish dried and in the methods of handling and pre-drying treatment employed but the essential preservation process is practiced by the reduction of moisture to a safe level with application of either sun or dry air.

Horner (1997) described drying techniques under three categories; (i) air or contact drying, (ii) vacuum drying and (iii) freeze drying. Doe (2002) categorized dried fish products into 'fully dried' and 'partly dried' products. The former have been dried until their moisture content is close to uniform and  $a_w$  is close to or below 0.75 and have a shelf-life between one week and several months. Partly dried fish have a shelf-life of up to one week and are usually kept refrigerated before consumption. A number of dried fish products (DFPs) are described by Doe (2002), Özden (2004) and Zaitsev *et al.* (2004).

#### **Health Hazards:**

Due to low moisture content of such products,  $a_w$  is also expected to be low for such products. As mentioned before,  $a_w$  directly affects bacterial growth of pathogens as well as histamine formers. Therefore, histamine poisoning presents low risk for DFPS. Although some reports indicated the involvement of histamine poisoning with DFPS (Tsai *et al.*, 2007) such involvement is suspected to occur due to temperature abuse of raw material. Pathogen risk also present low risk for products especially salted-dried fish products if  $a_w$  is below 0.8. On the other hand, dried products are susceptible to humid conditions and can easily absorb water if humidity level is high. Therefore, such products must be stored at low humid conditions that are suitable for product's  $a_w$  or vacuum packed in order to avoid environmental conditions. However, GMP and GHP must be applied efficiently to provide safe products. For monitoring purposes, measuring  $a_w$ , moisture content, relative humidity of air, time and temperature during drying and storage period are necessary. Doe (2002) suggested reducing  $a_w$  to 0.91 within 48 hours in order to prevent maggot infestation so as to prevent quality loss during processing. Such aim will also help to slow down the growth of pathogenic bacteria although much lower  $a_w$  is advisable for products that are not previously treated with adequate salt or with other preventive measures such as boiling/cooking.

Health risk of parasites has not been studied extensively for DFPS due to its their low risk level since such products are usually cooked before eating or heavily salted before drying. Therefore, parasite health risk is low for DFPS. However, some DFPS are known to be consumed without further cooking and salt content is not efficient to prevent parasite risk. Murrel (2002) reported that dry salting is lethal for anisakids within ten minutes; therefore, dry salting method is advisable to use such products to eliminate

parasite risk since acid conditions are not efficient to kill for such parasite. Freezing, heating or adequate combination of salt content and storage time can be used as treatment procedures for killing living parasites in dried or salted-dried products.

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