# Processing Parameters Needed to Control Pathogens in Cold-Smoked Fish

A Report of the Institute of Food Technologists
for the Food and Drug Administration
of the U.S. Department of Health and Human Services

submitted March 29, 2001

IFT/FDA Contract No. 223-98-2333
Task Order 2

# Table of Contents

Additional copies of this issue of *Journal of Food Science*, which includes this supplement, are available while supplies last. Send check or money order (U.S. dollars, U.S. bank) for $30 made out to "Institute of Food Technologists", to the IFT Customer Service Department, 221 N. LaSalle St., Suite 300, Chicago, IL 60601. The information in this supplement is also available for ***free*** at the IFT Website (www.ift.org) in PDF format, and at the FDA Website (www.fda.gov) in HTML format.

## SCIENTIFIC AND TECHNICAL PANEL

### Panel Chair and Senior Science Advisor

**Frank F. Busta, Ph.D.**
University of Minnesota

### Panel Members

**Gleyn E. Bledsoe, Ph.D., C.P.A.**
Northwest Indian College

**George J. Flick, Jr., Ph.D.**
Virginia Polytechnic Institute and State University

**Lone Gram, Ph.D.**
Danish Institute for Fisheries Research

**Daniel Herman, M.S.**
National Fisheries Institute

**Michael L. Jahncke, Ph.D.**
Virginia Tech and
Virginia Seafood Agricultural Research and Extension Center

**Donn R. Ward, Ph.D.**
North Carolina State University

# Preface

On September 30, 1998, the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services signed a five-year contract with the Institute of Food Technologists (IFT) to provide scientific review and analysis of issues in food safety, food processing and human health. Under the terms of the contract, FDA assigns IFT task orders, categorized as comprehensive or abbreviated reviews. IFT assembles Scientific and Technical Panels comprised of experts in the topic area to address the issues. The panels are charged with providing scientific and technical review and analysis, not with setting policy.

This report is IFT's response to Task Order #2: Processing Parameters Needed to Control Hazards in Cold-Smoked Fish. The Background and Scope of Work that FDA provided to IFT are included. In October 1999, IFT assembled a Scientific and Technical Panel. This panel was comprised of experts in food microbiology, HACCP, and seafood microbiology. The panel met in person and via conference calls throughout 2000 and into 2001. IFT also consulted with the Science Advisory Board that advises IFT on the FDA contract and on the individual task orders

The Institute of Food Technologists greatly appreciates the efforts of the Scientific and Technical Panels, the Science Advisory Board, the many reviewers, staff and others who made this report possible. Compensation for such an effort pales in comparison to the time, effort and expertise expended.

IFT is especially grateful to the FDA staff for their tremendous cooperation, communication and assistance at every stage of this project. IFT submitted this report to the agency in the hopes that the report makes a contribution to the understanding of the methods to control public health hazards that may derive from the consumption of cold-smoked fish.

# Background Provided by FDA to IFT

For centuries, smoking has been a popular way to preserve fish. The applications of salt, smoke and, in some products, nitrate imparts a characteristic texture and flavor that is enjoyed throughout the world. With the advent of refrigeration, these products now contain less salt and smoke and have higher concentrations of moisture. In addition, packaging systems such as vacuum packaging with high barrier films have extended shelf life.

The recent outbreak due to *Listeria monocytogenes* in hot dogs has prompted the Agency to evaluate the ready-to-eat products under its jurisdiction such as smoked fish as a potential source of this foodborne pathogen.

*L. monocytogenes* is a nonsporeforming, psychrotrophic bacterium that causes the disease, listeriosis. In humans, the primary manifestations of listeriosis are meningitis, abortion and prenatal septicemia. Immuno-compromised individuals, pregnant women and infants are most at risk. The estimated annual incidence of foodborne listeriosis in the United States is 1850 cases and 425 deaths. Although foodborne listeriosis is rare, the associated mortality rate is as high as 20% among those at risk.

Sporadic cases and outbreaks of listeriosis associated with seafood products have been reported: a 1980 outbreak (29 cases, 9 deaths) in New Zealand associated with fish or molluscan shellfish; an outbreak (9 cases) in Connecticut caused by contaminated shrimp; a case in which fish was implicated; a case in which smoked cod roe was implicated, 3 cases in Tasmania caused by smoked mussels, and 9 cases of listeriosis in Sweden suspected to have been caused by a gravid cold-smoked rainbow trout. FDA surveys of domestic and imported cooked, ready-to-eat seafood products found *L. monocytogenes* in crabmeat samples and smoked fish samples. Over 17% of the cold-smoked products were positive for the organism.

The focus of this task order is on cold-smoked fish. Hot-smoked fish receives a cook, for example, 62.8 °C (145 °F) for 30 min that should inactivate vegetative pathogens. The issue of *L. monocytogenes* in hot-smoked fish is the need to prevent recontamination after the cook through plant sanitation and other methods. Cold-smoked product, however, is not "cooked" and if the incoming product or the facility is contaminated with *L. monocytogenes* there is no inactivation or inhibition step. The Agency's interest is primarily in *L. monocytogenes*, however, other pathogens may be of concern particularly during the cold smoking at temperatures between 21.1 to 37.8 °C (70 to 100 °F), optimum temperatures for the growth of many pathogens, for extended periods of time, ranging from 12 h to 5 d.

Recommended salt levels and heat treatments used in cold and hot-smoked fish are intended to control *Clostridium botulinum* type E. In vacuum packaged products, 3.5% water phase salt is needed, and in air packed products, 2.5% wps. *L. monocytogenes*, however, is relatively tolerant of salt, so concentrations adequate to control *C. botulinum* type E have relatively little effect. The organism can grow fairly well in cold-smoked fish with 6% water phase salt at refrigerated temperatures. During hot-smoking, products are normally heated to an internal temperature of 62.8 °C (145 °F) for 30 min to inactivate vegetative pathogens. FDA's Fish and Fishery Products Hazards and Controls Guide recommends that during cold smoking, the smoker temperature be restricted to no more than 32.2 °C (90 °F) which is intended to allow the survival of spoilage organisms that would multiply and spoil the product before the production of *C. botulinum* toxin.

*L. monocytogenes* and nonproteolytic strains of *C. botulinum* are psychrotrophs and can grow at refrigeration temperatures at low as 1.1 °C (34 °F) and 3.3 °C (38 °F) respectively. Salted and/or smoked products have a longer refrigerated shelf life than raw fish, which gives extra time for psychrotrophic organisms to grow to significant levels even when stored at FDA recommended temperatures.

Because cold-smoked products do not receive a heat treatment during processing adequate to inactivate vegetative pathogens, *L. monocytogenes*, if present, may survive. There is also concern that the cold-smoking process of 32.2 °C (90 °F) for times varying from 12 h to 5 d may allow the proliferation of pathogens during the cold-smoking step itself. There is a question of whether *L. monocytogenes*, as well as toxin producers like *Staphylococcus aureus* and histamine producing species of bacteria may proliferate and result in food poisoning.

There is some evidence that *L. monocytogenes* enters the processing plant on raw material and during processing there are a number of opportunities for *L. monocytogenes* to be transferred from the exterior of the fish to cut surfaces of fillets, that is, contact with contaminated skin sides, filleting knives, gloves, brine, other equipment. In addition, the interior of the fish may be inoculated with injection systems using recirculated brines. *L. monocytogenes* on the internal areas of the flesh will be protected from the application of smoke and the organism could multiply at temperatures used during cold smoking. There may be a bacteriocidal effect of smoke on *L. monocytogenes* that remains on the surface of the product.

The inhibitory effect of smoke needs to be characterized as well as the best methods for application of smoke. The inhibitory effects of salt, nitrite, and sodium lactate have been investigated and while these preservatives have little effect when used alone, there is some evidence that when used in combination, there is an inhibitory effect on low levels of *L. monocytogenes* Other factors that may provide an inhibitory effect include pH control, water activity, and competitive microorganisms.

In hot-smoked products, *L. monocytogenes* is usually assumed to be a result of postprocessing contamination. There is some evidence that *L. monocytogenes* can survive on the surface of salmon fillets processed to an internal temperature of 83 °C (181 °F) without application of smoke. Other factors that may affect the survival of *L. monocytogenes* include the formation of a "pellicle," where the surface dries before the application of smoke which decreases the inhibitory effect of smoke. The use of liquid smoke may also provide an inhibitory effect.

## Current Policy

FDA's *L. monocytogenes* policy is based on the potentially severe public health consequences and the characteristics of the organism, that is, the organism causes human illness, death in over 20% of the cases, can grow at refrigeration temperatures, and the infectious dose is unknown. Currently, the Agency is

conducting a risk assessment on *L. monocytogenes*

Under the current policy, the detectable presence of *L. monocytogenes* in ready-to-eat food is considered to be a hazard to health. The limit of sensitivity of the analytical method is 1 colony-forming-unit (cfu) per 25 g (0.04 cfu per gram).

Because of FDA's policy, most processors do not test their end-products for the presence of *L. monocytogenes* Processors may use environmental sampling in their plants in place of and as predictors of the presence of *L. monocytogenes*, but sampling of end product is often avoided.

FDA requests recall of any ready-to-eat food in which *L. monocytogenes* is detected using present methodology.

# Scope of Work (as Assigned by FDA to IFT)

Independently and not as an agent of the Government, the Contractor shall furnish the necessary materials, services, facilities, and otherwise do all things necessary for or incident to the performance of the work set forth herein.

The contractor shall review the scientific literature, shall consult with academic experts, and shall consider the requirements of other governmental bodies to address the following specific questions:

1. Are the times and temperatures used during cold smoking conducive to the outgrowth of pathogens and histamine producing species of bacteria? What is the range of time and temperatures used by industry during cold smoking? Which pathogens are of concern? The contractor shall provide information on the various pathogens that might be expected to be present on seafood products. These pathogens would include but are not limited to: *L. monocytogenes, S. aureus, C. botulinum*, organisms capable of producing histamine in scombroid species and any other organism that may serve as a foodborne pathogen. The contractor shall do an in-depth review of how these organisms are inhibited (or not inhibited) in smoked fish products and define the critical control points important to each of them.

2. The contractor shall do an in-depth review on the processing parameters that may contribute to *L. monocytogenes* contamination of product, for example, incoming product, chlorination of rinse water, injection brining systems, recirculation of brine, etc.

3. The contractor shall do an in-depth review on the options available to eliminate or inhibit those organisms of public health concern in smoked fish products. The contractor shall evaluate the various preservatives (for example, the inhibitory effect of wood smoke, liquid smoke, salt, nitrite and sodium lactate) used alone or in combination and the levels needed as appropriate inhibitors to pathogen growth. The contractor shall include the influence of the time and method of application of such preservatives. In addition, the contractor shall evaluate the suitability of other controls (for example, pH, water activity, competitive microflora) on the prevention of outgrowth during processing and during subsequent finished product storage.

4. The contractor shall provide information on recommended levels of heat or preservatives, alone or in combination, that processors can use to establish critical limits for processing a cold-smoked product that is free from *L. monocytogenes* and bacterial toxins. The contractor shall review appropriate corrective actions that can be taken when critical limits are exceeded.

5. The contractor shall provide information on how processors can validate the adequacy of the above levels in their processing systems and how to verify that their process is adequate on an ongoing basis.

6. The contractor shall evaluate the various packaging options (for example, oxygen permeable packaging, vacuum packaging, modified atmosphere, controlled atmosphere) and their effect on the inhibition of spoilage bacteria and the outgrowth of pathogens. The contractor shall define the term oxygen permeable packaging as it relates to inhibiting the outgrowth of *C. botulinum* and other pathogens (for example, what characteristics must be present for a product to be considered "air" packaged). Products packed in high barrier film without a vacuum being pulled may become anaerobic rapidly due to the growth of aerobes in the product and the subsequent production of carbon dioxide.

7. The contractor shall evaluate methods to control *L. monocytogenes* on the incoming product. Are there good vessel/harvest/handling practices or microbiological monitoring procedures that will prevent contamination of incoming product with *L. monocytogenes* What are these practices and/or procedures?

8. While the scope of this task order is specific to cold-smoked fishery products, the contractor shall provide any information in the literature on hot-smoked fish that is germane. If the contractor finds sufficient information demonstrating that the time and temperature of the hot-smoke is inadequate to eliminate *L. monocytogenes* from hot-smoked products, it shall be noted in the review.

# Executive Summary

The overall purpose of this report is to evaluate the published and unpublished data concerning likely hazards of public health concern derived from the consumption of cold-smoked fish. In 1997, the U.S. Food and Drug Administration (FDA) mandated the application of the Hazard Analysis and Critical Control Point (HACCP) principles to the processing of domestically produced and imported fishery products. Although HACCP, with its focus on science, holds great promise for minimizing the risk of foodborne disease, the application of HACCP principles to foods and food processes such as cold-smoked fish is challenging. In certain cases, no useful strategies may be available to completely eliminate the identified food safety hazards. Specifically, FDA asked the Institute of Food Technologists (IFT) selected panel to provide an in-depth review of pathogens that might be found in cold-smoked fish products, to identify processing parameters that may contribute to pathogen growth, and to review options available to eliminate or inhibit foodborne pathogens in smoked fish products. FDA also asked for a review of the safety of current fish harvesting and handling practices as well as an evaluation of packaging options and their influence on survival or growth of the organisms of concern.

To address these issues, the report begins by describing the situation with regard to the safety of consuming cold-smoked fish. Focusing attention on cold-smoked finfish, the panel reviewed the most significant and likely to occur hazards in cold-smoked products—*Listeria monocytogenes*, *Clostridium botulinum*, human parasites, and biogenic amines. For each hazard, the report evaluates the effectiveness of methods for eliminating or preventing contamination in the processing environment, identifies possible control points for each step in the process, and, where there is scientific evidence, offers processing parameters that would control the hazard. The evaluation also takes into account control points during harvesting, packaging, storage, distribution, and use by the consumer. Based on these findings, the panel offers information for reducing the risk of hazard in cold-smoked fish products. The report also identifies research needs for further investigating control methods for the hazards reviewed in this report.

Control methods for some of the hazards of the cold-smoked fish product are difficult to determine, due to the many variables and unknown factors contributing to its potential contamination (the ubiquitous pathogen *L. monocytogenes* is a case in point). The definitions of cold-smoked fish are, themselves, vague. The Association of Food and Drug Officials (AFDO) and Codex Alimentarius define it as follows: "Cold process smoked fish means a smoked fish that has been produced by subjecting it to smoke at a temperature where the product undergoes only incomplete heat coagulation of protein." Cold-smoked fish is categorized as "lightly preserved" in Europe. Lightly preserved fish products contain low levels of salt and added preservatives, and must be stored and distributed at refrigeration (≤ 5 °C, 41 °F) or frozen temperatures. Because it would have been almost impossible to do an in-depth review of all the different ways in which fish are cold-smoked, for the purpose of this task order the panel chose to base the discussions on the general process described in the flowchart on the following page.

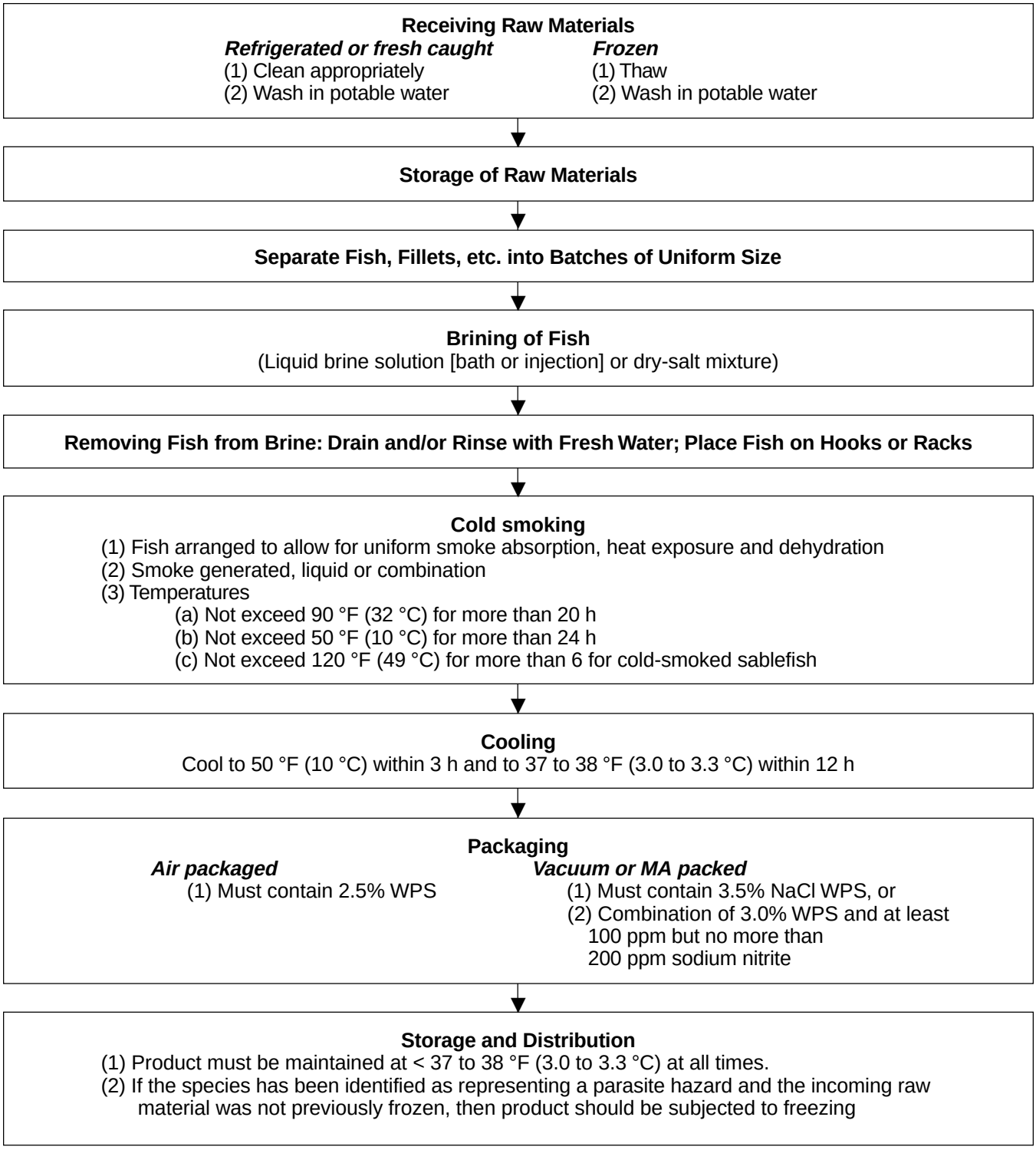
Cold-smoked fish products are consumed as ready-to-eat with no heat treatment. Because of the absence of a "killing" step, other parameters such as salting become of utmost importance to minimize the risk of foodborne hazards. Salting and drying are crucial steps to achieve the proper finished product water phase salt level. Drying times, after salting, range from 1 to 6 h at 20 to 28 °C (68 to 82 °F), smoking parameters at a maximum of 30 °C (86 °F) range from 3 to 6 h, and shelf life ranges from 3 to 6 wk at 5 °C (41 °F). The recommended smoke chamber temperature combinations must not exceed 90 °F (32 °C) for more than 20 h, 50 °F (10 °C) for more than 24 h, or 120 °F (48 °C) for more than 6 h (for cold-smoked sablefish). It is important that the product not be subjected to so much heat that the number of spoilage organisms is significantly reduced. Competitive inhibition may be important in cold-smoked products because the heat applied is insufficient to inactivate or damage the *C. botulinum* spores. However, although an important consideration, relying on the competitive flora to restrict growth of *C. botulinum* or to indicate spoilage before the product becomes hazardous is not an effective or reproducible control point, and cannot be trusted to control safety. According to current U.S. HACCP regulations, a suggested critical limit for air-packaged products is at least 2.5% NaCl, and for modified atmosphere-packaged products at least 3.5% NaCl, or a combination of 3.0% water phase salt and at least 100, but not more than 200 ppm, of sodium nitrite (for fish species where nitrite is permitted). All products must be maintained at 37 to 38 °F (3.0 to 3.3 °C) if no other controls are present (that is, an adequate NaCl concentration and the application of smoke). If adequate concentrations of NaCl and smoke are present, the product must be maintained at ≤ 40 °F (4.4 °C).

The harvesting environment is one of the factors that make it difficult for processors to control contamination in the smoked fish product. The microbial flora associated with freshly harvested fish is principally a function not of the fish species but of the environment in which the fish are caught. Although this generalization appears simple, there is great diversity in aquatic environments (that is, fresh, salt, estuarine, cold, tropical, temperate, coastal, open ocean, polluted, and pristine) and therefore the indigenous microbial populations of fish can vary significantly. The microflora on a fish product is a function of the indigenous flora and the microflora of the processing environment. Typically, the term "processing environment" is limited to that of an actual processing plant. Any handling of fish, and the associated sanitary practices from the point of harvesting, however, has the potential to contribute to the microflora on the final product. Consequently, the presence or absence of foodborne pathogens on a fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment, as well as any lethality associated with the actual process of preserving the fish.

The most universal means of preserving fish quality is chilling (ice or mechanical refrigeration systems). As the temperature on the surfaces of fish is reduced below optimal, bacterial growth begins to slow. Given the microbial diversity typical of fish, it is not surprising that chill temperatures impact some microbial species more dramatically than others. The growth of some species is totally inhibited while others grow, albeit more slowly. Consequently, the rather diverse microflora will shift until just a few species predominate, due to the selective pressure of the chilled environment. Furthermore, the salting, drying, and smoking pro-

cesses described above often reduce the numbers of microorganisms and cause a change in the spoilage microflora. Aerobic storage of cold-smoked fish at refrigerated temperatures results in the development of spoilage microflora consisting mostly of *Pseudomonas* spp. and yeast. During vacuum or $CO_2$-packing, lactic acid bacteria rapidly become the dominant microflora. The nature of the microorganisms that cause spoilage and the manner in which the interactions among the organisms influence the spoilage scenario are not yet fully understood. This is important because these spoilage bacteria may help control pathogens.

As indicated, the processes used for cold smoking of fish—from receiving the raw material to the processing, storage, and distribution of the smoked product—are not exceptionally rigorous. Thus, there is concern that some foodborne pathogens, if present, could survive. Organisms of primary concern are *L. monocytogenes* and *C. botulinum*. In addition, the extensive handling provides opportunities for other foodborne pathogens to contaminate products if insufficient attention is given to Good



Manufacturing Practices (GMPs).

*Listeria monocytogenes* and other *Listeria* species have been isolated from fishery products on a regular basis since the late 1980s. Although there have not been any large outbreaks of listeriosis, sporadic cases have been linked to smoked mussels and cold-smoked trout. *Listeria monocytogenes* can survive the cold smoking process and is capable of growing at the temperature-NaCl combinations of the final product, although in naturally contaminated products the growth is slow. An increase in the incidence of listeriosis over the next decades acquired from all food products is likely due to the increasing numbers of susceptible people (pregnant women, infants, the elderly, and immunocompromised). Although listeriosis occurs infrequently, at an annual rate of 2 to 10 per million, the fatality rate usually ranges from 20 to 30% in the highly susceptible groups. Consequently, keeping concentrations of *L. monocytogenes* in cold-smoked fish at extremely low levels is imperative to minimize the risk. To that effect, conforming to GMPs is essential.

With respect to *C. botulinum*, its prevalence is widespread but its incidence is low. The concerns with psychrotrophic nonproteolytic *C. botulinum* are not associated with the mere presence of the organism or its spores. Packaging environment and temperature significantly influence risk factors associated with *C. botulinum*. Because refrigeration temperatures alone will inhibit the growth of proteolytic strains, control could be established by maintaining temperatures below 3.0 to 3.3 °C (37 to 38 °F) throughout distribution, retail storage, and by the consumer. Maintaining temperatures consistently below 3.0 to 3.3 °C (37 to 38 °F) is not a realistic expectation. Consequently, a combination of both low temperature control [ < 4.4 °C (40 °F)] and salt (3.5% water phase of NaCl) are vital. Because there are no reports in the scientific literature linking cold-smoked fish to an outbreak of botulism, it is assumed that the combination of NaCl and low temperature is sufficient for control of the hazard.

As the processes employed in the cold smoking of fish are not rigorous, not only are such pathogens as *L. monocytogenes* and *C. botulinum* a potential hazard, but survival of human parasites is also a distinct possibility. Fish species carrying parasites that are known to be pathogenic to humans must be frozen to a specific internal temperature and for an amount of time at some stage during processing. Farmed salmon reared on pelletized feed are not subject to the freezing requirement because the feed is considered void of parasites due to the feed processing method.

Biogenic amines, such as histamine, are byproducts of bacterial growth on the surfaces of susceptible species of harvested fish and may cause scombroid toxicity. If scombrotoxin-forming fish are temperature-abused prior to cooling, levels of biogenic amines can rise. Lightly preserved fish products, such as cold-smoked fish, however, have not been linked epidemiologically to foodborne disease caused by scombroid toxicity.

In summary, the control of hazards in cold-smoked fish products requires careful attention by processors. The HACCP concept developed for controlling foodborne disease is based on a simple yet fundamental premise: "identify and control." With respect to cold-smoked fish, hazard analysis suggests that biological hazards may exist (that is, *L. monocytogenes, C. botulinum*), but a definitive control point is either problematic (that is, temperature control for *C. botulinum*); or nonexistent (that is, kill step for *L. monocytogenes*). The concerns associated with *C. botulinum* in smoked fish are not new. Thus, its prescribed treatment in processing is well established. At the processing level, vacuum packaging is acceptable if barriers (that is, 3.5% NaCl, smoke, and chill temperature control) are in place. Less salt is needed to inhibit growth of the psychrotrophic (nonproteolytic) *C. botulinum* types B, E, and F at chilled fish temperatures than at higher temperatures. Moreover, reduced pH in combination with salt enhances the inhibition of the organism. *L. monocytogenes*, on the other hand, is a relatively new concern. Although much has been learned about *L. monocytogenes*, the dose/response relationship of the organism for humans is not yet known. The United States policy has a "zero tolerance" (nondetectable, by the current methods) for *L. monocytogenes*. Using a quantitative risk assessment approach, a group of researchers concluded that unless rigorously enforced, the "zero tolerance" was not better for food safety than a specification at a level of ≤ 100 per gram at time of consumption. Clearly, the United States "zero-tolerance" policy for *L. monocytogenes* in ready-to-eat products is a significant issue for the cold-smoked fish industry.

After numerous panel discussions and consultations with outside reviewers, the panel made conclusions on the scientific status of these important issues and listed possibilities for further research. Following is a list of the main conclusions and research areas that would need further attention.

## Conclusions

The following conclusions are based on a thorough analysis and evaluation of the current science on control methods of human health hazards that may be associated with the consumption of cold-smoked fish.

### *Listeria monocytogenes*

- Given the ubiquitous nature of *L. monocytogenes*, the lack of listericidal steps in the cold-smoking procedure, and the ability of the organism to become established in the processing environment and recontaminate products, it is not possible to produce cold-smoked fish consistently free of *L. monocytogenes*. This is not unique to cold-smoked fish because this microorganism can be isolated from a wide range of ready-to-eat (RTE) foods.
- By adhering strictly to Good Manufacturing Practices (GMPs) and Good Hygienic Practices (GHPs) it is possible to produce cold-smoked fish with low levels of *L. monocytogenes*, preferably at < 1 cell/g at the time of production.
- Growth of *L. monocytogenes* in naturally contaminated fish products is significantly slower than predicted by models (using combinations of pH, NaCl, temperature, and lactate) and inoculation studies.
- Prevention of growth of *L. monocytogenes* in cold-smoked fish cannot be guaranteed not to occur using current combinations of NaCl and low temperature; however, growth can be prevented by freezing, by addition of certain additives (for example, nitrite), or by use of bioprotective bacterial cultures.
- If the organism cannot be eliminated and growth-inhibiting steps are not introduced, the hazard can be controlled by limiting shelf life (at 4.4 ° C, 40° F) to ensure that no more than 100 cells/g are present at time of consumption. Time limits may need to be established by each processor because the time limit should reflect the initial level of the organism in freshly produced product.
- Some countries, such as Australia, warn pregnant women about listeriosis and offer a list of food items to be avoided during the pregnancy. Labeling cold-smoked fish as well as other RTE foods in this risk category, indicating that these products may constitute a health hazard for immuno-compromised individuals and pregnant women could be considered.
- There is no control point during the cold-smoking process that will guarantee the elimination of *L. monocytogenes* on the final product; however, the occurrence of *L. monocytogenes* on the finished cold-smoked fish products of processors can be minimized by: 1) obtaining the primary product from known sources (for example, those with a history of noncontaminated fish); 2) following strict adherence to GMPs to prevent recontamination

during processing; and 3) inhibiting growth of any survivors by marketing the product frozen, or by using salt and other preservatives that can inhibit growth at refrigerated temperatures.

### *Clostridium botulinum*

- Psychrotrophic *C. botulinum* occurs naturally in the aquatic environment, so its presence in low numbers on fresh fish must be anticipated. Spores may also be isolated infrequently from cold-smoked fish, although numbers, if present, are low. Given this low number, the probability of germination and toxin production is low but present.
- Experiments with naturally contaminated hot-smoked fish produced from fish with high levels of *C. botulinum* show that toxin may be formed under conditions of temperature abuse.
- Toxin production by psychrotrophic *C. botulinum* is controlled with a combination of a moderate level of NaCl (3.5% NaCl WPS) and storage at chill temperature (< 4.4 °C, < 40 °F) for at least 4 wk. Based on the scientific data and because commercially produced cold-smoked fish has never been reported as a source of botulism, it is reasonable to conclude that the salt and cold keep the hazard under adequate control.
- Based on a range of model studies in broth and inoculation studies with hot- or cold-smoked fish, it can be concluded that a combination of 3.5% NaCl (as water phase salt) and chill storage (4.4 °C, 40 °F), allowing for short time periods of elevated temperatures up to 10 °C (50 °F), will prevent toxin formation in reduced oxygen packaging cold-smoked fish for several weeks beyond its sensory shelf life.
- As a general safeguard, salting to 3.5% for chilled stored cold-smoked fish is essential for reduced oxygen packaged (ROP) cold-smoked fish. In addition, the requirement for chilling with a sufficient salt concentration is an option for consideration in national or international regulations (for example, E.U. directives).
- For air-packaged products, levels of NaCl can, theoretically, be reduced; however, scientific data that support this argument do not exist and are needed before any reduction is recommended. Even when not packed under vacuum or modified atmosphere, pockets of anaerobic conditions may be created where slices of fish overlap or where aerobic spoilage bacteria consume the oxygen present.
- To control *C. botulinum* growth and toxin production in ROP products the following considerations are indicated: (1) A minimum 3.5% water phase salt concentration in the thickest part of the fillet for vacuum or modified atmosphere packaged fish, or a combination of at least 3% water phase salt and a nitrite level of 100 to 200 ppm is necessary for the control of *C botulinum* growth and toxin formation (Note: nitrite is not allowed in products sold in Europe, and is only allowed in the United States for sable, salmon, shad, chub, and tuna). (2) Packages containing refrigerated, cold-smoked fish should be labeled, "Keep Refrigerated at 40 º F (4.4 °C) or below. (3) Packages containing frozen, cold-smoked fish should be labeled, "This product must remain frozen until thawed at refrigeration temperatures and shall not be refrozen," and (4) Products should not be packaged in reduced oxygen packaging by the retailer.

### Biogenic amines

- The majority of species that are cold-smoked have not been identified by the scientific community as causing scombrotoxin illness. Therefore, the risk of foodborne illness is limited in the majority of cold-smoked products available in the marketplace.
- Only relatively high and sometimes controversial concentrations of histamine have usually resulted in illness. The contribution of other biogenic amines to the onset of symptoms is not well understood.
- Most scombrotoxin results from extrinsic, rather than intrinsic, spoilage through the growth of certain bacteria, generally members of the family *Enterobacteriaceae*. Some bacteria are capable of producing greater quantities of decarboxylase enzymes than others.
- Certain processing operations, such as freezing, salting, or smoking may be capable of inhibiting or inactivating biogenic amine-producing microorganisms; however, microorganism growth with potential toxin production may occur after thawing and postprocessing.
- Under certain conditions addition of lactic acid-producing microorganisms suppresses the growth of biogenic amine-forming microorganisms.
- Vacuum packaging does not prevent growth of biogenic amine-forming microorganisms.
- While biogenic amine-forming microorganisms may grow at refrigeration temperatures, generally the minimal temperature for growth is lower than the minimal temperature for toxin production.
- The most effective methods of preventing biogenic amine formation are handling and processing under sanitary conditions, rapid cooling of the fish, and continued refrigeration from harvest through consumption.
- To minimize the level of biogenic amines in species susceptible to histamine formation, temperature control is important throughout the process, particularly during the storage and transportation before cold smoking, the cooling step, and the final product storage, distribution, retail, and consumer steps. The temperatures required for the control of *C. botulinum* may be appropriate to control production of biogenic amines.
- Much of the published scientific research on scombrotoxin utilized fish samples obtained from processing facilities and retail food stores. Only a limited number of studies followed samples from harvest through analysis. Also, sensory analyses were not always incorporated into microbiological and analytical chemical studies. There is a lack of reports describing comprehensive and integrated projects.

### Parasites

- Some of the fish species used for cold-smoked processing are either intermediate or final hosts to parasites. For this reason, assuring the harvesting of parasite-free fish in the wild is difficult.
- Some aquacultured fish are considered free of parasites (if their feeding regime has not been supplemented with raw fish) because their diet can be controlled using net-pens, closed recycled systems or an equivalent system, and commercially pelleted diets; consequently, these control measures must be carefully considered and applied. An analysis of the potential control points for parasites in aquacultured fish is beyond the scope of this report.
- Freezing raw fish prior to smoking remains the most effective way to insure that viable parasites are not present in cold-smoked products consumed by the public. It is essential, therefore, that raw fish potentially containing viable parasites be frozen and held in that state for a period of time that assures destruction of all viable parasites in that fish species.

## Research needs

The following is a list of research areas that the panel suggests needs further attention:

### *Listeria monocytogenes*

- Conduct epidemiological investigations to determine if and to what extent cold-smoked fish is involved in cases of listeriosis. Despite prediction of a risk, only a limited number of cases have been associated with cold-smoked fish.

- Assess virulence potential of *L. monocytogenes* isolated from cold-smoked fish.
- Measure behavior of *L. monocytogenes* in naturally contaminated products. *Listeria monocytogenes* appears to grow more slowly and to lower numbers than anticipated based on model predictions and inoculation trials. An understanding of which factors cause these differences may be used to design appropriate control measures in the product.
- Determine the robustness and applicability of alternative growth inhibitory measures such as bioprotective cultures, bacteriocins, lactate and others.
- Determine how *L. monocytogenes* becomes established in smoke houses and processing facilities. Several studies show that particular DNA types become established in niches in the processing environments. Research is needed to evaluate what parameters determine which types reside—whether it be particular adhesion properties, or particular resistance properties, or other factors.
- Investigate the source of contamination in smoke houses and processing environments in order to introduce procedures specifically targeted at eliminating or limiting introduction of the organism.
- Identify GMP practices that would minimize the contamination and growth of *L. monocytogenes*.
- Determine the effectiveness of intervention strategies to reduce or eliminate *L. monocytogenes*, such as using chlorinated water to thaw and rinse incoming fish, and for rinsing fish following the brining operation.
- Develop cleaning and disinfection procedures targeted at adhered or established cells for removal of *L. monocytogenes* from surfaces.
- Determine if particular types of surfaces reduce numbers of adhering *L. monocytogenes* or if particular treatments (that is, spraying with lactic acid bacteria or lactate) can reduce surface contamination by minimizing adhesion and biofilm formation.
- Evaluate the robustness and sensory acceptability of the various procedures under investigation (that is, bioprotection, lactate, and so on) for the elimination of the possibility of growth in the product.
- Determine the effect of postprocessing methods such as irradiation and high pressure to eliminate *L. monocytogenes* in cold-smoked fish.

### *Clostridium botulinum*

- Evaluate growth and toxin production in naturally contaminated cold-smoked fish products to validate models and predictions for growth and toxin production.
- Determine the influence of redox-potential, various concentrations of trimethylamine oxide (TMAO), and NaCl on toxin production by psychrotrophic *C. botulinum* in gadoid and nongadoid species.
- Determine the potential facilitation by TMAO on formation of nitrosamines, if nitrite is added, during cold smoking.
- Identify processing conditions and gas transmission rates of films under various time and temperature conditions for products to be considered "air packaged." Determine the Oxygen Transmission Rate (OTR) needed for a product with 2.5% salt concentration to provide equivalent safety compared with cold-smoked reduced oxygen-packaged products (ROP).
- Conduct challenge studies on air-packaged, cold-smoked fish in films with OTRs between 7000 and 10000 cc/$m^2$/24 h and compare to unpackaged cold-smoked fish.
- Establish minimum water phase salt concentrations required to inhibit growth and toxin formation by *C. botulinum* in air-packaged and unpackaged cold-smoked fish.
- Determine the shelf life of the product relative to product quality as well as safety under different packaging methods and storage temperatures.
- Determine appropriate sell-by dates and evaluate the use of time-temperature indicators to ensure a safe product.

### Biogenic amines

- Determine the influence of ROP on the inhibition of biogenic amine production by Gram-negative bacteria.
- Define the minimum temperatures for growth and biogenic amine production of biogenic amine-forming microorganisms.
- Identify practical temperatures that would minimize the levels of biogenic amines in all steps of the production chain and in the final product.
- Determine the effect of salt and redox potential on the formation of biogenic amines on the final product.
- Determine the impact of the interrelationship(s) among histamine, putrescine, cadaverine, and perhaps other biogenic amine concentrations in scombrotoxin and their effects on subsequent host responses.
- Investigate the effects of various cold-smoked fish processes (water phase salt concentrations, process times and temperatures) on biogenic amine formation.
- practical methods for cold-smoked fish processors to determine the histamine/scombrotoxin risk in the raw material used for smoking.
- Apply new processes, such as irradiation, modified atmospheres, or high pressure, to reduce specific groups of microorganisms to determine if control of those responsible for biogenic amine formation reduces the hazard.
- Evaluate the effects of harvesting methods and postharvest handling practices on biogenic amine formation under varying environmental conditions.
- Identify specific methods for representative and effective sampling and for accurate and precise analysis of biogenic amines.

### Parasites

- Describe possible alternative freezing procedures that are or could be effective for inactivation of various fish parasites.
- Establish the kinetics and lethal effect of specific regimes of freezing on various fish parasites.
- Evaluate alternative processing procedures, such as high pressure and X-ray or e-beam irradiation for control of various fish parasites.

# CHAPTER I
# Description of the Situation

DONN R. WARD

## 1. Introduction

On December 18, 1997, the U.S. Food and Drug Administration (FDA) implemented a dramatic change in the manner with which domestically produced and imported fishery products are regulated. The new regulations mandated the application of the Hazard Analysis and Critical Control Point (HACCP) principles to the processing of seafood. Many compelling motivations are driving the use of HACCP, but four of the most prominent driving forces are that HACCP (1) is focused on food safety, (2) is science-based, (3) relies on preventive controls rather than retrospective end-product testing, and (4) focuses control on those food safety hazards that are reasonably likely to occur. In essence, HACCP requires food processors to understand the safety hazards associated with the food, the process, as well as distribution and marketing conditions, and to use appropriate controls so that any identified hazard(s) is prevented, eliminated, or reduced to acceptable levels. Although HACCP holds great promise for minimizing the risk of foodborne disease, application of HACCP principles to a few foods and food processes is challenging because no useful strategies are available to control some identified food safety hazards. Cold-smoked fish and the processes generally used for this product are examples of foods and processes that pose such challenges. Although the cold-smoking process may be applied to seafood commodities other than finfish, this report is limited to cold-smoked finfish. When applicable, the report refers to hot-smoked fish, but an in-depth analysis of hot-smoked fish—a completely different product from cold-smoked fish—is outside the scope of this report.

## 2. Cold-smoked fish

### 2.1 Definition

The definitions associated with cold-smoked fish are vague. For example, both the Codex Alimentarius Commission (1979) and the Association of Food and Drug Officials (AFDO 1991) use the following definition:

Cold process smoked fish means a smoked fish that has been produced by subjecting it to smoke at a temperature where the product undergoes only incomplete heat coagulation of protein.

Given the nonspecific nature of the Codex and AFDO definition, one would expect to find a wide variety of processing parameters being used by industry. In fact, processors do use different parameters, which in turn impart specific sensory attributes to their products. In certain markets there are distinct preferences for cold-smoked fish with specific sensory characteristics (that is, salty/dry or low salt/moist).

Gram and Huss (2000) categorized cold-smoked fish as "lightly preserved." In Europe, this group includes fish products preserved by low levels of salt (< 6% NaCl in the water phase) and, for some products, the addition of preservatives (sorbate, benzoate, $NO_2$, or smoke). The pH of the products is high (> 5.0) and they are often packaged under vacuum and must be stored and distributed at refrigeration (≤ 5 °C, 41 °F) or frozen temperatures. These products are typically consumed as ready-to-eat with no heat treatment. The authors further reported that drying times, after salting, ranged from 1 to 6 at 20 to 28 °C (68 to 82 °F), and smoking parameters had a maximum of 30 °C (86 °F) for 3 to 6 h, and shelf life (based on sensory evaluation) ranging from 3 to 6 wk at 5 °C (41 °F).

According to current U.S. HACCP regulations, a suggested critical limit for air-packaged product is at least 2.5% NaCl (water phase in the loin muscle), for vacuum-packaged or modified atmosphere-packaged product at least 3.5% NaCl (water phase in the loin muscle), or a combination of 3.0% water phase salt (WPS) and at least 100, but not more than 200 ppm, of sodium nitrite (allowed in the United States for sable, salmon, shad, and chub). The application of smoke, which can be "normal" (wood generated), liquid, or a combination of both, is typically done in the following time and smoke chamber temperature combinations: a) not to exceed 90 °F (32 °C) for more than 20 h, b) not to exceed 50 °F (10 °C) for more than 24 h, or c) not to exceed 120 °F (48 °C) for more than 6 h (for cold-smoked sablefish). All products must be maintained at 37 to 38 °F (3.0 to 3.3 °C) at all times, if no other controls are present (that is, an adequate NaCl concentration and the application of smoke). If adequate concentrations of NaCl and smoke are present, the product must be maintained at ≤ 40 °F (4.4 °C). These formulation and process scenarios are based on the need to inhibit the germination of *Clostridium botulinum* spore germination, growth, and toxin formation. The interplay of the inhibitory effects of salt, temperature, smoke, and nitrite is complex. Control of the brining or dry salting process is significant to ensure that there is sufficient salt in the finished product; however, preventing *C. botulinum* type E (and nonproteolytic types B and F) toxin production is made even more complex by the fact that adequate salt levels are often not achieved during brining. Therefore, proper drying is also important to achieve the finished product WPS level (the concentration of salt in the water portion of the fish flesh) needed to inhibit the growth and toxin formation of *C. botulinum*.

Interestingly, current FDA guidance (FDA 1998) for cold-smoked fish indicates that it is important that the product not be subjected to so much heat that the number of spoilage organisms is significantly reduced. Spoilage organisms are necessary to compete with the growth and toxin formation of *C. botulinum* type E and nonproteolytic types B and F. Thus, competitive inhibition may be important in cold-smoked products because the heat applied during the process is insufficient to inactivate or damage the *C. botulinum* spores and may not even affect the vegetative cell form. Packaging may have a significant influence on the efficacy of competitive inhibition. It is likely that the lactic acid bacteria, which will dominate in a nonoxygen storage environment, may in some circumstances inhibit *C. botulinum*. In an oxygen storage environment, however, a mix of surviving aerobic and facultative anaerobic spoilage organisms could create anaerobiosis, which would facilitate growth and toxin formation by *C. botulinum*. Relying on the competitive flora to restrict growth of *C. botulinum* or to indicate spoilage, however, is not an effective or reproducible control point and cannot be trusted to control safety.

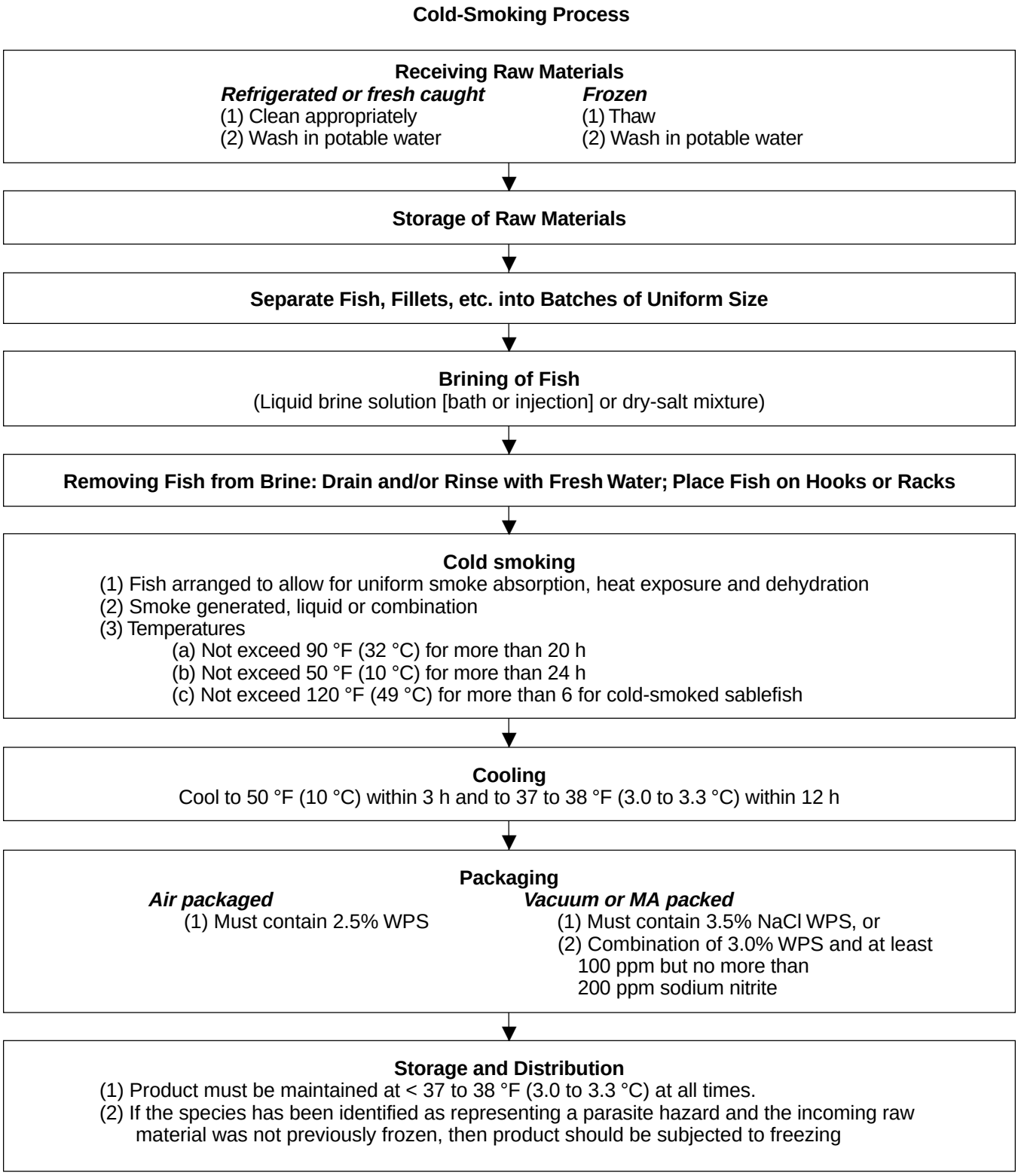
### 2.2. Generalized description of the process

The following flowchart is a generalized description of the cold-smoking process, and as such, it does not account for other process variations encountered in actual industry practice.

### 2.3. Microbiology of products

The microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish are caught, not the fish species (Shewan 1977). Although this generalization appears simple, there is great diversity in aquatic environments (that is, fresh, salt, estuarine, cold, tropical, temperate, coastal, open ocean, polluted, and pristine) and therefore, the indigenous microbial populations of fish can vary significantly.

The microflora on temperate-water fish is predominately psychrotrophic or psychrophilic, Gram-negative bacteria belonging to the genera *Pseudomonas, Moraxella, Acinetobacter, Alcaligenes,*



*Shewanella,* and *Flavobacterium. Aeromonas* spp. are characteristic of freshwater fish, whereas *Vibrio* spp. are typical of marine waters. Additionally, several types of *C. botulinum* are found in the aquatic environment; however, the nonproteolytic *C. botulinum*, especially strains producing type E toxin (called *C. botulinum* type E), is truly indigenous, particularly in the temperate and subarctic zones (Huss 1980). While spores of *C. botulinum* are found in most sediments and on fish from around the world, the limited quantitative data available suggest that the numbers are low, usually less than 100 cfu/g. Higher numbers have been reported from the Great Lakes and the Sound of Scandinavia (Dodds 1993).

Since the bacterial flora on freshly caught fish is a reflection of the environment in which the fish is caught, it is not surprising that aquacultured fish are more likely to be contaminated with certain nonindigenous species. This is due to the closer proximity of fish farms to human and animal populations and the waste generated by each. Research has demonstrated that *Listeria monocytogenes* is a frequent isolate from surface waters (up to 62% positive samples) and polluted seawater (up to 33% positive samples), while it is not isolated from unpolluted ocean waters and spring water (Huss and others 1995).

The microflora on a fish product is a function of the indigenous flora and the microflora of the processing environment. Typically, the term "processing environment" is limited to that of an actual processing plant. Any handling of fish, and the associated sanitary practices from the point of harvesting, however, have the potential to contribute to the microflora on the final product. Consequently, the presence or absence of foodborne pathogens on a fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment, as well as any lethality associated with the actual process.

The most universal means of preserving fish quality is chilling (ice or mechanical refrigeration systems). As the temperature on the surfaces of fish is reduced below optimal, bacterial growth begins to slow. Given the microbial diversity typical of fish, it is not surprising that chill temperatures impact some microbial species more dramatically than others. The growth of some species is totally inhibited while the growth of other species proceeds, albeit more slowly. Consequently, the rather diverse microflora will shift until just a few species predominate, due to the selective pressure of the chilled environment. The two groups that will ultimately become dominant during aerobic ice storage are *Pseudomonas spp.* and *Shewanella putrefaciens* (Gram and others 1987; Levin 1968).

The salting and drying/smoking processes often reduce the numbers of microorganisms and cause a change in the spoilage microflora. While autolytic changes are believed to be the cause of some of the textural changes observed in vacuum-packed, cold-smoked fish during chill storage (Truelstrup Hansen and others 1996), microorganisms are responsible for the unpleasant off-odors and flavors that develop. Aerobic storage of cold-smoked fish at refrigerated temperatures results in the development of spoilage microflora consisting mostly of *Pseudomonas* spp. and yeast. Growth directly on the product may become so pronounced that microbial colonies can actually be observed without magnification. During vacuum- or $CO_2$-packing, lactic acid bacteria rapidly become the dominant microflora. Typically, their numbers increase from $10^2$ to $10^7$–$10^8$ cells/g within 2 wk (Truelstrup Hansen and others 1996; Jorgensen and others 2000; Civera and others 1995). Gram-negative bacteria, such as psychrotrophic *Enterobacteriaceae* or marine vibrios, are often part of the microbial community as well. The spoilage of this product is complex. For example, the nature of the microorganisms that cause spoilage and the manner in which the interactions among the organisms influence the spoilage scenario are not yet fully understood (Gram and Huss 2000). This is important because these spoilage bacteria may be expected to help control pathogens.

## 3. Potential health hazards

The processes used for cold smoking of fish are not exceptionally rigorous; thus, there is concern that some foodborne pathogens, if present, could survive. Organisms of primary concern are *C. botulinum* (psychrotrophic nonproteolytic type B, E, and F), and *L. monocytogenes*. In addition to contributing to pathogen survival, the extensive handling of products following the cold-smoking process provides ample opportunities for other foodborne pathogens (that is, *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus, Vibrio parahaemolyticus,* and *Vibrio cholerae)* to contaminate and survive in the products if insufficient attention is given to Good Manufacturing Practices (GMPs), Sanitation Standard Operating Procedures (SSOPs), and hygienic practices of plant employees. While noting that negligence in sanitary processing and employee hygienic practices could nullify any HACCP control strategies, there is little history of classic pathogens such as *Salmonella* sp., *Shigella* sp., and *S. aureus* occurring in cold-smoked fish products. The reasons for this vary but may include the fact that these pathogens are poor competitors (for example, *S. aureus*), or that they may be injured or reduced by the cold-smoking process or the high salt concentration of the product. Therefore, in this document, these organisms are not included in the discussion of potential health hazards that are reasonably likely to occur.

The Food and Agriculture Organization (FAO 1999) reported that *L. monocytogenes* and other *Listeria* species have been isolated from fishery products on a regular basis since the late 1980s. *Listeria monocytogenes* can survive the cold-smoking process and is capable of growing at the temperature-NaCl combinations of the final product. Studies of inoculated vacuum-packed cold-smoked fish have shown that the organism may grow from $10^3$ cfu/g to $10^7$–$10^8$ cfu/g in 2 to 4 wk. However, the growth in naturally contaminated products is significantly slower and levels above $10^4$ cfu/g are rarely detected, even at end of shelf life. Interestingly, given the relatively high incidence in ready-to-eat and heat-treated fishery products, there have not been any large outbreaks of listeriosis due to the consumption of contaminated fishery products. A couple of sporadic cases, however, have been linked to lightly preserved fish products such as smoked mussels and cold-smoked trout (Brett and others 1998; Miettinen and others 1999). The FAO report did suggest, however, that an increase in the incidence of listeriosis over the next decades acquired from all food products is likely due to the increasing numbers of susceptible people. Highly susceptible groups include pregnant women, infants, the elderly, and immuno-compromised people. Although listeriosis occurs infrequently, at an annual rate of 2 to 10 per million, the fatality rate usually ranges from 20 to 30% in the highly susceptible groups (Farber and Peterkin 2000). In an effort to understand the extent of the *L. monocytogenes* food safety problem, the United States Department of Health and Human Services' FDA's Center for Food Safety and Applied Nutrition in collaboration with the U.S. Department of Agriculture's Food Safety and Inspection Service and the Centers for Disease Control and Prevention conducted a risk assessment on listeriosis associated with various foods, including smoked fish. The risk assessment presents an estimate of the level of exposure of consumers to *L. monocytogenes* and its relationship to public health. Because the period of request for public comments is still open at the time of this writing, after which the draft assessment may be subject to revision, we did not include any material from this risk assessment document. Once

the risk assessment is finalized, we anticipate that the results from it will become invaluable information in support of future scientific evaluation of public health hazards in ready-to-eat foods, such as cold-smoked fish. The draft risk assessment is available at http://www.foodsafety.gov/~dms/lmrisk.html.

With respect to *C. botulinum*, the concerns with psychrotrophic nonproteolytic *C. botulinum* are not associated with the mere presence of the organism or its spores. The organism is found both in freshwater and saltwater species of fish; hence, its prevalence is widespread but its incidence is low. Packaging environment and temperature significantly influence risk factors associated with *C. botulinum*. Since *C. botulinum* is an anaerobe, packaging that eliminates or reduces the oxygen concentration enhances the opportunity for germination, growth, and toxin production of the organism. This is especially true when competing organisms, such as the aerobic spoilage microflora, are suppressed due to the anaerobic environment. Because refrigeration temperatures alone will inhibit the growth of proteolytic *C. botulinum* and of nonproteolytic *C. botulinum*, appropriate temperature control could be an important control measure. Control could be established by maintaining temperatures below 3.0 to 3.3 °C (37 to 38 °F) throughout distribution, retail storage, and by the consumer to inhibit growth of all nonproteolytic and proteolytic strains. Maintaining temperatures consistently below 3.0 to 3.3 °C, however, is not a realistic expectation, based on current distribution, warehousing, retailing, and consumer handling practices. Consequently, a combination of both low temperature control and salt are vital. Because there are no reports in the scientific literature linking cold-smoked fish to an outbreak of botulism, it is speculated that the combination of NaCl and low temperature has been sufficient for control of the hazard.

The food safety concerns associated with cold-smoked fish are not limited to microbiological hazards. Consideration must also be given to the likelihood of another biological hazard—parasites—surviving the processes employed in cold smoking. As stated earlier, the processes employed in the cold smoking of fish are not rigorous; thus, parasite survival is a distinct possibility. Therefore, species carrying parasites that are known to be pathogenic to humans must be frozen at some stage during processing. Current FDA guidance indicates that freezing at the following temperatures will kill parasites of concern: 4 °F (–20 °C), measured internally or externally, for 7 d, or –31 °F (–35 °C), measured internally, for 15 h (FDA 1998). European legislation requires that raw fish used in the production of matjes-herring and cold-smoked fish (wild salmon, herring, mackerel, sprat, cod, and halibut) be frozen at least 24 h at –20 °C (EEC 1991). In the United States and Europe, farmed salmon reared on pelletized feed are not subject to the freezing requirement because the feed is considered void of parasites due to the feed processing method.

Within the context of HACCP, the food safety hazard associated with high levels of histamine is classified as a chemical hazard. Biogenic amines, like histamine, are byproducts of bacterial growth on the surfaces of susceptible species of harvested fish. Some surface bacteria excrete enzymes capable of decarboxylating amino acids, particularly histidine to produce histamine. Once formed, biogenic amines are quite stable and are not destroyed or eliminated by any of the steps associated with the processing of cold-smoked fish. The most significant factor associated with formation of biogenic amines is temperature. If freshly caught fish are cooled rapidly and maintained at cold temperatures, levels remain low. If, however, the fish are temperature-abused prior to cooling, levels of biogenic amines can rise, even under subsequent refrigerated conditions, due to the activity of the preformed decarboxylase enzymes released by the bacteria during the period of temperature abuse. Histamine may be formed in lightly preserved fish products. Levels between 3 and 240 ppm have been detected in cold-smoked salmon (Jørgensen and others 2000). The high levels are above the FDA histamine guidance level of 50 ppm (FDA 1998) and of the 100 ppm by the European regulation for *Scombroidae* and *Clupeidae* (EEC 1991). Also, the high levels exceed the European maximum limit of 200 ppm. Lightly preserved fish products, such as cold-smoked fish, however, have not been linked epidemiologically to foodborne disease caused by scombroid toxicity (Gram and Huss 2000).

## 4. The dilemma

The HACCP concept is based on a simple yet fundamental premise of "identify and control." Specifically, HACCP requires that all food safety hazards associated with the food and with the processes used in manufacturing the food, that are reasonably likely to cause illness or injury in the absence of control, must be identified. Once all the food safety hazards are identified, a control procedure must be established to prevent, eliminate, or reduce the hazards to acceptable levels. If a control option cannot be identified in the existing process, HACCP ideology dictates that the process must be modified to create a control opportunity. With respect to cold-smoked fish, hazard analysis suggests that biological hazards may exist (that is, *L. monocytogenes, C. botulinum*), but a definitive control point is either problematic (that is, temperature control for *C. botulinum*) or nonexistent (that is, kill step for *L. monocytogenes*). Suggestions that the process be modified, such as adding additional salt or a terminal heating step, are generally not welcome by producers because such changes would significantly alter the sensory attributes of the final product and result in the loss of customers.

The concerns associated with *C. botulinum* in smoked fish are not new. Since the early 1960s, FDA has grappled with the issue of this organism in fishery products; specifically, those products packaged in vacuum or modified atmospheres. Prior to the implementation of the HACCP regulation, FDA discouraged the use of these forms of packaging on both fresh and processed products. In fact, the FDA's Food Code (FDA 1997) and AFDO's Retail Guidelines for Refrigerated Foods in Reduced Oxygen Packages (AFDO 1990) specifically prohibit these forms of packaging at the retail level, unless the products are frozen before, during, and after packaging. Nonetheless, at the processing level vacuum packaging is acceptable if barriers (that is, 3.5% NaCl, smoke, and chill temperature control) are in place. Since the advent of the HACCP rule, however, there has been a dramatic shift in responsibility. Under HACCP, FDA is requiring smoked fish processors to document that the *C. botulinum* hazard is being controlled. The Fish & Fisheries Products Hazards and Controls Guide (FDA 1998) indicates that control can be accomplished through a combination of temperature, salt, packaging, and, where appropriate, preservatives (nitrite). According to Gram and Huss (2000), less salt is needed to inhibit growth of the psychrotrophic (nonproteolytic) *C. botulinum* types B, E, and F at chilled fish temperatures than at higher temperatures. Moreover, reduced pH in combination with salt enhances the inhibition of the organism.

While *C. botulinum* has been of concern for many years, *L. monocytogenes* is a relatively new concern. *Listeria monocytogenes* has been isolated from fishery products on a regular basis (FAO 1999) although no clear contamination route is known (Eklund and others 1995). Additionally, it survives both the salting and the cold-smoking processes and is capable of growth at refrigeration temperatures (Hudson and Mott 1993). The prevalence of *L. monocytogenes* in cold-smoked fish is highly variable. Jørgensen and Huss (1998) found the organism to range from < 1.4% to 100% in cold-smoked salmon from three production sites. In the context of HACCP control, Huss and others (1995) indicated that since the contamination source is not known and the preservation steps do

not prevent growth, no definitive critical control point can be identified. The problem is further complicated by FDA's current policy of "zero-tolerance" (nondetectable on samples by the current methods [AOAC 1995] in 25 g sample) for *L. monocytogenes* on ready-to-eat products. This policy is a continuation of the agency's initial response to the public health concerns that ensued when *L. monocytogenes* was first implicated as the causative agent in a foodborne disease outbreak in the mid 1980s. At that time, little was known about the organism, its pathogenicity, or infectious dose. Although much has been learned, the dose/response relationship of the organism for humans is not yet known. Based on the reported numbers of *Listeria* in contaminated foods responsible for epidemic and sporadic foodborne cases, however, there is little evidence that a very low number of *L. monocytogenes* in foods causes disease (FAO 1999). In fact, the FAO (1999) reported that data indicates that a per capita human exposure to doses of *L. monocytogenes* exceeding 1000 cfu (total ingested dose) is likely to occur several times each year. Despite this exposure, however, the total incidence of invasive listeriosis is estimated to be somewhere between 2 to 10 cases per million population per annum in countries where data are available. Ross and others (2000), using a quantitative risk assessment approach, concluded that unless the "zero tolerance" was rigorously enforced, this standard was not better for food safety compared to a level of ≤ 100 per gram at time of consumption.

The United States is not alone in its "zero-tolerance" criteria for ready-to-eat products. Austria, Australia, New Zealand, and Italy currently require the absence of *L. monocytogenes* in a 25 g sample. Germany, Netherlands, and France have a tolerance of less than 100 cfu/g at the point of consumption, while Canada and Denmark have a tolerance of less than 100 cfu/g for some foods and zero tolerance for other foods (that is, those with extended shelf life that can support the growth of the organism).

Clearly, the "zero-tolerance" policy (nondetectable, by the current methods [AOAC 1995] in 25 g sample) for *L. monocytogenes* in ready-to-eat products is a significant issue for the cold-smoked fish industry. Scientific evidence indicates that finding *L. monocytogenes* in cold-smoked fish is merely a question of "when" not "if." Some studies have demonstrated significant plant-to-plant variation in detecting positive samples; however, plants that may have been negative on one sampling date have produced positive findings on another date (Jørgensen and Huss 1998; Fonnesbech Vogel, Ojeniyi and others 2001).

## 5. Summary

As previously noted, cold-smoked fish presents a dilemma. As a food, there is little question that cold-smoked fish has been a part of our dietary heritage for centuries. Only recently, however, have serious questions of safety been raised. Is the product safe? If yes, why? If no, why not? Can the process be modified to establish a greater margin of safety? Will the modified product be acceptable? For a relatively minor seafood commodity, cold-smoked fish introduces some rather significant challenges.

To address the safety issues with cold-smoked fish, this report is structured in sections. The first sections discuss the potential hazards: *L. monocytogenes*, *C. botulinum* toxin, biogenic amines, and parasites. The last section addresses the hazards, control points, and processing parameters for each step of the process, from harvesting to consumption.

## References


[AFDO] Association of Food and Drug Officials. 1990. Retail guidelines for refrigerated food in reduced oxygen packages. J Assoc Food Drug Of 54(5):80–4.

[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. [York (PA)]: Association of Food and Drug Officials. 7 p.

[AOAC] Association of Official Analytical Chemists. 1995. FDA Bacteriological Analytical Manual (BAM), 8th ed. Gaithersburg (MD): AOAC Int.

Brett MSY, Short P, McLauchlin J. 1998. A small outbreak of listeriosis associated with smoked mussels. Int J Food Microbiol 43:223–9.

[CAC] Codex Alimentarius Commission. 1979. Recommended International Code of Practice for Smoked Fish. Rome: Codex Alimentarius Commission. CAC/RCP 25–1979.

Civera T, Parisi E, Amerio GP, Giaccone V. 1995. Shelf-life of vacuum-packed smoked salmon: microbiological and chemical changes during storage. Arch Lebensmittelhyg 46:13–7.

Dodds KL. 1993. *Clostridium botulinum* in the environment. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum*: ecology and control in foods. New York: M Dekker. p 21–52.

EEC. 1991. Council directive 91/493/EEC of 22nd July 1991 laying down the health conditions for the production and the placing on the market of fishery products. Off J Eur Comm(Nr L268):15–32.

Eklund MW, Poysky FT, Paranjpye RN, Lashbrook LC, Peterson ME, Pelroy GA. 1995. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. J Food Prot 58(5):502–8.

[FAO] Food and Agriculture Organization. 1999 May. Report of the FAO expert consultation on the trade impact of *Listeria* in fish products. Rome: FAO. FAO Fisheries Report nr 604. 34 p.

Farber JM, Peterkin PI. 2000. *Listeria monocytogenes*. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of foods. Gaithersburg (MD): Aspen. p 1178–1232.

[FDA] Food and Drug Administration. 1998. Fish & Fisheries Products Hazards & Controls Guide. 2nd ed. Washington, D.C.: FDA, Office of Seafood. 276 p.

[FDA] Food and Drug Administration. 1999. Food Code. Washington, DC: U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration.

Fonnesbech Vogel B, Ojeniyi B, Ahrens P, Due Skov L, Huss HH, Gram L. 2001. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Appl Environ Microbiol. Forthcoming.

Gram L, Huss HH. 2000. Fresh and processed fish and shellfish. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Gaithersburg (MD): Aspen. p 472–506.

Gram L, Trolle G, Huss H. 1987. Detection of specific spoilage bacteria from fish stored at low 40 º C) and high 4.4 ° C) temperature. Int J Food Microbiol 4:65–72.

Hudson JA, Mott SJ. 1993. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cold-smoked salmon under refrigeration and mild temperature abuse. Food Microbiol 10:61–8.

Huss HH. 1980. Distribution of *Clostridium botulinum*. Appl Environ Microbiol 39:764–9.

Huss HH, Ben Embarek PK, From Jeppesen V. 1995. Control of biological hazards in cold-smoked salmon production. Food Control 6(6):335–40.

Jorgensen LV, Dalgaard P, Huss HH. 2000. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. J Agric Food Chem 48:2448–53.

Jorgensen LV, Huss HH. 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. Int J Food Microbiol 42:127–31.

Levin RE. 1968. Detection and incidence of specific species of spoilage bacteria on fish. 1. Methodology. Appl Microbiol 16:1734–7.

Miettinen MK, Siitonen A, Heiskanen P, Haajanen H, Bjorkroth KJ, Korkeala HJ. 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. J Clin Microbiol 37(7):2358–60.

Ross T, Todd E, Smith M. 2000. Exposure assessment of *Listeria monocytogenes* in ready-to-eat foods: preliminary report for joint FAO/WHO expert consultation on risk assessment of microbiological hazards in foods. Rome: Food and Agriculture Organization of the United Nations. 242 p.

Shewan JM. 1977. The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. Proceedings of the conference on handling, processing, and marketing of tropical fish. London: Tropical Products Institute. p 51–60.

Truelstrup Hansen L, Gill T, Drewes Rontved S, Huss HH. 1996. Importance of autolysis and microbiological activity on quality of cold-smoked salmon. Food Res Int 29:181–8.

CHAPTER II

# Potential Hazards in Cold-Smoked Fish: *Listeria monocytogenes*

LONE GRAM

## Scope

This section outlines what is currently known about the prevalence and behavior of *Listeria monocytogenes* during the cold-smoking process, including its survival in the processing environment and its presence and growth in the final product. Control methods in both the final product and the environment are discussed. While the section provides some of the data relevant for a science-based risk assessment, it does not give complete information on all four components: hazard identification, hazard characterization, exposure assessment, and risk characterization. Recent texts by Farber and others (1996), Farber and Peterkin (2000), Buchanan and others (1997), and Notermans and others (1998) cover these issues. Finally, based on the scientific data, approaches to minimize the presence and growth of *L. monocytogenes* in cold-smoked fish are considered. Lists of conclusions and research areas that need future attention are also included.

## 1. Introduction

*Listeria monocytogenes* is a Gram-positive, foodborne pathogen. It is widely distributed in the environment and occurs naturally in many raw foods. *Listeria monocytogenes* is psychrotrophic and halotolerant (Seeliger and Jones 1986) and can, under otherwise optimal conditions, grow in the range of 1 to 45 °C (34 to 113 °F) and between 0 and 10% NaCl. As a consequence it may grow in many food products with extended shelf lives (Barakat and Harris 1999; Rørvik and others 1991). Products that do not receive a heat treatment by the consumer, including ready-to-eat (RTE) products such as cheeses, meat, and fish delicatessen products, may contain high levels of *L. monocytogenes* when eaten, and many of these types of foods have been associated with listeriosis (McLauchlin 1997). In general, populations in foods are low (0 to $10^3$ cfu/g with 90 to 99% being below $10^2$ cfu/g and less than 1% being between $10^3$ and $10^4$ cfu/g) (Teufel and Benzulla 1993; Jørgensen and Huss 1998; Farber and Peterkin 2000); however, higher concentrations ($10^5$ to $10^7$ cfu/g) have been reported (Farber and Peterkin 1991; Teufel and Bendzulla 1993).

*Listeria monocytogenes* may be divided into 13 different serotypes, all of which may cause listeriosis. The vast majority of cases, however, is caused by serotypes 1/2a, 1/2b and 4b (Farber and Peterkin 2000). The ingestion of high numbers of *L. monocytogenes* is a significant threat to health for people in risk groups such as immuno-compromised, elderly, fetuses, and newborn babies. In these groups, the mortality from listeriosis is high, typically 20 to 30% (McLauchlin 1997). Because *L. monocytogenes* can readily be isolated from smoked fish and because inoculation trials have demonstrated significant growth in such products, the risk of getting listeriosis from consuming these types of products must be taken seriously.

Cold-smoked fish is an RTE product and such products have been linked to sporadic cases of listeriosis. Epidemiological evidence suggests that listeriosis has been associated with smoked mussels (Brett and others 1998), "gravad" trout (Ericsson and others 1997), and smoked trout (Miettinen, Siitonen and others 1999). In the latter case (Miettinen, Siitonen and others 1999), the outbreak was not the classical invasive listeriosis, but cold-smoked trout was associated with febrile gastroenteritis in five healthy people. There are, to date, no reports in the literature linking listeriosis cases/outbreaks in the United States to cold-smoked fish. Although cold-smoked fish, as discussed below, potentially is a high risk product with respect to listeriosis, case-control studies have linked listeriosis to a number of other products such as uncooked hotdogs, undercooked chicken, paté and cold cuts, and cheeses (Elliot and Kvenberg 2000; Rocourt and others 2000).

The virulence of *L. monocytogenes* varies with the strain. Although no single test can predict the pathogenicity of a strain, a combination of tests is used to indicate virulence potential. These tests determine presence of genes coding for listeriolysin and actin polymerizing protein, adherence to CaCo2 cells, plaque formation on mouse L-cells, and infection of chick embryos. Wiedmann and others (1997) suggested that *L. monocytogenes* should be subdivided into three different lineages, of which mainly lineage I contains virulent strains. In a recent study Norton, Timothe and others (2000) found that the majority (63.2%) of *L. monocytogenes* isolated from cold-smoked salmon belonged to lineage II, whereas clinical isolates and 30% of fish isolates belonged to lineage I. These findings suggest that the pathogenicity potential of isolates from cold-smoked fish may be lower than predicted from its prevalence.

## 2. Prevalence in water, raw fish, and smoked fish

*Listeria monocytogenes* is a ubiquitous organism (Farber and Peterkin 2000). Although its natural niche is probably soil and vegetation, it can readily be isolated from fresh and marine waters. Ben Embarek (1994) reviewed a number of studies and found that the prevalence in a variety of water bodies (river waters, seawater, surface water, spring water) varied from 0 to 62%. Notably, the highest number of positive samples was found in waters exposed to runoff from agricultural or urban areas, whereas waters such as spring water or free ocean waters were negative for the organism (Table II-1). Fenlon and others (1996) investigated river waters in the United Kingdom and found 17 of 36 samples to be positive for *L. monocytogenes*, with levels in positive samples ranging from 10 to 350 cfu/liter.

*Listeria monocytogenes* is commonly present in certain waters, as demonstrated by the following research findings. Farber (1991) reported the presence of *L. monocytogenes* in salmon from the United States, Chile, Norway, and Canada. The prevalence of *L. monocytogenes* in raw fish, as reported in published studies, is low, ranging from 0–1% to 10% (Johansson and others 1999; Autio and others 1999; Jemmi and Keusch 1994; Jinneman and others 1999; Weagant and others 1988). Only 1.3% of 781 samples of Japanese fish contained *L. monocytogenes* (Iida and others 1998), and none of 60 raw salmon sampled in Japan was positive for the bacterium (Jin and others 1994). The literature reviewed by Ben Embarek (1994) indicated large variations, with 0 to 50%

**Table II-1. Prevalence of *Listeria* spp. in freshwater, seawater and sediments**

| Sampling location | Nr of samples | % positive for *Listeria* spp. | % positive for *L. monocytogenes* |
|---|---|---|---|
| **Freshwater** | | | |
| river, domestic animals (USA) | 37 | 81 | 62 |
| not specified (UK) | 7 | 100 | nd |
| river, populated (UK) | 36 | nd | 47 |
| canals, lakes (Holland) | 180 | nd | 37 |
| ground water (Switzerland) | 12 | 0 | 0 |
| spring water (Switzerland) | 12 | 0 | 0 |
| **Seawater** | | | |
| coastal area (USA) | 3 | 33 | 33 |
| shellfish growing area (USA) | 70 | 3 | nd |
| not specified (Holland) | 43 | 0 | 0 |
| used for salmon transport (Norway) | 21 | 52 | 14 |
| around salmon farm (Norway) | 8 | 0 | 0 |
| **Sediments** | | | |
| freshwater (USA) | 46 | 30 | 17 |
| freshwater (USA) | 15 | 20 | 0 |

Modified from Ben Embarek 1994; Jemmi and Keusch 1994; Huss and others 1995.
nd = not determined

**Table II-2. Prevalence of *Listeria* spp. and *Listeria monocytogenes* in live or newly slaughtered fish**

| Sampling location | Nr of samples | % positive for *Listeria* spp. | % positive for *L. monocytogenes* |
|---|---|---|---|
| **Freshwater** | | | |
| skin of live trout (Switzerland) | 45 | 33 | 11 |
| channel catfish (USA) | 4 | 100 | nd |
| slaughtered trout (Switzerland) | 27 | 22 | 15 |
| **Seawater** | | | |
| salmon, at harvest (Norway) | 10 | 0 | 0 |
| salmon, at processing plant (Norway) | 18 | 0 | 0 |
| salmon (Faroe islands) | 18 | nd | 1 |
| frozen salmon (received at plant) (USA) | 65 | nd | 34 |
| salmon (USA, Chile, Norway, Canada, Scotland) | 32 | nd | 10 |

Modified from Farber 1991; Ben Embarek 1994; Jemmi and Keusch 1994; Eklund and others 1995; Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001.
nd = not determined

**Table II-3. Prevalence of *Listeria* spp. *and Listeria monocytogenes* in cold-smoked fish**

| Sampling location | Nr of samples | % positive for *Listeria* spp. | % positive for *L. monocytogenes* |
|---|---|---|---|
| ***Salmon*** | | | |
| Norway | 33 | nd | 9 |
| Norway | 40 | 80 | 33 |
| Norway | 65 | 11 | 11 |
| Italy | 63 | 0–100 | 0–29 |
| Italy | 165 | nd | 19 |
| Switzerland | 100 | nd | 24 |
| Switzerland | 64 | nd | 6 |
| New Zealand | 12 | nd | 75 |
| UK | 22 | 14 | nd |
| USA | 61 | nd | 79 |
| Iceland | 13 | 23 | 0 |
| Canada, USA, Chile, Scotland, Norway | 32 | nd | 31 |
| Japan (origin ?) | 76 | 30 | 16 |
| Denmark | 188 | nd | 34 |
| ***Trout*** | | | |
| Switzerland | 49 | 4 | 2 |
| ***Species not specified*** | | | |
| USA | 291 | nd | 18 |
| USA | 96 | nd | 7 |
| Switzerland | 324 | nd | 14 |

Modified from Ben Embarek 1994; Nilsson 1999; Norton, McCamey and others 2000.
nd = not determined

of fresh fish samples positive. Unpublished information indicates that the prevalence in fish in mud freshwater ponds or fish in seawater nets close to land runoff can be as high as 100%. Based on published data, the frequency appears to be low; however, surveys are needed to evaluate the prevalence and levels on raw, live fish from different sources. To the panel's knowledge, quantitative data are not available on live, fresh fish. In areas where the prevalence is low, it must be assumed that levels are very low (< 10 *L. monocytogenes*/g). It could be speculated that fish reared in waters close to agricultural runoff would carry a higher load of *L. monocytogenes* than fish cultured in waters free from such soil and vegetation sources. As shown in Table II–2, this can be neither confirmed nor excluded based on current data.

After cold smoking, there is an increase in the percentage of samples containing *L. monocytogenes*. The variation is high, ranging from 0 to 100% (Ben Embarek 1994; Eklund and others 1995; Dillon and others 1994; Heinitz and Johnson 1998; Jørgensen and Huss 1998; Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001). Given this vast range, average values are meaningless, but typical prevalence is between 15 and 40%. While Eklund and others (1995) found positive samples from all of 6 cold-smoked salmon plants sampled, other authors have seen a large plant-to-plant variation. The prevalence in products such as cold-smoked salmon seems to be similar in several countries. Thus, in Danish samples from 10 smoke houses, the number of positive samples of finished product varied from 0 to 100%, whereas a survey of 6 United States smoke houses showed *L. monocytogenes* in the product in 50 to 100% of the samples (Eklund and others 1995). Heinitz and Johnson (1998) reported that an average of 17.5% of cold-smoked fish (291 samples) and 8.1% of hot-smoked fish (234 samples) from the United States contained *L. monocytogenes*, and 7.3% of 96 cold-smoked fish samples from 5 United States smoke houses were positive (Norton, McCamey and others 2000). Similar levels have been reported from Switzerland (Jemmi 1990), where 12.2% were positive; from Norway, where 9% were positive (Rørvik and Yndestad 1991); and from a range of other studies (Ben Embarek 1994). Jin and others (1994) did not detect the bacterium in raw salmon; however, they found 16% of smoked salmon positive for *L. monocytogenes* (see Table II–3 for a selection of data).

Jørgensen and Huss (1998) reported that products from some smoke houses repeatedly were free from the organism while samples from other smoke houses always were positive. More recently (Fonnesbech Vogel, Jorgensen and others 2001; Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001), the bacterium could be detected from plants and products that were negative in the former study by Jørgensen and Huss (1998), indicating that although eliminated during some periods, the bacterium can be re-introduced in processing plants and, therefore, in the final product.

Although the prevalence of *L. monocytogenes* in cold-smoked fish is often high, numbers of microorganisms are typically low. For instance, Eklund and others (1995) found that although as many as 48 out of 61 samples of U.S. cold-smoked salmon contained *L. monocytogenes,* numbers of the microorganism ranged from 0.3 to 34.3 cells/g with a mean of 6.2 cells/g. Similarly low levels were found in Danish cold-smoked salmon, where 34 of 64 samples were positive, with 28 of them containing fewer than 10 cells/g, 5 samples containing between 10 and 100 cells/g, and one sample containing between 100 and 1000 cells/g (Jørgensen and Huss 1998). In a survey of smoked fish (not specified hot- or cold-smoked) on the German market, 27 samples (of 380) were positive for *L. monocytogenes,* with 5 samples containing fewer than 1 cell/g, 14 samples containing between 1 and 100 cells/g, and 4 samples containing between 100 and $10^4$ cells/g. In four

samples, levels exceeding $10^4$ cells/g were found (Teufel and Benzulla 1993; Notermans and others 1998). The levels of *L. monocytogenes* found in cold-smoked fish are not different from those in other RTE food products (Table II–4).

**Table II-4. Concentration of *Listeria monocytogenes* in ready-to-eat food products on the German market**

| | | % samples (of positives) containing different levels of *L. monocytogenes* in cfu/g | | | |
|---|---|---|---|---|---|
| Type of ready-to-eat product | % positive | 0.04 to 1 | 1 to $10^2$ | $10^2$ to $10^4$ | > $10^4$ |
| Meat products | 23.1 | 59 | 34 | 6 | 1 |
| Fish products | 9.4 | 64 | 23 | 6 | 6 |
| Cheese | 3.1 | 58 | 23 | 16 | 3 |
| Salads | 5.2 | 60 | 37 | 4 | 0 |

Based on testing of 7063 samples and modified from Teufel and Benzulla 1993; Notermans and others 1998

## 3. Effect of various processing steps

This section describes the parameters and steps of the fish-smoking process that may influence the survival and growth of *L. monocytogenes*. It has been suggested that rinsing of the thawed and brined fish is important to reduce numbers of *L. monocytogenes*; however, no data on the effect of this procedure were found in the scientific literature, and consequently the effect of such steps cannot be evaluated. One U.S. processor reported that the raw material (frozen, thawed fish) are " treated with a pH control at 12.5," which eliminates the bacterium. This processor further treats the finished product with chlorine dioxide prior to packing. These two steps, combined with chlorine-dioxide disinfection of the plant, enables the processor to produce cold-smoked salmon free of *L. monocytogenes*. Bremer and Osborne (1998) reported that rinsing raw fish (salmon) in chlorine solution with 200 ppm free chlorine caused a significant reduction of surface-inoculated *L. monocytogenes*; however, this treatment could not ensure listeria-free raw materials. The procedure was not evaluated on naturally contaminated salmon, so it is not known if this treatment would be more or less efficient on a naturally present population.

### 3.1. Freezing

A freezing step often is included during the smoking process. Either the raw fish or the smoked product is frozen. El-Kest and others (1991) found that freezing (at –18 °C, –0.4 °F) *L. monocytogenes* in buffer caused a one log reduction, whereas freezing in nutrient broth resulted in a 50% reduction. It is well known that lipids and dry matter protect bacteria against freezing damage. It must be assumed that fish, particularly fatty fish, offers a good protection and that reduction of numbers of *L. monocytogenes* due to freezing is marginal. Preliminary work at the Danish Institute for Fisheries Research found that growth of *Listeria monocytogenes* in cold-smoked salmon was not affected by an initial freezing period. It is, however, not known if prolonged frozen storage, either of the raw fish or packed product, can affect subsequent growth at chill temperature of *L. monocytogenes* in the smoked-fish product.

### 3.2. Salting/drying

Salting of fish before smoking is done either by brine-injection, bath brining, or dry salting. As *L. monocytogenes* is a halotolerant bacterium, salting is not likely to reduce the number of microorganisms. On the contrary, several studies have isolated the organism from brine (Eklund and others 1995), needles used for brine injection (Fonnesbech Vogel 2000; personal communication; unreferenced), and in fish flesh that had been injected with contaminated brine (Eklund and others 1995). Autio and others (1999) found that the brining step caused a major increase in contamination of *L. monocytogenes* during cold-smoked trout processing. Salt levels (salt in water phase) in the final product range from 3% to in a few cases as high as 12%, although salt levels typically range from 3.5% to 5% (Truelstrup Hansen and others 1998; Jørgensen and others 2000;). This level of salt (3.5%–5%) has no inhibitory effect on the bacterium (Peterson and others 1993). At levels above 6% NaCl and with a low initial inoculum, growth is prevented at 5 °C (Peterson and others 1993); however, this level of salt is generally too high for consumer preferences. The level of salt may also affect the growth of an accompanying lactic acid bacterial flora, and high levels (> 5.5%) can significantly delay growth of the lactics, thereby reducing their potential inhibitory effect against *L. monocytogenes* (Nilsson 2001; personal communication; unreferenced).

### 3.3. Smoking process

After salting, the fish is often dried and smoked. The hot-smoking process (usually 60 °C for 30 min) is sufficient to kill *L. monocytogenes* (Jemmi and Keusch 1992; Ben Embarek and Huss 1993). Eklund and others (1995) found that cells of *L. monocytogenes* inoculated into the fish flesh could grow during a cold-smoking process (20 to 30 °C [68 to 90 °F]). If cells of *L. monocytogenes* were inoculated on the surface of the fish, cold smoking at 22 to 30 °C (72 to 90 °F) for 18 h caused a 2 log reduction in numbers (Eklund and others 1995). Smoke must be applied to the product before the surface dries, otherwise *L. monocytogenes* will be embedded under the pellicle where the effect of smoke is markedly reduced (Eklund 2001, personal communication; unreferenced). Consistent with the results by Eklund and others (1995), Autio and others (1999) did not find a significant increase in the number of positive samples during the cold-smoking step of trout. In cold smoking of salmon, no positive samples were detected immediately following the smoking procedure of 16 h at 22 °C (Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001). In a Norwegian study, *L. monocytogenes* was detected in salmon samples before filleting and after salting but not immediately after cold smoking (Rørvik and others 1995). In a subsequent study, Rørvik (2000) reported that 54% of 200 samples of salmon were positive for *L. monocytogenes* just before smoking, whereas only 9.5% were positive after smoking. Of 11 samples that contained between 10 and 300 *L. monocytogene*/g before smoking, none was above 10 cfu/g after smoking. In conclusion, the studies indicate that short-term cold smoking (< 24 h, as recommended by the Association of Food and Drug Officials [AFDO] guidelines, AFDO 1991) reduces rather than increases numbers of *L. monocytogenes*.

Some processors use a drying procedure combined with addition of liquid smoke instead of the more traditional smoke-generated process. The potential inhibitory properties of liquid smoke will vary depending on type of wood, method of preparation, and target organism. In a study of 7 commercial smoke preparations (Suñen 1998), large variations in minimum inhibitory concentration (MIC) values against a range of microorganisms were seen. *Listeria monocytogenes* was inhibited by two preparations (one liquid and one solid), both high in aldehydes, phenols, furan, and pyran derivatives and acids, whereas other preparations had no effect even at high concentrations (> 8%). In conclusion, any antilisterial effect of liquid smoke will depend on the particular product in use.

## 4. Growth in refrigerated smoked fish

Innumerable studies have documented that cold-smoked fish is an excellent substrate for *L. monocytogenes*. When inoculated, the organism will grow to high numbers even when stored at 5 °C (41 °F) under vacuum (Farber 1991; Rørvik and others

**Table II-5. Growth of *Listeria monocytogenes* in naturally contaminated cold-smoked salmon stored at 5 °C**

| Product characteristics | | | | | | | *L.m.* growth during storage log(cfu/g) | | | Average growth rate log(cfu/g)/wk | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lot | storage time (d) | WPS (%) | WPL (g/l) | pH | Initial TVC log(cfu/g) | Initial *L.m.* (log(MPN/g) | observed | highest number in single packs[1] | predicted | observed | predicted |
| A | 8 | 5.0 | 6.7 | 6.2 | 3.6 | 0.6 | 0.8 | 2–3 | 2.2 | 0.7 | 1.9 |
| B | 14 | 3.5 | 9.4 | 6.2 | < 2 | < 0 | 0.8 | 2.3 | 4.5 | 0.4 | 2.3 |
| C | 14 | 4.0 | 7.8 | 6.2 | 2.8 | < 0 | 0.8 | 2–3 | 4.3 | 0.4 | 2.2 |
| D | 16 | 5.0 | 8.8 | 6.1 | 5.2 | 0.9 | 1.1 | 2–3 | 1.7 | 0.5 | 0.7 |
| E | 16 | 4.2 | 8.2 | 6.1 | 6.1 | 0.8 | 0.5 | 1–2 | 3.7 | 0.2 | 1.6 |
| F | 20 | 4.1 | 7.1 | 6.2 | 4.8 | < 0 | 2.1 | 2.4 | 4.7 | 0.7 | 1.6 |
| G | 21 | 3.9 | 10.4 | 6.1 | 5.5 | 0.6 | 0.4 | 1–2 | 5.7 | 0.1 | 1.9 |
| H | 21 | 5.4 | 8.5 | 6.2 | 5.9 | < 0 | 0.3 | 1–2 | 3.8 | 0.1 | 1.3 |
| I | 21 | 5.2 | 9.3 | 6.3 | 6.0 | 0.9 | 0.9 | 3.2 | 5.0 | 0.3 | 1.7 |
| J | 21 | 8.9 | 8.8 | 6.3 | 3.9 | < 0 | 0 | < 1 | 0 | 0 | 0 |
| K | 23 | 4.4 | 9.2 | 6.2 | 3.9 | < 0 | 0 | < 1 | 6.2 | 0 | 1.9 |
| L | 43 | 3.7 | 11.1 | 6.2 | 2.5 | < 0 | 0 | < 1 | 7.7 | 0 | 1.3 |
| M | 49 | 5.8 | 7.5 | 6.2 | 3.6 | < 0 | 1.1 | 2.7 | 7.7 | 0.2 | 1.1 |

1.

Numbers indicated as a range, that is, 2 to 3, were determined by a semiquantitative method. Numbers indicated with one decimal point were determined by a quantitative method.
Modified from Dalgaard and Jørgensen 1998. Predictions based on Food MicroModel using lot characteristics. WPS = water phase salt; WPL = water phase lactate

1991; Jemmi and Keusch 1992; Hudson and Mott 1993; Jin and others 1994; Nilsson and others 1997, 1999). Some variation in the growth rates was reported. For example, some studies have found that levels may increase from $10^3$ to $10^5$ logs in a few weeks (Farber 1991; Jin and others 1994; Nilsson and others 1997, 1999), while in other studies a similar increase takes several weeks (Rørvik and others 1991; Hudson and Mott 1993). Variations in strains and preculture conditions (that is, with or without adaptation to the saline, cold environment) may explain some of these differences.

One should be cautious, though, about drawing definite conclusions from inoculated studies which significantly overestimate both real growth rates and maximum cell numbers. Growth in naturally contaminated samples of cold-smoked fish does not follow the predictions from the inoculation studies (Dalgaard and Jørgensen 1998). Jørgensen and Huss (1998) reported that initial numbers of *L. monocytogenes* in cold-smoked salmon were < 10 cells/g (53 out of 64 positive) and only 2 samples (of 32 positive) contained between $10^3$ and $10^4$ cfu/g after 3 to 7 wk of storage. Cortesi and others (1997) did not find growth of *L. monocytogenes* in naturally contaminated cold-smoked salmon stored up to 5 wk at 2 or 10 °C (36 or 50 °F). In 380 samples of smoked fish, 27 were positive for *L. monocytogenes* (Teufel and Bendzulla 1993). Of these, 4 samples contained numbers between 100 and 10000, and 4 samples contained levels above 10000 cells of *L. monocytogenes*/g.

Current predicting models for growth of *L. monocytogenes* are based on combinations of salt, temperature, and atmosphere. The appropriateness of these models for cold-smoked fish is questionable, because other parameters may influence the behavior of the organism, that is, structure, smoking, drying, and associated microflora. Also, the physiological state of cells naturally contaminating the product (having been exposed to drying, freezing, cleaning, and sanitizing agents) is likely to be different than that of cells used for inoculation experiments. A combination of these factors may explain the discrepancy seen between levels predicted by models and actual levels reached in fish products.

Following the growth of *L. monocytogenes* in 10 different batches of cold-smoked salmon, both maximum numbers and average growth rate were much lower than predicted by Food MicroModel (UK) based on the water phase salt (WPS), pH, and atmosphere (Table II-5). As outlined below, several factors may explain this lack of growth, one being the competitive action of the natural lactic acid bacterial flora. Rørvik and others (1991) also noted that even inoculated strains grew slowly if salmon with a high background flora was used for the study.

Keeping initial numbers low is important in order to limit numbers at, for example, time of spoilage. Thus, Rørvik (2000) reports that concentrations of *L. monocytogenes* in stored product (3 wk at 5 °C, 41 °F) were below 100 cfu/g if levels on the freshly produced product were less than 100/g. In contrast, if levels in the freshly produced product were 300–400 cfu/g, *L. monocytogenes* grew to $3 \times 10^4$ cfu/g during the same storage period.

## 5. Source of contamination

Listeriosis seems to be caused almost exclusively by industrialized, highly processed foods. Therefore, in recent years, much attention has been focused on the prevalence of the bacterium in raw materials and food products and to tracing its contamination routes in modern food processing plants. An important prerequisite for control of *L. monocytogenes* is the knowledge and understanding of its niches during food production. Several studies have shown that *L. monocytogenes* is able to reside in food processing plants ranging from poultry production (Wenger and others 1990; Lawrence and Gilmour 1995; Ojeniyi and others 1996), meat processing industries (Nesbakken and others 1996; Giovannacci and others 1999), ice cream plants (Miettinen, Bjorkroth, Korkeala and others 1999), shrimp peeling operations (Destro and others 1996), " gravad" (Autio and others 1999), and smoked-fish (Rørvik and others 1995; Norton, McCamey and others 2000; Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001θ) processing plants.

Early studies of contamination routes depended solely on isolating and counting the organism at different places along the processing line (Eklund and others 1995; Jemmi and Keusch 1992). Recent studies have been greatly facilitated by the use of molecular typing methods of high discriminatory power. These have included Pulsed Field Gel Electrophoresis (PFGE) (Autio and others 1999; Destro and others 1996; Miettinen, Bjorkroth, Korkeala 1999; Ojeniyi and others 1996), ribotyping (Norton, McCamey and others 2000; Norton, Timothe, and others 2000) and Randomly Amplified Polymorphic DNA (RAPD) profiles (Lawrence and Gilmour 1995; Destro and others 1996; Wagner and others 1999; Fonnesbech Vogel, Jorgensen and others 2001; Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001). Comparing

DNA-types of *L. monocytogenes* isolates from raw fish to finished products allows identification of spots along the whole processing line where contamination of finished product occurs.

Conclusions from published studies attempting to identify the source of *L. monocytogenes* contamination vary. In studies of meat products, some authors (Giovannacci and others 1999) found that the raw materials were the source of product contamination with *L. monocytogenens*. Eklund and others (1995) reached a similar conclusion in their study of cold-smoked salmon where the raw fish entering the plant was identified as the primary source of *L. monocytogenes*. Several other studies (Autio and others 1999; Johansson and others 1999; Wenger and others 1990; Rørvik and others 1995; Fonnesbech Vogel and others 2001a,b; Dauphin and others 2001) have found that the major source of direct product contamination is the process environment and equipment (Autio and others 1999; Johansson and others 1999; Wenger and others 1990; Rørvik and others 1995; Fonnesbech Vogel, Jorgensen and others 2001θ; Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001). Rørvik and others (1995) investigated 475 samples from raw fish, water, products, and environment of a cold-smoked salmon processing plant. *Listeria monocytogenes* could not be detected in any of 50 raw fish samples, whereas almost one third of samples from the smokehouse products and environment were positive. Using multilocus enzyme electrophoresis (MEE) to compare the strains, a particular electrophoretic type (ET6) that was found in the finished product was repeatedly isolated from the smokehouse environment. Similar conclusions were reached by Autio and others (1999), who used PFGE to compare strains of *L. monocytogenes* isolated from a cold-smoked trout processing plant. In this study prevalence on raw fish was also low, with only 1 in 60 samples being positive. The PFGE type of these raw fish strains was different from the dominant type found in the product (type I). On the other hand, type I was isolated from several areas of the plant, pointing to the environment as a source of contamination. Dauphin and others (2001) used PFGE to trace contamination of *L. monocytogenes* in three cold-smoked salmon processing plants. In plant I, one pulsotype dominated in the plant and was detected in the product even though no *L. monocytogenes* were found in the raw fish. In plant II, 87% of the raw fish were contaminated; however, no *L. monocytogenes* were detected in the final product.

In a recent study, *L. monocytogenes* strains were isolated from products from 6 Danish smoke houses, some of which were sampled on two occasions with a time interval of 6–8 mo (Jørgensen and Huss 1998). RAPD-typing of these strains revealed that different DNA-types were found in products from different smoke houses, but that the same DNA-type was found in products from the same smoke house over this period of time (Fonnesbech Vogel, Jorgensen, and others 2001θ). These data indicated that the same type or clone persisted in a particular smoke house over time. A similar conclusion was reached by Norton, McCamey and others (2000) who visited 5 United States smoke houses over a 6-mo period. Using ribotyping, the authors found that each smoke house harbored its own specific ribotype(s) of *L. monocytogenes* (Table II-6).

Two out of the 6 Danish smoke houses were selected for further study over a 4-year period (Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001). Raw fish and environmental samples were tested at two repeated visits. As shown in Table II-7, in plant II, one DNA-type dominated in the finished product over the 4-year period. That particular DNA-type was also isolated from the slicing lines, whereas other DNA-types were found in the raw fish handling room. In plant I, the prevalence of *L. monocytogenes* was lower than in plant II (Table II-8). The pattern was somewhat similar to the pattern of plant I, in that one particular DNA-type dominated the slicing areas and was re-isolated on the finished product. That type, however, was not found on the raw fish. In both plants, experiments were conducted in which samples were taken of 18 marked fish from beginning of processing until finished product. *Listeria monocytogenes* was not detected in the raw fish but could be found immediately after slicing (Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001). In a similar study, Norton, Timothe and others (2000) sampled from different U.S. smoke houses on repeated visits. Ribotyping showed that in one plant, a particular ribotype isolated from finished product could be traced to both the raw fish and the processing line, whereas in another plant the ribotype found in the product was only isolated from the processing environment, not from the raw fish.

**Table II-6. Prevalence of *Listeria monocytogenes* ribotypes in each processing facility over five sampling visits**

| | % prevalence | | |
|---|---|---|---|
| Ribotype | Processor B (129 samples) | Processor C (173 samples) | Processor D (229 samples) |
| 1039C | 0.0 | 0.0 | 10.0 |
| 1042B | 0.8 | 1.2 | 0.4 |
| 1042C | 6.2 | 0.6 | 0.4 |
| 1044A | 0.0 | 2.3 | 3.1 |
| 1045 | 5.4 | 0.0 | 0.9 |
| 1046B | 0.0 | 2.3 | 0.0 |
| 1053 | 0.0 | 0.6 | 1.7 |
| 1062 | 0.8 | 0.6 | 2.6 |

Norton, McCamey, and others 2000

The majority of published studies suggest that the processing line (with a "colonized" *L. monocytogenes* flora) is the immediate, direct source of *L. monocytogenes* in cold-smoked fish, as opposed to raw fish being the direct source of contamination in the final product. None of the studies, however, has determined the initial source of *L. monocytogenes*. *Listeria monocytogenes* may originate from the raw fish, which seeds the processing line, but may also be introduced by staff (2 to 6% of healthy individuals are carriers of the bacterium [cited from Rocourt and others, 2000]), or transporting devices entering the processing area. As mentioned above, however, some studies also implicate raw fish as the direct source of final product contamination. Based on current data, it is not possible to determine which of these are most important. Given the ubiquitous nature of *L. monocytogenes*, it is likely that many sources contribute to the entrance of the organism into the processing environment and that the most important source of immediate product contamination is the processing environment.

In support of this last scenario, *L. monocytogenes* is capable of adhering to food processing surfaces like stainless steel (Hood and Zottola 1997; Jeong and Frank 1994). In addition, *L. monocytogenes* cells in the adhering state may be more resistant to cleaning and disinfecting procedures than cells in the planktonic state (Wirtanen and Mattila-Sandholm 1992; Norwood and Gilmour 2000). For example, Fonnesbech Vogel, Ojeniyi, Ahrens and others (2001) repeatedly isolated the same organism from cleaned and disinfected smoke houses. Such resistance may explain the finding that the same DNA-types of *L. monocytogenes* can persist in a food processing plant over years (Miettinen, Bjorkroth, Korkeala 1999).

## 6. Control of *Listeria monocytogenes*

### 6.1. Control in the processing environment

The complete and indefinite erradication of *L. monocytogenes* from processing environments in which RTE foods are produced is currently considered impossible. As outlined above, this is the

**Table II–7. Number of samples, number of *Listeria monocytogenes* positive and Randomly Amplified Polymorphic DNA (RAPD) type of *L. monocytogenes* of processing plant II. P = product, R = raw fish, R-E = Raw fish environment, S-E = smoking environment, SI-E = Slicing environment. X = Unique types; isolated only once each (Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001)**

| | | Number of isolates with RAPD type | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RAPD type by HLWL 85 | | 1995 | 1996 | Nov. 1998 | | | | | | March 1999 | | | | | |
| | | P | P | R | R-E | S-E | SI-E1 | SI-E2 | P | R | R-E | S-E | SI-E1 | SI-E2 | P |
| 2 | | — | — | — | 4 | — | 3 | — | 7 | — | — | — | — | — | — |
| 3 | | — | — | — | 1 | — | — | — | — | — | 3 | — | — | — | — |
| 5 | | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 6 | | — | — | — | 36 | — | 1 | — | — | — | — | — | — | — | — |
| 7 | | 4 | — | — | 4 | — | — | — | — | — | 5 | — | — | — | 6 |
| 12 | | 25 | 12 | — | 1 | — | 63 | 37 | 7 | — | — | — | 6 | 2 | 2 |
| 13 | | — | — | — | 1 | — | 1 | — | — | — | — | — | — | — | — |
| 15 | | — | — | — | 6 | — | 10 | 1 | 1 | — | 6 | — | 3 | 1 | 7 |
| 110 | | — | — | — | — | — | — | 1 | — | — | — | — | — | — | — |
| x | | — | — | — | 2 | — | 2 | — | — | — | 3 | — | — | — | — |
| Nr of samples | positive for *L. monocytogenes* | 31 | 12 | 0 | 55 | 0 | 80 | 39 | 15 | 0 | 17 | 0 | 9 | 3 | 15 |
| | total | ?? | 20 | 36 | 239 | 8 | 150 | 147 | 40 | 12 | 105 | 2 | 75 | 100 | 48 |

**Table II-8. Number of samples, number of *Listeria monocytogenes* positive and Randomly Amplified Polymorphic DNA (RAPD) type of *L. monocytogenes* of processing plant I. P = product, R = raw fish, R-E = Raw fish environment, S-E = smoking environment, SI-E = Slicing environment. X = Unique types; isolated only once each (Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001)**

| | | Number of isolates with RAPD type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RAPD type by HLWL 85 | | 1995 | 1996 | Oct. 1998 | | | | | March 1999 | | | | |
| | | P | P | R | R-E | S-E | Sl.-E. | P | R. | R-E | S-E | Sl-E | P |
| 2 | | — | — | 1 | 25 | 5 | 7 | 8 | — | 2 | — | — | — |
| X | | — | — | — | 4 | — | 2 | — | — | 3 | — | — | 1 |
| Nr of samples | positive for *L. monocytogenes* | 0 | 0 | 1 | 29 | 5 | 9 | 8 | 0 | 5 | 0 | 0 | 1 |
| | total | 20 | 20 | 15 | 197 | 60 | 211 | 45 | 14 | 151 | 6 | 135 | 35 |

case not only for cold-smoked fish but for also for meat products. Therefore, even cooked RTE meat products, where a heat-step which kills *L. monocytogenes* is included, have a high prevalence of the organism. This high prevalence in cooked RTE products is attributed to postprocess contamination (Tompkin and others 1999). Recontamination following a heating step or increase in contamination on, for example, cold-smoked fish during processing is often the consequence of the establishment of *L. monocytogenes* in a particular niche, or "hot spot," in which the bacteria multiply and spread downstream (Tompkin and others 1999; Autio and others 1999; Fonnesbech Vogel, Ojeniyi, Ahrens 2001).

Under the HACCP concept, a critical control point is a point or process at which the organism of concern is eliminated, or combinations of preserving factors can guarantee that growth of the organism does not occur. Cold smoking does not include such a control point for *L. monocytogenes*. Therefore, reduction of *L. monocytogenes* to the lowest possible levels must rely on prerequisite programs adhering strictly to Good Hygienic Practices (GHPs) and Good Manufacturing Practices (GMPs). For more details, see guidelines by Tompkin and others (1999) and the Food and Agriculture Organization (FAO 1999) specifically for control of recontamination with *L. monocytogenes* in RTE foods. In general, focus must be on education of staff, cleaning and sanitation, redesign of equipment, and proper flow and separation in the processing plant.

In support of the implementation of these guidelines, Fonnesbech Vogel, Ojeniyi, Ahrens and others (2001) found that the prevalence of *L. monocytogenes* could be dramatically reduced in a smoke house by strictly adhering to GMPs and by targeting spots where the organism had been found to reside with appropiate cleaning and disinfection procedures. Despite the isolation of sporadic positive samples, long-time persistence was eliminated, as indicated by the variations of RAPD types.

The following are areas of upmost importance in the environmental control of *L. monocytogenes*:

**6.1.1. Training of staff** to understand the necessity of hygienic handling and avoidance of recontamination is of major importance. Industry experiences indicate that the more aware the staff is of the problem and hygienic practices, the lower the prevalence of *L. monocytogenes*.

**6.1.2. Reduction or elimination** of *L. monocytogenes* in the niches in which it becomes established is a continuous effort. Only a limited range of thorough investigations on *L. monocytogenes* contamination has been carried out in the smoked-fish industry; however, these indicate that particular attention should be paid to the following points:

- brine
- injection needles
- slicers

Several approaches may be used to reduce or eliminate the organism when found. Quaternary ammonium compounds have been found efficient against *L. monocytogenes*. Peracetic acid and peroctanoic acid have been effective against *L. monocytogenes* in biofilms (Tompkin and others 1999). If possible, disinfection using steam (that is, 80 °C, 176 °F for 60 min) is effective in eliminating the organism in processing plants (Autio and others 1999; Tompkin and others 1999).

Other areas along the processing line where the product comes in direct contact with surfaces, for example conveyor belts, should also be closely monitored. Experience has shown that floors and particularly floor drains often harbor *L. monocytogenes*. Attention must be paid to cleaning these areas. Spreading citric acid powder on the floor (to pH of 5) may reduce numbers (Tompkin and others 1999). This procedure, however, should

only be used if corrosion or other damage to equipment can be avoided. Foot baths with disinfectants can help reduce the spread of the bacterium in this particular environment.

**6.1.3. Monitoring of contamination** with *L. monocytogenes* must be part of the quality check of the processing plant. Some plants may not want to test for the pathogen directly. In those cases, *Listeria* spp. has been found to be a useful indicator organism.

It is the opinion of this panel that cold-smoked fish products consistently free from *L. monocytogenes* cannot be produced; however, by adhering to GMPs (including training staff), it is possible to reduce prevalence. Smoke houses with strict adherence to GMPs are capable of producing cold-smoked salmon with very low levels of *L. monocytogenes*, often less than 1 cell/g. Although not eliminated, such low levels would ensure that the number of *L. monocytogenes* does not increase to above 100 cfu/g at time of consumption given that appropriate temperature (5 °C) and time (3 to 4 wk) limits are met.

### 6.2 Prevention of growth in the product

Based on levels of *L. monocytogenes* in smoked fish and the number of cases of listeriosis in Germany, Buchanan and others (1997) developed a dose-response relationship for the organism and listeriosis. Although a threshold value could not be indicated, a linear relationship was seen between the logarithm of (assumed) ingested numbers and of the logarithm of probability of disease. It was concluded that the risk of contracting listeriosis was extremely low from foods containing low numbers (< 100 cfu/g). In addition, the model predicted that 198 of 200 yearly cases of listeriosis resulted from ingestion of samples containing $10^4$ (or more) *L. monocytogenes*/g. Based on their analysis, the authors concluded that "the initial focus for risk-management decisions should be the prevention of the growth of this pathogen in foods to high levels" (Buchanan and others 1997).

Because the combination of salt and temperature used in smoked-fish products is not sufficient to guarantee "no growth," several studies have evaluated the growth-inhibitory effect of principles other than salt and low temperature. Lately, several physical methods (irradiation, high pressure, pulsed light) have been suggested as general decontamination procedures (Lucore and others 2000; Thayer and Boyd 1999; Rowan and others 1999); however, these procedures remain to be evaluated in real products and therefore will not be addressed in this section. The following text focuses on procedures and principles that have been evaluated against *L. monocytogenes* in substrates mimicking fish or in real products.

#### 6.2.1 Frozen storage

Frozen storage is an efficient way to completely prevent growth of *L. monocytogenes*. If the product is vacuum-packaged, frozen storage will have few adverse effects on sensory quality (that is, lipid oxidation). One study has reported growth of *L. monocytogenes* at –1.5 °C (29 °F) in roast beef, but the generation time at this temperature was 129 h and other studies have found minimum temperature for growth at 0.1°C to 1.1 °C (32 to 34 °F) (Farber and Peterkin 2000).

#### 6.2.2. Carbon dioxide

Carbon dioxide has an inhibitory effect on *L. monocytogenes*. Levels of 70 to 100% $CO_2$ cause a significant increase in lag phase as well as a reduction in growth rate at chill temperatures (Hendricks and Hotchkiss 1997; Nilsson and others 1997; Szabo and Cahill 1998). Given the limited growth in naturally contaminated samples, it is likely that $CO_2$ packing would offer complete elimination of growth of the organism at low temperatures. The use of 70% $CO_2$ for packaging cold-smoked salmon was found not to impart any changes in sensory quality (Nilsson and others 1997). A predictive model describing the influence of $CO_2$, pH, NaCl and incubation temperature on growth of *L. monocytogenes* has been developed and validated in selected foods (Fernandez and others 1997).

Regardless of its potential to control *L. monocytogenes*, the vast majority of smoked fish plants today use vacuum packing. Changing to $CO_2$-packing would require investing in new equipment. Also, the volume/pack would increase, which may increase transportation costs. For these reasons, $CO_2$-packing currently may not be a feasible control method. Nonetheless, the panel recommends investigating the technological requirements to introduce this packaging method on a broader scale.

#### 6.2.3. Nitrite

Nitrite (at 200 ppm) has, in combination with salt, an inhibitory effect against *L. monocytogenes* at 5 °C (41 °F), where no increase in cell numbers was seen when nitrite was added. In contrast, at 10 °C (50 °F) growth occurred almost at the same rate with and without nitrite (Pelroy, Peterson, Paranjpye and others 1994). Sodium nitrite used in combination with salt and sodium lactate increased the inhibitory effect (Pelroy, Peterson, Holland and others 1994).

#### 6.2.4. Lactate

Experiments have been conducted on lactate added to raw, salted salmon. Growth of *L. monocytogenes* was completely inhibited by 2% lactate at 5 °C (41 °F), whereas 3% lactate was required to inhibit growth at 10 °C (50 °F) (Pelroy, Peterson, Holland and others 1994). While lactate is used as a flavor enhancer in some products, it is not known what the sensory effect of lactate would be on smoked fish. Also, it may be difficult to adsorb 2% lactate into the water phase of the fish.

#### 6.2.5. Sorbate

Sorbate is Generally Recognized as Safe (GRAS) and therefore may be used as an additive. No studies have been published on the effect of sorbate on growth of *L. monocytogenes* in cold-smoked fish. El-Shenawy and Marth (1988) reported that 0.05% sorbate at pH 5.6 caused a marked reduction in growth rate of *L. monocytogenes* at 4 °C (39 °F). In control samples growth increased from $10^3$ to $10^8$ cells in 24 d, whereas a count of $10^6$ was reached after 45 d when sorbate was added. In vacuum-packed crayfish stored at 4 °C, 0.3% potassium sorbate caused a lag phase of 2 d in *L. monocytogenes*, but growth rate was not affected (Dorsa and others 1993). In different types of meat sausage products (which typically include salt and nitrite), addition of potassium sorbate delays or inhibits growth of the organism (Hu and Shelef 1996). As with lactate, the application of sorbate may be difficult from a technological point of view.

#### 6.2.6. Bacteriocins

Innumerable studies have determined the antilisterial effect of lactic acid bacteria and their bacteriocins. For a recent overview of this, see Farber (2000). *Listeria monocytogenes* has been inhibited by nisin and divercin in fish products, by nisin and enterocin in dairy products, by nisin and pediocin in meat products, and by pediocin in vegetable products (Nilsson 1999). Nilsson and others (1997) found that the addition of 1000 ppm nisin to cold-smoked salmon caused an initial reduction in numbers of *L. monocytogenes*, but after 2 wk, growth in vacuum packs at 5 °C (41 °F) resumed as in the control. Duffes and others (1999) similarly found that nisin caused a significant delay in growth of *L. monocytogenes* on vacuum-packaged, cold-smoked salmon. The inhibition was less pronounced at 8 °C (46 °F) than at 4 °C (39 °F). Combining nisin with 70% $CO_2$ packaging eliminated

growth of the bacterium in cold-smoked salmon (Nilsson and others 1997). This was later explained by the synergistic effect of nisin and $CO_2$ both acting on the cell membrane of the bacterium (Nilsson and others 2000). French studies have demonstrated that bacteriocins from *Carnobacterium* spp. are efficient in preventing growth of *L. monocytogenes* on cold-smoked salmon (Duffes and others 1999) (Figure II-1). While the inhibitory effect of several bacteriocins against *L. monocytogenes* is well documented, practical application of bacteriocins will be hampered by several aspects. First, only some countries allow the use of bacteriocins for certain products. Second, bacteriocins' stability and activity in food products are unpredictable (bacteriocins may be degraded by proteases or absorb to food matrix components). Finally, resistance to bacteriocins in *L. monocytogenes* has been shown to occur quite readily.

### 6.2.7. Background microflora

Several studies have shown that growth of *L. monocytogenes* in smoked fish is hampered by a high background microflora (Rørvik and others 1991). The deliberate use of nonpathogenic, nonspoiling lactic acid bacteria to control *L. monocytogenes* is a promising area of targeted preservation. Growth of *L. monocytogenes* could be significantly delayed in brined shrimp by the addition of a *Lactobacillus saké/curvatus* strain (From Jeppesen and Huss 1993). In a pork product, an *L. saké* strain caused a reduction in maximum cell density of *L. monocytogenes* of 6 log units compared to the control (lactic acid bacteria was not added to the control) (De Martinis and Franco 1998). Despite strong antilisterial activity, the strain used in brined shrimp was not suited for use on cold-smoked salmon, as it produced pronounced sulfur off odors. In contrast, *Carnobacterium piscicola* and *Carnobacterium divergens* have no adverse effects on the sensory quality (Duffes and others 1999; Nilsson and others 1999; Paludan-Müller and others 1998). Several laboratories have shown that carnobacteria inhibit growth of *L. monocytogenes* in food products (Buchanan and Klawitter 1992) and that growth of *L. monocytogenes* in cold-smoked salmon can be completely inhibited by addition of *C. piscicola* or *C. divergens* (Duffes and others 1999; Nilsson and others 1999).

## 7. Conclusions

Based on the scientific data available, the panel concludes that:

- Given the ubiquitous nature of *L. monocytogenes*, the lack of listericidal steps in the cold-smoking procedure, and the ability of the organism to become established in the processing environment and recontaminate products, it is not possible to produce cold-smoked fish consistently free of *L. monocytogenes*. This is not unique to cold-smoked fish because this microorganism can be isolated from a wide range of ready-to-eat (RTE) foods.
- By adhering strictly to GMPs and GHPs, it is possible to produce cold-smoked fish with low levels of *L. monocytogenes*, preferably at < 1 cell/g at the time of production.
- Growth of *L. monocytogenes* in naturally contaminated fish products is significantly slower than predicted by models (using combinations of pH, NaCl, temperature, and lactate) and inoculation studies.
- Prevention of growth in cold-smoked fish cannot be guaranteed using current combinations of NaCl and low temperatures; however growth can be prevented by freezing. Other alternatives (for example, lactate, nitrite or bioprotective cultures) are currently being researched.
- If the organism cannot be eliminated and growth inhibiting steps are not introduced, the hazard must be controlled by limiting shelf life (at 4.4 °C, 40 °F) to ensure that no more than 100 cells/g are present at time of consumption. The limitation of shelf life has been suggested by several investigators (Rørvik 2000, Nørrung 2000, Farber 2000). Time limits may need to be established by each processor, reflecting the initial level of the organism in freshly produced product.
- Some countries, such as Australia, warn pregnant women about listeriosis and offer a list of food items to be avoided during the pregnancy. Labels on cold-smoked fish as well as other RTE foods indicating that these products may constitute a health hazard for immuno-compromised individuals and pregnant women could be considered.

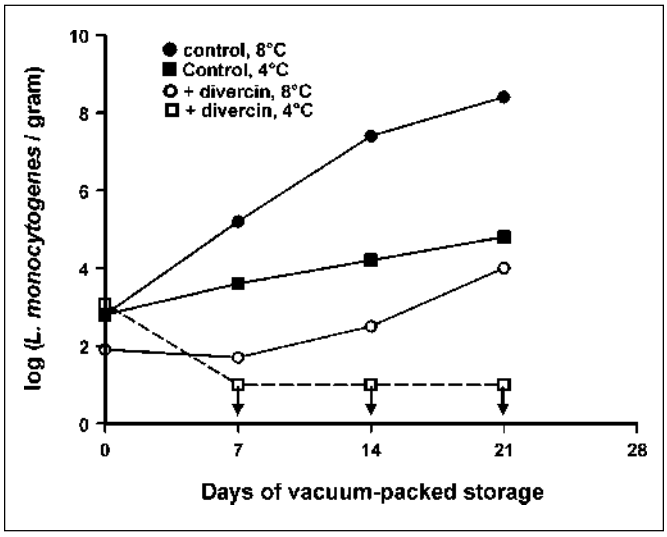



**Figure II-1—Growth of *Listeria monocytogenes* in vacuum-packed, cold-smoked salmon at 4 or 8 °C with or without the addition of crude bacteriocin from a *Carnobacterium divergens* (data from Duffes and others 1999)**

## 8. Research needs

The panel concluded that research is needed to:

- Conduct epidemiological investigations to determine if and to what extent cold-smoked fish is involved in cases of listeriosis. Despite prediction of a risk, only a limited number of cases have been associated with cold-smoked fish.
- Assess the virulence potential of *L. monocytogenes* isolated from cold-smoked fish.
- Measure behavior of *L. monocytogenes* in naturally contaminated products. *Listeria monocytogenes* appears to grow more slowly and to lower numbers than anticipated based on model predictions and inoculation trials. An understanding of which factors cause these differences may be used to design appropriate control measures in the product.
- Determine the robustness and applicability of alternative growth inhibitory measures such as bioprotective cultures, bacteriocins, lactate and others.
- Determine how *L. monocytogenes* becomes established in the smoke houses and processing facilities. Several studies show that particular DNA-types become established in niches in the processing environments. Research is needed to evaluate what parameters determine which types reside; whether it be particular adhesion properties, particular resistance properties, or other factors.
- Investigate the source of contamination in smoke houses and processing environments in order to introduce procedures specifically targeted at eliminating or limiting introduction of the organism.
- Identify GMP practices that would minimize the contamination and growth of *L. monocytogenes*.

- Determine the effectiveness of intervention strategies to reduce or eliminate *L. monocytogenes*, such as using chlorinated water to thaw and rinse incoming fish, and for rinsing fish following the brining operation.
- Develop cleaning and disinfection procedures targeted at adhered or established cells for removal of *L. monocytogenes* from surfaces.
- Determine if particular types of surfaces reduce numbers of adhering *L. monocytogenes* or if particular treatments (that is, spraying with lactic acid bacteria or lactate) can reduce surface contamination by minimizing adhesion and biofilm formation.
- Evaluate the robustness and the sensory acceptability of various procedures under investigation (that is, bioprotection, lactate, and so on) for the elimination of growth in the cold-smoked fish product.
- Determine the effectiveness of postprocessing methods such as irradiation and high pressure for the elimination of *L. monocytogenes* in cold-smoked fish.

## References


[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. [York (PA)]: Association of Food and Drug Officials. 7 p.

Autio T, Hielm S, Miettinen M, Sjoberg A-M, Aarnisalo K, Bjorkroth J, Mattila-Sandholm T, Korkeala H. 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Appl Environ Microbiol 65(1):150–5.

Barakat RK, Harris LJ. 1999. Growth of *Listeria monocytogenes* and *Yersinia enterocolitica* on cooked modified-atmosphere-packaged poultry in the presence and absence of a naturally occurring microbiota. Appl Environ Microbiol 65(1):342–5.

Ben Embarek PK. 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. Int J Food Microbiol 23:17–34.

Ben Embarek PK, Huss HH. 1993. Heat resistance of *Listeria monocytogenes* in vacuum packaged pasteurized fish fillets. Int J Food Microbiol 20:85–95.

Bremer PJ, Osborne CM. 1998. Reducing total aerobic counts and *Listeria monocytogenes* on the surface of king salmon (*Oncorhynchus tshawytscha*). J Food Prot 61:849–54.

Brett MSY, Short P, McLauchlin J. 1998. A small outbreak of listeriosis associated with smoked mussels. Int J Food Microbiol 43:223–9.

Buchanan RL, Damert WG, Whiting RC, van Schothorst M. 1997. Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for *Listeria monocytogenes* levels and incidence of listeriosis. J Food Prot 60(8):918–22.

Buchanan RL, Klawitter LA. 1992. Effectiveness of *Carnobacterium piscicola* LK5 for controlling the growth of *Listeria monocytogenes* Scott A in refrigerated foods. J Food Saf 12:219–36.

Cortesi ML, Sarli T, Santoro A, Murru N, Pepe T. 1997. Distribution and behavior of *Listeria monocytogenes* in three lots of naturally-contaminated vacuum-packed smoked salmon stored at 2 and 10° C. Int J Food Microbiol 37:209–14.

Dalgaard P, Jorgensen LV. 1998. Predicted and observed growth of *Listeria monocytogenes* in seafood challenge tests and in naturally contaminated cold-smoked salmon. Int J Food Microbiol 40:105–15.

Dauphin G, Ragimbeau C, Malle P. 2001. Use of PFGE typing for tracing contamination with *Listeria monocytogenes* in three cold-smoked salmon processing plants. Int J Food Microbiol 64:51–61.

De Martinis ECP, Franco BDGM. 1998. Inhibition of *Listeria monocytogenes* in a pork product by a *Lactobacillus sake* strain [research communication]. Int J Food Microbiol 42:119–26.

Destro MT, Leitao MFF, Farber JM. 1996. Use of molecular typing methods to trace the dissemination of *Listeria monocytogenes* in a shrimp processing plant. Appl Environ Microbiol 62(2):705–11.

Dillon R, Patel T, Ratnam S. 1994. Occurrence of *Listeria* in hot and cold-smoked seafood products. Int J Food Microbiol 22:73–7.

Dorsa WJ, Marshall DL, Semien M. 1993. Effect of potassium sorbate and citric acid sprays on growth of *Listeria monocytogenes* on cooked crawfish (*Procambarus clarkii*) tail meat at 4 °C. Lebensm Wiss u Technol 26:480–2.

Duffes F, Corre C, Leroi F, Dousset X, Boyaval P. 1999. Inhibition of *Listeria monocytogenes* by in situ produced and semipurified bacteriocins on *Carnobacterium* spp. on vacuum-packed, refrigerated cold-smoked salmon. J Food Prot 62(12):1394–1403.

Eklund MW, Poysky FT, Paranjpye RN, Lashbrook LC, Peterson ME, Pelroy GA. 1995. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. J Food Prot 58(5):502–8.

El-Kest SE, Yousef AE, Marth EH. 1991. Fate of *Listeria monocytogenes* during freezing and frozen storage. J Food Sci 56(4):1068–71.

Elliot EL, Kvenberg JE. 2000. Risk assessment used to evaluate the US position on *Listeria monocytogenes* in seafood. Int J Food Microbiol 62:253–60.

El-Shenawy MA, Marth EH. 1988. Inhibition and inactivation of *Listeria monocytogenes* by sorbic acid. J Food Prot 51:842–7.

Ericsson H, Eklow A, Danielsson-Tham ML, Loncarevic S, Mentzing LO, Persson I, Unnerstad H, Tham W. 1997. An outbreak of listeriosis suspected to have been caused by rainbow trout. J Clin Microbiology 35(11):2904–7.

[FAO] Food and Agriculture Organization. 1999 May. Report of the FAO expert consultation on the trade impact of *Listeria* in fish products. Rome: FAO. FAO Fisheries Report nr 604. 34 p.

Farber JM. 1991. *Listeria monocytogenes* in fish products. J Food Prot 54(12):922–4, 934.

Farber JM. 2000. Present situation in Canada regarding *Listeria monocytogenes* and ready-to-eat seafood products. Int J Food Microbiol 62:247–51.

Farber JM, Peterkin PI. 1991. *Listeria monocytogenes*, a foodborne pathogen. Microbiol Rev 55:476–511.

Farber JM, Peterkin PI. 2000. *Listeria monocytogenes*. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of foods. Gaithersburg (MD): Aspen. p 1178–1232.

Farber JM, Ross WH, Harwig J. 1996. Health risk assessment of *Listeria monocytogenes* in Canada. Int J Food Microbiol 30:145–56.

Fenlon DR, Wilson J, Donachie W. 1996. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. J Appl Bacteriol 81:641–50.

Fernandez PS, George SM, Sills CC, Peck MW. 1997. Predictive model of the effect of CO2, pH, temperature and NaCl on growth of *Listeria monocytogenes*. Int J Food Microbiol 37:37–45.

Fonnesbech Vogel B, Jorgensen LV, Ojeniyi B, Huss HH, Gram L. 2001. Diversity of *Listeria monocytogenes* isolates from cold-smoked salmon produced in different smoke houses as assessed by randomly amplified polymorphic DNA analyses. Int J Food Microbiol 65:83–92.

Fonnesbech Vogel B, Ojeniyi B, Ahrens P, Due Skov L, Huss HH, Gram L. 2001. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Appl Environ Microbiol. Forthcoming.

Giovannacci I, Ragimbeau C, Queguiner S, Salvat G, Vendeuvre J-L, Carlier V, Ermel G. 1999. *Listeria monocytogenes* in pork slaughtering and cutting plants use of RAPD, PFGE, and PCR-REA for tracing and molecular epidemiology. Int J Food Microbiol 53(1999):127–40.

Heinitz ML, Johnson JM. 1998. The incidence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* in smoked fish and shellfish. J Food Prot 61(3):318–23.

Hendricks MT, Hotchkiss JH. 1997. Effect of carbon dioxide on the growth of *Pseudomonas fluorescens* and *Listeria monocytogenes* in areobic atmospheres. J Food Prot 60(12):1548–52.

Hood SK, Zottola EA. 1997. Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int J Food Microbiol 37:145–53.

Hu AC, Shelef LA. 1996. Influence of fat content and preservatives on the behavior of *Listeria monocytogenes* in beaker sausage. J Food Saf 16:175–81.

Hudson JA, Mott SJ. 1993. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cold-smoked salmon under refrigeration and mild temperature abuse. Food Microbiol 10:61–8.

Huss HH, Ben Embarek PK, From Jeppesen V. 1995. Control of biological hazards in cold-smoked salmon production. Food Control 6(6):335–40.

Iida T, Kanzaki M, Nakama A, Kokubo Y, Maruyama T, Kaneuchi C. 1998. Detection of *Listeria monocytogenes* in humans, animals, and foods. J Vet Med Sci 60(12):1341–3.

Jemmi T. 1990. Zum vorkommen von *Listeria monocytogenes* in importierten geraucherten und fermentierten fischen. Arch Lebensmittelhyg 41:107–9.

Jemmi T, Keusch A. 1992. Behavior of *Listeria monocytogenes* during processing and storage of experimentally contaminated hot-smoked trout. Int J Food Microbiol 15:339–46.

Jemmi T, Keusch A. 1994. Occurrence of *Listeria monocytogenes* in freshwater fish farms and fish-smoking plants. Food Microbiol 11:309–16.

Jeong DK, Frank JF. 1994. Growth of *Listeria monocytogenes* at 10° C in biofilms with microorganisms isolated from meat and dairy processing environments. J Food Prot 57(7):576–86.

Jeppesen V, Huss HH. 1993. Antagonistic activity of two strains of lactic acid bacteria against *Listeria monocytogenes* and *Yersinia enterocolitica* in a model fish product at 5° C. Int J Food Microbiol 19:179–86.

Jin M, Kusunoki K, Ikejima N, Arai T, Irikura Y, Suzuki K, Hirata I, Kokubo Y, Maruyama T. 1994. Incidence of *Listeria moncytogenes* in smoked salmon. Jpn J Food Microbiol 11(2):107–11.

Jinneman KC, Wekell MM, Eklund MW. 1999. Incidence and behavior of *Listeria monocytogenes* in fish and seafood. In: Ryser ET, Marth ELH, editors. *Listeria*, listeriosis and food safety. New York: M Dekker. p 601–30.

Johansson T, Rantala L, Palmu L, Honkanen-Buzalski T. 1999. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. Int J Food Microbiol 47:111–9.

Jorgensen LV, Dalgaard P, Huss HH. 2000. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. J Agric Food Chem 48:2448–53.

Jorgensen LV, Huss HH. 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. Int J Food Microbiol 42:127–31.

Lawrence LM, Gilmour A. 1995. Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Appl Environ Microbiol 61(6):2139–44.

Lucore LA, Shellhammer TH, Yousef AE. 2000. Inactivation of *Listeria monocytogenes* Scott A on artificially contaminated frankfurters by high-pressure processing. J Food Prot 63(5):662–4.

McLauchlin J. 1997. The pathogenicity of *Listeria monocytogenes*: a public health perspective. Rev Med Microbiol 8(1):1–14.

Miettinen MK, Bjorkroth KJ, Korkeala HJ. 1999. Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. Int J Food Microbiol 46:187–92.

Miettinen MK, Siitonen A, Heiskanen P, Haajanen H, Bjorkroth KJ, Korkeala HJ. 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. J Clin Microbiol 37(7):2358–60.

Nesbakken T, Kapperud G, Caugant DA. 1996. Pathways of *Listeria monocytogenes* contamination in the meat processing industry. Int J Food Microbiol 31:161–71.

Nilsson L. 1999. Control of *Listeria monocytogenes* in cold-smoked salmon by biopreservation [DPhil thesis]. Lyngby: Technical University of Denmark, Danish Institute for Fisheries Research. 136 p.

Nilsson L, Chen Y, Chikindas ML, Huss HH, Gram L, Montville TJ. 2000. Carbon dioxide and nisin act synergistically on *Listeria monocytogenes*. Appl Environ Microbiol 66(2):769–74.

Nilsson L, Gram L, Huss HH. 1999. Growth control of *Listeria monocytogenes* on cold-smoked salmon using a competitive lactic acid bacteria flora. J Food Prot 62(4):336–42.

Nilsson L, Huss HH, Gram L. 1997. Inhibition of *Listeria monocytogenes* on cold-smoked salmon by nisin and carbon dioxide atmosphere. Int J Food Microbiol 38:217–27.

Norrung B. 2000. Microbiological criteria for *Listeria monocytogenes* in foods under special consideration of risk assessment approaches. Int J Food Microbiol 62:217–21.

Norton DM, McCamey MA, Gall KL, Scarlett JM, Boor KJ, Wiedmann M. 2000. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry and implications for control strategies. Forthcoming.

Norton DM, Sue D, Timothe J, Scarlett JM, Boor KJ, Wiedmann M (Cornell Univ, Ithaca, NY). 2000. Characterization and pathogenic potential of *L. monocytogenes* isolates from the smoked fish industry [abstract]. Abstract submitted for presentation at the 2000 Annual Meeting of the American Society of Microbiology (ASM).

Norwood DE, Gilmour A. 2000. The growth and resistance to sodium hypochlorite of *List-*

*eria monocytogenes* in a steady-state multispecies biofilm. J Appl Microbiol 88:512–20.
Notermans S, Dufrenne J, Teunis P, Chackraborty T. 1998. Studies on the risk assessment of *Listeria monocytogenes*. J Food Prot 61(2):244–8.
Ojeniyi B, Wegener HC, Jensen NE, Bisgaard M. 1996. *Listeria monocytogenes* in poultry and poultry products: epidemiological investigations in seven Danihs abattoirs. J Appl Bacteriol 80:395–401.
Paludan-Muller C, Dalgaard P, Huss HH, Gram L. 1998. Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum- and modified packed cold-smoked salmon stored at 5° C. Int J Food Microbiol 39:155–66.
Pelroy GA, Peterson ME, Holland PJ, Eklund MW. 1994. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium lactate. J Food Prot 57(2):108–13.
Pelroy GA, Peterson ME, Paranjpye R, Almond J, Eklund M. 1994. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium nitrite and packaging method. J Food Prot 57(2):114–9.
Peterson ME, Pelroy GA, Paranjpye RN, Poysky FT, Almond JS, Eklund MW. 1993. Parameters for control of *Listeria monocytogenes* in smoked fishery products: sodium chloride and packaging method. J Food Prot 56(11):938–43.
Rocourt J, Jacquet C, Reilly A. 2000. Epidemiology of human listeriosis and seafood. Int J Food Microbiol 62:197–209.
Rorvik LM. 2000. *Listeria monocytogenes* in the smoked salmon industry. Int J Food Microbiol 62:183–90.
Rorvik LM, Caugant DA, Yndestad M. 1995. Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. Int J Food Microbiol 25:19–27.
Rorvik LM, Yndestad M. 1991. *Listeria monocytogenes* in foods in Norway. Int J Food Microbiol 13:97–104.
Rorvik LM, Yndestad M, Skjerve E. 1991. Growth of *Listeria monocytogenes* in vacuum-packed, smoked salmon during storage at 4° C. Int J Food Microbiol 14:111–8.
Rowan NJ, MacGregor SJ, Anderson JG, Fouractre RA, McIlvaney L, Farish O. 1999. Pulsed-light inactivation of food-related microorganisms. Appl Environ Microbiol 65:1312–5.
Seeliger HPR, Jones D. 1986. Genus Listeria Pirie 1940, 383al. In: Sneath PHA, Mair SN, Sharpe ME, Holt JG, editors. Bergey's Manual of Systematic Bacteriology. 9th ed. Baltimore (MD): Williams and Wilkins. p 1235–45.
Sunen E. 1998. Minimum inhibitory concentration of smoke wood extracts against spoilage and pathogenic micro-organisms associated with foods. Letts Appl Microbiol 27:45–8.
Szabo EA, Cahill ME. 1998. The combined affects of modified atmosphere, temperature, nisin and ALTA[tm] 2341 on the growth of *Listeria monocytogenes*. Int J Food Mircobiol 43:21–31.
Teufel P, Bendzulla C. 1993. Bundesweite Erhebung zum vorkommen von *L. monocytogenes* in Lebenmitteln. Berlin: Bundesinstitut fur gesundheitlichen Verbraucherschutz und Veterinarmedizin. NB.
Thayer DW, Boyd G. 1999. Irradiation and modified atmosphere packaging for the control of *Listeria monocytogenes* on turkey meat. J Food Prot 62:1136–42.
Tompkin RB, Scott VN, Bernard DT, Sveum WH, Gombas KS. 1999. Guidelines to prevent postprocessing contamination from *Listeria monocytogenes*. Dairy, Food Environ San 19(8):551–62.
Truelstrup Hansen L, Drewes Røntved S, Huss HH. 1998. Microbiological quality and shelf life of cold-smoked salmon from three different processing plants. Food Microbiology 15:137–50.
Wagner M, Maderner A, Brandl E. 1999. Development of a multiple primer RAPD assay as a tool for phylogenetic analysis in *Listeria* spp. strains isolated from milkproduct associated epidemics, sporadic cases of listeriosis and dairy environments. Int J Food Microbiol 52:29–37.
Weagant SD, Sado PA, Colburn KG, Torkelson JD, Stanley FA, Krane MH, Shields SC, Thayer CF. 1988. The incidence of *Listeria* species in frozen seafood products. J Food Prot 51:655–7.
Wenger JD, Swaminathan B, Hayes PS, Green SS, Pratt M, Pinner RW, Schuchat A, Broome CV. 1990. *Listeria monocytogenes* contamination of turkey franks: evaluation of a production facility. J Food Prot 53(12):1015–9.
Wiedmann M, Bruce JL, Keating C, Johnson AE, McDonough PL, Batt CA. 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infec Immun 65(7):2707–16.
Wirtanen G, Mattila-Sandholm T. 1992. Removal of foodborne biofilms-comparison of surface and suspension tests. Part I. Lebensm Weiss Technol 25(1):43–9.

CHAPTER III

# Potential Hazards in Cold-Smoked Fish: *Clostridium botulinum* type E

LONE GRAM

## Scope

*Clostridium botulinum* type E is an indigenous organism in the aquatic environment and is the type mainly associated with botulism from seafood products. This section outlines what is currently known about the organism and its behavior in cold-smoked fish. Major findings of importance to the safety of cold-smoked fish and the control of *C. botulinum* type E are included. While the section provides some of the data relevant for a science-based risk assessment, it does not give complete information on all four components: hazard identification, hazard characterization, exposure assessment, and risk characterization. Recent book chapters about the organism in Lund and Peck (2000) and Dodds and Austin (1997) cover these issues in greater depth. This section ends with lists of conclusions and research needs.

## 1. Introduction

*Clostridium botulinum* comprises a range of Gram-positive, anaerobic, spore-forming bacteria that produce botulinum neurotoxin. The bacteria are vastly different on a phylogenetic scale and only similar in toxins and disease pattern. The toxins (as well as the microorganism) have been divided into types A, B, C, D, E, F, and G depending on their antigenic properties. Only the confusion that would arise upon renaming them has prevented a redistribution of the different types into different bacterial species (Lund and Peck 2000; Dodds and Austin 1997).

Botulism may be caused by four different scenarios: a) foodborne botulism which results from ingestion of toxin preformed in foods, b) infant botulism in which spores are ingested, germinate, and lead to toxin formation in the intestine, c) wound botulism and d) adult botulism caused by intestinal germination and growth. In general, botulism occurs today only rarely; indeed, the latter scenario is extremely rare. The botulinum neurotoxins, however, are the most potent toxins known. Before anti-toxins for treatment became available, mortality was as high as 75%. Due to the severity of the disease, research has been directed to understanding the ecology, toxin production, and pathogenicity of the different types and strains. Much has been learned about the ecology of these organisms and about prevention of growth and toxin-production in foods. Two of the *C. botulinum* groups are responsible for foodborne botulism. Group I *C. botulinum* is proteolytic and mesophlic, and strains produce toxins of types A, B or F. Group II *C. botulinum* is nonproteolytic and psychrotrophic, and strains produce toxins of types B, E or F. Group II *C. botulinum,*, and in particular strains that produce neurotoxin of type E, is a major concern for cold-smoked fish. In this document, these strains will be referred to as *C. botulinum* type E. One reason for the emphasis on strains that produce type E toxin is that these strains appear to be truly aquatic. It is important to recognize that controls for *C. botulinum* type E will also be valid for strains of Group II *C. botulinum* that produce toxins of types B or F.

Many research groups focused their activities on *C. botulinum* type E following a range of outbreaks of botulism from hot-smoked freshwater fish in the 1960s and 1970s. The more general findings described below are cited from chapters by Lund and Peck (2000), Dodds and Austin (1997), Eklund (1992), and Huss (1981).

*Clostridium botulinum* type E is a Gram-positive, anaerobic, spore-forming foodborne pathogen. As mentioned, it is part of the psychrotrophic, nonproteolytic group of *C. botulinum*, which also comprises *C. botulinum* types B and F. Disease is caused by a neurotoxin produced in the food product. Spores are widely distributed in the aquatic environment and occur naturally, albeit at low levels, in many raw aquatic foods (Lund and Peck 2000). The organism is strictly anaerobic and sensitive to oxygen. *Clostridium botulinum* type E can grow and produce toxin at temperatures of 3.0 to 3.3 °C (37 to 38 °F) or in up to 5% NaCl (water phase salt, WPS). Toxin production at these limits, however, requires that all other growth conditions are optimal. Thus, for example, toxin production is inhibited at lower salt concentrations than 5% if the temperature is lower than optimum. The tolerance to lowering of water activity depends on the solute; thus, the $a_w$ minimum is 0.97 when NaCl is used and 0.94 when glycerol is used. The organism is acid sensitive and does not grow below pH 5.0. In conclusion, as a consequence of its capabilities to grow at low temperature and its resistance to salt, it may, if anoxic conditions are created, germinate and produce toxins in many food products with extended chilled shelf lives and cause foodborne botulism.

The toxins produced by all of the different *C. botulinum* types are heat labile. The heat resistance of *C. botulinum* type E toxin depends on the pH; the toxin is destroyed by moderate heating at neutral pH and is more resistant at lower pH values (pH 4.0–5.0). Thus, the toxin was destroyed after 5 min at 60 °C (140 °F) in a cooked meat medium (pH 7.5) (cited from Huss 1981) but at 62 °C (144 °F) and 65 °C (149 °F) in meat broth (pH 6.2) (Abrahamsson and others 1965). Woodburn and others (1979) investigated the heat inactivation of several botulinum toxins and found that in canned corn at pH 6.2, a 3D reduction occurred in 2 min at 74 °C (165 °F). In phosphate buffer, a similar reduction occurred in 1 min at pH 6.8 but took 6 min if 1% gelatin was added to the buffer (Woodburn and others 1979). In general, heat treatments used for food preparation are sufficient to destroy the toxin; however, it would not be considered acceptable to rely on cooking to inactivate any *C. botulinum* toxin that may be present in food. Type E toxin is resistant to NaCl; it is stable for weeks in 26% NaCl (Huss 1981).

*Clostridium botulinum* type E can be isolated from water, aquatic sediments, and aquatic organisms. In general, numbers of the microorganism in fish are low, ranging from 1 to 2 spores to a few hundred per kg. Some studies have found higher levels, such as 2000 to 3000 spores/kg (Lund and Peck 2000). Because the disease caused by *C. botulinum* type E is an intoxication, low numbers of spores are not considered a hazard as such, but products allowing germination, growth, and toxin production

must be evaluated carefully to determine the combinations of parameters that can either eliminate the organism, for example by heat treatment, or guarantee that growth and toxin production do not occur within the shelf life of the product.

The risk of toxin formation of *C. botulinum* in cold-smoked fish must be taken seriously, because the processing of cold-smoked fish does not involve a heating step that eliminates the spores (or any vegetative cells). Cold-smoked fish is often vacuum-packed. Low levels of salt and low temperatures are used only as a means of preservation. Numerous studies, models and predictions (see 4.3 and 4.4 below), however, have documented that appropriate combinations of NaCl (water activity) and low temperature are sufficient to guarantee that no growth of the organism occurs within the shelf life of the product under vacuum. Considering the amount of the scientific evidence, the combination of salt and refrigeration can be considered critical parameters to control *C. botulinum* growth and toxin production when stored under vacuum.

Lightly preserved fish products, particularly fermented fish, have been linked to cases of botulism (Lund and Peck 2000). In most cases, the cause of the disease has been linked to home processing or faulty processing by small producers. Hot-smoked fish has led to several outbreaks. As outlined by Eklund (1992), however, the products were typically underprocessed, often with NaCl of less than 1%; vacuum-packed; and grossly temperature-abused during distribution. In contrast, the panel is unaware of outbreaks of botulism involving cold-smoked fish.

## 2. Prevalence in water, raw fish, and smoked fish

*Clostridium botulinum* type E is a truly indigenous organism of the aquatic environment (Table III-1 and Dodds and Austin 1997). Spores are detected at different levels in sediments. In some areas of the world, such as Great Britain and Ireland, *C. botulinum* type B is more commonly isolated. It has been shown in studies on the Pacific Coast of the United States that *C. botulinum* type E is the most frequently isolated type off the coast of Alaska, Washington and Oregon. Off the coast of South Carolina, however, nonproteolytic *C. botulinum* types B and F were isolated more frequently than *C. botulinum* type E (Eklund 1992). Due to the widespread occurrence in aquatic environments, *C. botulinum* type E can also be isolated from fresh fish (Table III–2). Levels are typically low, although high levels have been detected in one sample of Danish fish (Huss and others 1974). In this case, fish were sampled from trout reared in mud ponds during the time when wet feed was allowed. Both of these factors are believed to have increased numbers of *C. botulinum*.

Only a limited number of studies have determined real-life prevalence of *C. botulinum* in cold-smoked fish. Heinitz and Johnson (1998) sampled from 201 vacuum-packaged smoked fish and shellfish products and did not detect spores of *C. botulinum* in any package. Nielsen and Pedersen (1967) found 1.7% positive samples in cold-smoked salmon with a level of < 1 spore/kg. Hyytiä and others (1998) found that 3% of 64 samples of cold-smoked rainbow trout were positive, with numbers between 40 and 290 spores/kg. In the latter case (Hyytiä and others1998), detection was based on PCR of the E toxin gene, whereas the other studies relied on classical mouse test detection of the toxin, which may contribute to the different results obtained. In hot-smoked fish studies from the mid 1960s, between 1% and 20% of samples were positive with levels of 1–20 MPN/kg. Hot-smoked fish seem to present a higher risk than cold-smoked fish with respect to *C. botulinum* (Southcott and Razzell 1973). As outlined by Eklund (1992), this may be due to severe underprocessing. Also, the heating step in the hot-smoking process may act as a spore-activating step, inducing germination and posing a greater risk than without a heating step.

**Table III–1. Prevalence of *Clostridium botulinum* type E in freshwater and seawater sediments**

| Sampling location | Sample size, g | % positive | MPN[2]/kg |
|---|---|---|---|
| *Freshwater* | | | |
| Green Bay (USA) | 1 | 77 | 100000 to 36000000 |
| Rivers leading into Green Bay (USA) | ? | 5 to 50 | 10000 |
| Not specified (Denmark) | 10 | 86 | 20 to 800 |
| Lake Washington (USA) | 5 | 91 | 18400 to 35000 |
| *Seawater* | | | |
| Coastal area (Scandinavia) | 6 | 100 | More than 780 |
| Caspian Sea (Iran) | 2 | 17[1] | 93 |
| Pacific coast (USA) | 5 | 53 | ? |
| Coast (Canada) | 10 | 0.6 | 40 to 280 |
| Bellingham Bay (USA) | 5 | 50 to 93 | 540 to 32000 |
| Not specified (Denmark) | 10 | 92 | 800 to 350 000 |

[1]a few strains of type B isolated
[2]MPN, most probable number
Modified from Dodds 1993, Huss 1981, Huss 1980, Sugiyama and others 1970, Eklund and Poysky 1967

**Table III–2. Prevalence of *Clostridium botulinum* Type E in fish (modified from Dodds and Austin 1997, Huss 1981, Hyytiä and others 1998, Huss and others 1974, Nickerson and others 1967, Cann and others 1966)**

| Sampling location | Sample size, g | % positive | MPN[2]/kg |
|---|---|---|---|
| Whitefish | 10 | 12 (also C) | 14 |
| Flounder, vacuum-packed, frozen | 1.5 | 10 | 70 |
| Rockfish, dressed | 10 | 100 | 2400 |
| Cod, whiting, flounder | ? | 0.40 | — |
| Salmon, smoked | 20 | 2 (type B) | < 1 |
| Carp, salted | 2 | 63 | 490 |
| Cod, haddock a.o. | 10 intestines | 4.5 | 40 to 100 |
| Trout, farmed | whole fish | 5 to 100 | 340 to 5300 |
| Trout, herring | 80 to 234 | 0 | — |
| Herring | whole fish | 45 to 65 | 5 to 60 |
| Rainbow trout, cold-smoked | 100 to 200 | 3[1] | 40 to 290 |
| Rainbow trout, fresh | 100 to 200 | 15 to 20[1] | 30 to 1900 |

[1]Determined by PCR-detection of the E toxin gene
[2]MPN, maximum probable number

## 3. Growth in refrigerated smoked fish

Innumerable studies have documented that fish is an excellent substrate for *C. botulinum* type E. Several trials have specifically evaluated how the combination of low temperature and atmosphere (that is, $N_2$ or $CO_2$) influence toxin production as compared to spoilage patterns. The majority of these studies have been done with fresh (nonsalted, nonsmoked) fish. Recent reviews contain more information on these studies (Gram and Huss 2000, Lund and Peck 2000).

Similarly, a vast range of studies has shown that hot-smoked and cold-smoked fish are good substrates for *C. botulinum* and that the organisms may grow and produce toxin, depending on salt and temperature levels. As outlined in Chapter II, growth in naturally contaminated products may be different from growth in inoculated samples. Few studies have evaluated growth and toxin production in naturally contaminated samples of cold-smoked fish. Due to the large outbreaks in the 1960s, several trials have evaluated growth and toxin production in hot-smoked fish. Hot-smoking is a different process from cold smoking (60 to 70 °C [140 to 158 °F] in contrast to 20 to 30 °C [68 to 86] °F, internal temperatures). The resulting product is different in structure (that is, the proteins are heat coagulated) and microflora. Cann and Taylor (1979) prepared hot-smoked whole trout from fish naturally contaminated with *C. botulinum*. Levels of *C. botulinum* were not reported, but the trout were from a farm where the prevalence consistently was 80% or above (Cann and others

**Table III-3. Growth and toxin production by psychrotrophic *Clostridium botulinum* depending on salt, temperature, and pH**

| Inoculum | Medium | Temperature °C | Salt, % WPS | pH | Time to toxicity | Time to growth | Reference |
|---|---|---|---|---|---|---|---|
| $10^4$/ml | Two-phase meat medium | 5 °C | 2.0 | 6.2 | — | 4 wk | Graham and others 1997 |
| | | | 3.0 | 5.5 to 6.0 | — | > 13 wk | |
| | | | | 6.5 | 10 wk | 10 wk | |
| | | | 3.5 | 5.5 to 7.0 | > 13 wk | > 13 wk | |
| | | 10 °C | 3.0 | 6.0 | — | 2 wk | |
| | | | | 6.5 to 7.0 | — | 1 wk | |
| | | | 3.5 | 6.0 | — | 12 wk | |
| | | | | 6.4 to 7.0 | — | 2 wk | |
| $10^5$/ml | Meat medium | 5 °C | 2.5 | 6.5 | 24 to 27 d | 24 to 27 d | Graham and others 1996a |
| | | | 4.3 | 6.5 | > 104 d | > 104 d | |
| | | 8 °C | 2.5 | 6.5 | 8 to 9 d | 8 to 9 d | |
| | | | 4.3 | 6.5 | 14 to 17 d | 14 to 17 d | |
| $10^5$/ml | TPG medium | 8 °C | 2.5 | 7.0 | — | 14 d | Segner and others 1966 |
| | | | 3.0 | 7.0 | — | 14 to 16 d | |
| | | | 3.5 | 7.0 | — | 21 to 23 d | |
| $10^5$/ml | Peptone-sucrose-medium | 5 °C | 3.0 | 7.2 | — | > 180 d | Emodi and Lechowich 1969 |
| | | 10 °C | 4.5 | 7.2 | — | 31 d | |
| $10^2$/g and $10^4$/g | Hot-smoked salmon | 25 °C | > 3.8 | ns | > 2 wk | | Pelroy and others 1982 |
| Natural level | Hot-smoked fish | 10 °C | 2.0 | ns | 30 d[1] | | Cann and Taylor 1979 |
| | | | 3.0 | | > 30 d | | |
| $10^2$/g | | | 3.0 | | > 30 d | | |
| $10^3$/g | Hot-smoked fish | 15 °C | ? | ? | 5 d | | Huss and others 1980 |
| $3 \times 10^3$/g | Hot-smoked fish | 27 °C | 3.5 to 5.2 | ns | > 35 d | | Cuppett and others 1987 |
| $10^2$/g | Cold-smoked trout | 4 °C | 1.7 | 6.1 to 6.3 | > 28 d | | Dufresne 2000 |
| | | 8 °C | 1.7 | 6.2 to 6.3 | > 28 d | | |
| | | 12 °C | 1.7 | 6.4 to 6.5 | 14 d | | |
| ns[2] | Laboratory media | 5 °C | 2.0 | ns | 50 d | | McClure and others 1994 |
| | | | 3.0 | | 60 d | | |
| | | 10 °C | 3.0 | | 18 d | | |

[1]only ungutted fish
ns = not stated

1975). The fish were salted to different levels, vacuum-packed, and stored at 10 °C (50 °F). At 2.5% NaCl, no toxin was detected after 30 d of storage at 10 °C (50 °F). At 2.0% NaCl, only products produced from whole, ungutted fish became toxic. In addition, ungutted fish have caused botulism outbreaks in salted fish such as Kapchunka (Anonymous 1985, 1987).

In recent Finnish studies (Hyytiä and others 1999), vacuum-packed, cold-smoked rainbow trout inoculated with less than 10 *C. botulinum* spores/g became toxic after 3 wk at 8 °C (46 °F) or after 4 wk at 4 °C (39 °F) with a NaCl concentration of 3.2% (WPS). This is a rapid toxin formation compared to other studies, in particular considering the low spore level and the salt/temperature combinations (Table III-3). Surprisingly, the authors did not detect growth of the bacterium. Graham and others (1996a), who studied the growth and toxin production by *C. botulinum* using a high inoculum at different combinations of pH, NaCl, and heat activation, concluded that "on no occasion was toxin detected under conditions which did not result in visible growth." In a later study, Graham and others (1997) did detect toxin formation in a few samples where growth was not detectable as turbidity in the tube. The authors (Hyytiä and others 1999) offer some suggestions for the difference between their results and those of other studies (sampling problems, micro-niches, inconsistent NaCl distribution). Currently, no clear conclusions can be reached and further studies are required.

## 4. Effect of processing steps and preservation parameters

### 4.1. Freezing

Frozen storage does not affect spores but is an efficient way to completely prevent germination and growth of *C. botulinum*. If the product is vacuum-packaged, frozen storage will have little adverse effects on sensory quality (that is, lipid oxidation).

### 4.2. Cold smoking

Although the smoking process uses temperatures at which *C. botulinum* grows well, cold smoking is a highly aerated process; therefore, no growth of the organism occurs during this processing step.

### 4.3. Combinations of salt and low temperature

In combination, salt and low temperature are the two major factors controlling growth of psychrotrophic *C. botulinum* in cold-smoked fish. This section attempts to draw data and conclusions from a wide range of studies that have evaluated the inhibitory potential of these two factors. To draw conclusions, the following points on the methodology of the studies should be considered:

- Due to the technical difficulty in achieving low levels of spores, high numbers (often exceeding the natural levels by several orders of magnitude) have been used in most studies. This will overestimate the risk because probability of outgrowth becomes greatly reduced at low spore levels. The use of high inocula can, however, be justified as an introduction of a safety margin.
- Studies are based on inoculation trials. It is possible that growth of the natural population is slower or more inhibited than that of the inoculated strains. This has been documented for *L. monocytogenes* (see Chapter II). In contrast, culture collection strains may be less adapted to the food environment than naturally occurring strains.

Psychrotrophic *C. botulinum* will, under otherwise optimal conditions, grow in up to 5% WPS ($a_w \leq 0.97$); however, at reduced temperatures, less salt is tolerated. Also, pH influences salt tolerance. NaCl-concentrations of less than 5% are required to inhibit growth, as pH is reduced from neutral (pH ≤ 7.0) (Table III-3). The pH of cold-smoked salmon varies between 6 and 6.3. In general, pH of fish varies between 6 and 7. It has been

**Table III-4. Toxin production in hot-smoked herring surface inoculated with *Clostridium botulinum* type E spores ($10^3$/g). Some fish were also surface inoculated with $10^3$/g of spoilage bacteria (*Shewanella putrefaciens*). Fish were packed in air or vacuum-packed and stored at 15 °C (59 °F).**

| Spoilage bacterium | Packaging | Hg Eh, mV | $O_2$ mm | Nr of toxic samples (two sampled on each day) after storage at 15°C | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1[1] | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| - | Air | +250–0 | 120–80 | nt[2] | nt | nt | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 2 |
| - | vacuum | +200–0 | 30–20 | nt | 0 | 0 | 0 | 2 | 2 | 2 | nt | nt | nt | nt | nt | nt | Nt | nt | nt |
| + | Air | +200–+150 | 130–100 | nt | nt | 0 | 0 | 1 | 2 | 2 | 2 | nt | nt | nt | nt | nt | Nt | nt | nt |
| + | vacuum | +200–0 | 0 | nt | nt | 0 | 1 | 2 | 2 | 2 | 2 | nt | nt | nt | nt | nt | Nt | nt | nt |

[1]Days of storage
[2]nt = not tested
From Huss and others 1980

suggested that the high redox potential (Eh) in some fish (caused by the presence of trimethylamine oxide) can render the bacterium more susceptible to NaCl than under conditions of low Eh (Huss 1980).

Several studies have evaluated the time for growth and/or toxin production of psychrotrophic *C. botulinum* as related to temperature, salt and pH. A compilation of data relevant for cold-smoked fish is given in Table III-3. The studies can be divided into studies using laboratory media and, typically, high inocula (Segner and others 1966, McClure and others 1994; Graham and others 1996a, 1996b, 1997), and studies using fish, typically with lower inocula (Cann and Taylor 1979; Cuppett and others 1987; Dufresne and others 2000). The levels of spores used in fish inoculation studies, however, are still several magnitudes of order above natural levels (compare Tables III-2 and III-3).

The inhibition of the formation of botulinum toxin by psychrotrophic strains can be ensured using a minimum level of 5% NaCl (WPS). Combining NaCl control with chill storage can prevent toxin formation for certain periods of time. In laboratory media using high inocula ($10^4$–$10^5$ spores/ml), toxin growth is prevented for at least 2 to 3 wk at 2% to 2.5% NaCl and for at least 4 to 5 wk at 3% to 3.5% NaCl at 5 °C (41 °F). At 10 °C (50 °F), 2% to 2.5% NaCl prevents toxin growth for at least 1 wk and 3% to 3.5% NaCl for at least 1 to 2 wk. Some studies (McClure and others 1994, Emodi and Lechowich 1969) report longer "safe periods" than those just stated.

It is striking that studies using fish as substrate have shown that similar salt/temperature combinations result in significantly longer time to growth and toxin production. Thus, at 1.7% NaCl, toxin was not detected for 4 wk in cold-smoked trout stored at 4 °C (39 °F) or 8 °C (46 °F) (Dufresne and others 2000) and at 2.5% to 3.5% NaCl, toxin was not detected in hot-smoked trout stored at 10 °C (Cann and Taylor 1979). The model studies using laboratory media would point to very restricted safe storage times, for example, maximum 4 wk at 5 °C with 3.5% NaCl and only 1 wk at 10 °C with 3.5% NaCl. Based on the fish studies, the history of cold-smoked fish not being involved in cases of botulism, a level of 3.5% NaCl appears sufficient to prevent toxin formation for up to 4 wk at chill temperatures. Thus, the recommendation by the Advisory Committee on the Microbiological Safety of Foods (ACMSF 1992) was that 3.5% NaCl (WPS throughout the product) results in a safe (from psychtrotrophic *C. botulinum*) product for at least 4 wk at 4 °C to 5 °C. This panel therefore concludes that 3.5% NaCl (WPS) combined with a maximum storage time of 4 wk at 40 °F (4.4 °C) will result in *C. botulinum*-safe products of vacuum-packed, cold-smoked fish. Similar, although less specific in terms of storage time, conclusions have been reached by Eklund (1992) and Huss and others (1995).

### 4.4. Atmosphere

*Clostridium botulinum* is an anaerobic organism and is sensitive to oxygen. Sensitivity to redox potential (Eh) is not as pronounced. Therefore, growth and toxin production may occur at high Eh if compounds other than $O_2$ are used to establish a positive Eh (Lund and Peck 2000). Due to the intolerance to $O_2$, most attention has been paid to vacuum- and $CO_2$-packaged products. Many studies have documented that $O_2$ removal enhances toxin formation (Eklund 1992), but several studies have found that toxicity may also occur with oxygen present (Table III–4). Thus, Huss and others (1980) found that air-packaging delayed toxin formation by *C. botulinum* type E in hot-smoked herring stored at 15 °C (59 °F), compared to vacuum-packaging if the fish were handled under aseptic conditions and *C. botulinum* type E was able to grow and form toxin under 100% $O_2$ atmosphere. Kautter (1964) also reported that toxin could be produced without packaging. In fish contaminated with aerobic spoilage bacteria, toxicity occurred after 4 to 5 d when vacuum-packed, compared to 5 to 6 d when air-packed (Table III-4). Thatcher and others (1962) reported that hot-smoked fish packed in plastic wrappers had caused cases of botulism. They, therefore, investigated the influence of atmosphere on toxin formation in fish surface inoculated with $10^3$ spores/g. After 8 d at 30 °C (90 °F), both samples incubated under anaerobic and aerobic conditions were toxic. In a study of spoilage and botulinum toxin formation in cold-smoked trout, Dufresne and others (2000) found that at 8 °C (46 °F), fish packed in high $O_2$-transmission films became toxic before fish packed in low $O_2$-transmitting films (Table III-5 and 6). As implied in these studies, although there is no doubt that vacuum-packing and $CO_2$-packing may enhance toxin formation, aerobic packaging or the inclusion of $O_2$ in modified atmosphere packaging (MAP) cannot be relied upon as a safeguard. ACMSF (1992), an advisory body reporting to the Department of Health under the UK-Ministry of Agriculture, Fisheries, and Food (UK-MAFF), concluded on the safety hazards of *C. botulinum* in vacuum-packed foods: "It is now recognized that the growth of *C. botulinum* in foods does not depend on the total exclusion of oxygen, nor does the inclusion of oxygen as a packaging gas ensure that growth of *C. botulinum* is prevented. Anaerobic conditions may occur in microenvironments in foods that are not vacuum- or modified-atmosphere packaged. For example, in the flesh of fish, conditions which are favorable to toxin production can exist in air-packaged fish as well as in vacuum- or modified atmosphere-packaged fish."

In cold-smoked fish, aerobic conditions lead to faster spoilage than under vacuum- or MA-packaging (Table III–5). Under aerobic conditions pseudomonads, yeast, and some lactic acid bacteria develop, whereas anoxic packaging conditions result in development of a lactic acid bacteria flora with a minor component of Gram-negative bacteria. Typically, shelf life is reduced by a factor of 1.5 to 2 by aerobic storage as compared to vacuum-packed storage (Table III–6).

The United States requires that vacuum-packed, cold-smoked fish contain 3.5% NaCl (water phase) or 3.0% if combined with 200 ppm nitrite. Only 2.5% NaCl is required of aerobically packed fish,

**Table III-5. Shelf life (determined by sensory assessment) of cold-smoked fish depending on temperature and packaging atmosphere**

| | oxygen transmission | WPS | Shelf life, days | | | | |
|---|---|---|---|---|---|---|---|
| Packaging | cc/m²/d/atm | % | 4 °C | 5 °C | 8 °C | 10 °C | Reference |
| vacuum; riloten film 20/60 | 4[1] | 4–5 | | 21 to 28 | | 14 | From Jeppesen 1988 |
| aerobic; polyethylene 70 | "oxygen permeable" | 4–5 | 28 | 14 | | 7 to 14 | From Jeppesen 1988 |
| | 12[2] | 1.7 | 28 | | 14 | | Dufresne 2000 |
| | 2950[2] | 1.7 | 22 | | 13 | | |
| | 4920[2] | 1.7 | 18 | | 16 | | |
| | 10040[2] | 1.7 | | | 6 | | |

[1]at 75% RH, 25 °C
[2]at 0% RH, 24 °C
From Jeppesen 1988, Dufresne and others 2000

**Table III-6. Sensory shelf life (odor) and time to botulinum toxin formation (inoculated with $10^2$ spores of *Clostridium botulinum*/g) in cold-smoked trout with 1.7% NaCl (WPS) stored at 4 (39 °F) or 8 °C (46 °F) in packaging films with different oxygen transmission rate**

| Temp. | $O_2$-transmission cc/m²/d/atm at 24 °C, 0% RH | Shelf life, days | Nr of toxic samples (2 sampled) after 7, 14, 21 and 28 d | | | |
|---|---|---|---|---|---|---|
| | | | 7 | 14 | 21 | 28 |
| 4 °C | 12 | ~28 | 0 | 0 | 0 | 0 |
| | 2950 | ~28 | 0 | 0 | 0 | 0 |
| | 4920 | 22 | 0 | 0 | 0 | 0 |
| | 10040 | 18 | 0 | 0 | 0 | 0 |
| 8 °C | 12 | 14 | 0 | 0 | 0 | 0 |
| | 2950 | 13 | 0 | 0 | 0 | 0 |
| | 4920 | 16 | 0 | 0 | 0 | 1 |
| | 10040 | 6 | 0 | 0 | 0 | 1 |
| 12 °C | 12 | 11 | 0 | 1 | 2 | 1 |
| | 2950 | 12 | 0 | 1 | 2 | 2 |
| | 4920 | 11 | 0 | 1 | 2 | 2 |
| | 10040 | 6 | 0 | 1 | 2 | 2 |

Modified from Dufresne and others 2000

which spoil more rapidly. No clear definition of an aerobic pack exists. The more rapid spoilage—not the presence of oxygen—is relied upon as a safeguard against *C. botulinum*. Recent data by Dufresne and others (2000) showed that in aerobic-packaged, cold-smoked trout (with 1.7% WPS) stored at 8 °C (46 °F), toxin formation occurred more rapidly when packaged under high $O_2$-transmission than under low $O_2$-transmission. The data emphasize that although spoilage did occur more rapidly under the highest $O_2$ transmitting film (10000 cc/m2/d/atm at 24 °C, 0% RH), toxin formation also occurred more rapidly, and oxygen was no safeguard against botulinum toxin formation.

### 4.5. Nitrite

Nitrite has for some time been used in cured meats and some fish. It is an efficient anti-botulinogenic compound. The effect of nitrite is influenced by pH, NaCl, and temperature. The exact mechanism of its effect is not known. Nitrate, probably via conversion to nitrite, inhibits or delays botulinum toxin formation. The addition of nitrite may reduce the amounts of NaCl required to inhibit *C. botulinum* toxin formation (Pelroy and others 1982). NaCl concentration may be reduced in vacuum-packed fish by adding 200 ppm nitrite. Due to concerns raised about the potential carcinogenic effects of nitrosamines, however, there is some reluctance to increase use of this compound.

### 4.6. Lactate

Lactate is inhibitory to psychrotrophic *C. botulinum*. Meng and Genigeorgis (1993) found that the lag phase of $10^4$ spores/sample of turkey roll was prolonged from 8 h to 28 h at 8 °C (46 °F) when 2% lactate was added. The effect of lactate was more pronounced with the concurrent addition of NaCl; adding 2% NaCl to the 2% lactate increased the lag phase to 58 d.

### 4.7. Sorbate

Sorbate is a GRAS compound and may therefore be used in cold-smoked fish. In some studies, sorbate has been reported to inhibit spore germination, whereas others have found no effect of 1% sorbate (at pH 6.7) on germination (cited from Kim and Foegeding 1993). Vegetative cell growth is inhibited by sorbate, particularly at low pH. For instance, sorbic acid is most effective at pH < 6.0–6.5 (Lund and others 1987, cited by Kim and Foegeding 1993).

### 4.8. Role of background microflora

As indicated in Table III–4, the aerobic microflora of some products may serve to enhance the risk of toxin formation, probably due to the depletion of $O_2$ by the respiration of the nonbotulinum microflora (Abrahamsson and others 1965, Huss and others 1980, ACMSF 1992). In contrast, the growth of some other microorganisms may inhibit growth and toxin production by *C. botulinum*. Thus, Lyver and others (1998) reported that certain *Bacillus* isolates were inhibiting toxin production. Also, lactic acid bacteria, either by acid or by bacteriocin production, may inhibit growth and toxin formation of *C. botulinum* (Kim and Foegeding 1993). Relying on competition from naturally occurring background flora to restrict *C. botulinum*, however, is not an effective or reproducible way of preventing growth.

## 5. Conclusions

The following conclusions are based on a thorough analysis and evaluation of the current science on control methods of *C. botulinum* in cold-smoked fish:

- Psychrotrophic *C. botulinum* occurs naturally in the aquatic environment, so its presence in low numbers on fresh fish must be anticipated. Spores may also be isolated infrequently from cold-smoked fish, although numbers, if present, are low. Given this low number, the probability of germination and toxin production is low but present.
- Experiments with naturally contaminated hot-smoked fish produced from fish with high levels of *C. botulinum* show that toxin may be formed under conditions of temperature abuse.
- Toxin production by psychrotrophic *C. botulinum* is controlled with a combination of a moderate level of NaCl (3.5% NaCl WPS) and storage at chill temperature (< 4.4 °C, < 40 °F) for at least 4 wk. Based on the scientific data and because commercially produced cold-smoked fish has never been reported as a source of botulism, it is reasonable to conclude that the salt and cold keep the hazard under adequate control.
- Based on a range of model studies in broth and inoculation studies with hot- or cold-smoked fish, it can be concluded that a combination of 3.5% NaCl (WPS) and chill storage (4.4 °C, 40 °F), allowing for short periods of elevated temperatures up to 10 °C (50 °F), will prevent toxin formation in reduced oxygen packaging cold-smoked fish for several weeks beyond its sensory shelf life.
- As a general safeguard, salting to 3.5% (WPS) for chilled, stored cold-smoked fish is essential for reduced oxygen packaged (ROP) cold-smoked fish. The requirement for chilling with a

sufficient salt concentration is an option to considered in national or international regulations (for example, E.U. directives).

● For air-packaged products, levels of NaCl can, theoretically, be reduced; however, scientific data that support this argument do not exist and are needed before any reduction is recommended. Even when not packed under vacuum- or modified atmosphere, pockets of anaerobic conditions may be created where slices of fish overlap or where aerobic spoilage bacteria consume the oxygen present.

## 6. Research needs

The following is a list of research areas that the panel suggests need further attention:

● Evaluate growth and toxin production in naturally contaminated cold-smoked fish products to validate models and predictions for growth and toxin production.

● Determine the influence of redox potential, various concentrations of trimethylamine oxide (TMAO), and NaCl on toxin production by psychrotrophic *C. botulinum* in gadoid and nongadoid species.

● Determine the potential facilitation by TMAO on formation of nitrosamines, if nitrite is added, during cold smoking.

● Identify processing conditions and gas transmission rates of films under various time/temperature conditions for products to be considered "air packaged." Determine the oxygen transmission rates (OTR) needed for a product with 2.5% salt concentration to provide equivalent safety compared with cold-smoked ROP products.

● Conduct challenge studies on air-packaged, cold-smoked fish in films with OTR between 7000 and 10000 cc/$m^2$/24 h and compare to unpackaged cold-smoked fish.

● Establish minimum WPS concentrations required to inhibit growth and toxin formation by *C. botulinum* in air-packaged and unpackaged cold-smoked fish.

● Determine the shelf life of the product, relative to product quality as well as safety, under different packaging methods and storage temperatures.

● Determine appropriate sell-by dates and evaluate the use of time-temperature indicators to ensure a safe product.

## References


Abrahamsson K, De Silva NN, Molin N. 1965. Toxin production by *Clostridium botulinum*, type E, in vacuum-packed, irradiated fresh fish in relation to the changes to the associated microflora. Can J Microbiol 11:523–9.

[ACMSF] Advisory Committee on the Microbiological Safety of Foods. 1992. Report on vacuum packaging and associated processes. London (UK): Her Majesty's Stationery Office.

Anonymous. 1985. Botulism associated with commercially distributed Kapchunka-New York City. MMWR 34(35):546–7.

Anonymous. 1987. International outbreak of type E botulism associated with ungutted, salted whitefish. MMWR 36(49):812–3.

Cann DC, Taylor LY. 1979. The control of the botulism hazard in hot-smoked trout and mackerel. J Food Technol 14:123–9.

Cann DC, Taylor LY, Hobbs G. 1975. The incidence of *Clostridium botulinum* in farmed trout raised in Great Britain. J Appl Bacteriol 39:331–6.

Cann DC, Wilson BB, Shewan JM, Hobbs G. 1966. Incidence of *Clostridium botulinum* type E in fish products in the United Kingdom. In: Nature. p 205–6.

Cuppett SL, Gray JI, Pestka JJ, Booren AM, Price JF, Kutil CL. 1987. Effect of salt level and nitrite on toxin production by *Clostridium botulinum* type E spores in smoked great lakes whitefish. J Food Prot 50(3):212–7.

Dodds KL. 1993. *Clostridium botulinum* in the environment. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum*: ecology and control in foods. New York: M Dekker. p 21–52.

Dodds KL, Austin JW. 1997. *Clostridium botulinum*. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food Microbiology. Fundamentals and Frontiers: American Society for Microbiology. p 288–304.

Dufresne I, Smith JP, Liu JN, Tarte I, Blanchfield B, Austin JW. 2000. Effect of films of different oxygen transmission rate on toxin production by *Clostridium botulinum* type E in vacuum packaged cold and hot-smoked trout fillets. J Food Saf 20:251–68.

Eklund MW. 1992. Control in fishery products. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum*: Ecology and control in foods. New York: M Dekker. p 209–32.

Eklund MW, Poysky F. 1967. Incidence of *Cl. botulinum* type E from the pacific coast of the United States. In: Ingram M, Roberts TA, editors. Botulism 1966. [unknown]: Chapmann and Hall.

Emodi AS, Lechowich RV. 1969. Low temperature growth of type E *Clostridium botulinum* spores. 1. Effects of sodium chloride, sodium nitrite and pH. J Food Sci 34:78–81.

Graham AF, Mason DR, Maxwell FJ, Peck MW. 1997. Effect of pH and NaCl on growth from spores of nonproteolytic *Clostridium botulinum* at chill temperature. Letts Applied Microbiol 24:95–100.

Graham AF, Mason DR, Peck MW. 1996a. Inhibitory effect of combinations of heat treatment, pH, and sodium chloride on growth from spores of nonproteolytic *Clostridium botulinum* at refrigeration temperature. Appl Environ Microbiol 62(7):2664–8.

Graham AF, Mason DR, Peck MW. 1996b. Predictive model of the effect of temperature, pH, and sodium chloride on growth from spores of nonproteolytic *Clostridium botulinum*. Int J Food Microbiol 31:69–85.

Gram L, Huss HH. 2000. Fresh and processed fish and shellfish. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Gaithersburg (MD): Aspen. p 472–506.

Heinitz ML, Johnson JM. 1998. The incidence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* in smoked fish and shellfish. J Food Prot 61(3):318–23.

Huss HH. 1980. Distribution of *Clostridium botulinum*. Appl Environ Microbiol 39:764–9.

Huss HH. 1981. *Clostridium botulinum* type E and botulism [DSci thesis]. Lyngby (DK): Technical University, Technological Laboratory of the Ministry of Fisheries. 58 p.

Huss HH, Ben Embarek PK, From Jeppesen V. 1995. Control of biological hazards in cold-smoked salmon production. Food Control 6(6):335–40.

Huss HH, Pedersen A, Cann DC. 1974. The incidence of *Cl. botulinum* in Danish trout farms. II: Measures to reduce the contamination of the fish. J Food Technol 9:451–8.

Huss HH, Petersen ER. 1980. The stability of *Clostridium botulinum* type E toxin in salty and/or acid environment. J Food Technol 15:619–27.

Huss HH, Schaeffer I, Pedersen A, Jepsen A. 1980. Toxin production by *Clostridium botulinum* type E in smoked fish in Ireation to the measured oxidation reduction (Eh) potential, packaging method and the associated microflora. In: Connell JJ, editor. Advances in Fish Science and Technology: Fishing News Books Ltd. England. p 476–9.

Hyytia E, Hielm S, Korkeala H. 1998. Prevalence of *Clostridium botulinum* type E in Finnish fish and fishery products. Epidemiol Infect 120:245–50.

Hyytia E, Hielm S, Mokkila M, Kinnunen A, Korkeala H. 1999. Predicted and observed growth and toxigenesis by *Clostridium botulinum* type E in vacuum-packaged fishery product challenge tests. Int J Food Microbiol 47:161–9.

Jeppesen V. 1988. Results in report: " Predictive microbiology-measurement and control of food quality". Lyngby: Danish Institute for Fisheries Research, Dept. of Seafood Research.

Kautter DA. 1964. *Clostridium botulinum* type E in smoked fish. J Food Sci 29:843–9.

Kim J, Foegeding PM. 1993. Principles of Control. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum*. Ecology and control in foods. New York: M Dekker. p 121–76.

Lund BM, George SM, Franklin JG. 1987. Inhibition of type A and type B (proteolytic) *Clostridium botulinum* by sorbic acid. Appl Environ Microbiol 53:935-.

Lund BM, Peck MW. 2000. *Clostridium botulinum*. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of foods. Gaithersburg (MD): Aspen. p 1057–109.

Lyver A, Smith JP, Austin J, Blanchfield B. 1998. Competitive inhibition of *Clostridium botulinum* type E by Bacillus species in a value-added seafood product packaged under a modified atmosphere. Food Res Int 31(4):311–9.

McClure PJ, Cole MB, Smelt JPPM. 1994. Effects of water activity and pH on growth of *Clostridium botulinum*. J Appl Bacteriol Symp Suppl 76:105S–114S.

Meng J, Genigeorgis CA. 1993. Modeling lag phase of nonproteolytic *Clostridium botulinum* toxigenesis in cooked turkey and chicken breast as affected by temperature, sodium lactate, sodium chloride and spore inoculum. Int J Food Microbiol 19:109–22.

Nickerson JTR, Goldblith SA, DiGioia G, Bishop WW. 1967. The presence of *Cl. botulinum*, type E in fish and mud taken from the gulf of Maine. In: Ingram M, Roberts TA, editors. Botulism 1966. [unknown]: Chapman and Hall.

Nielsen SF, Pedersen HO. 1967. Studies of the occurrence and germination of *Cl. botulinum* in smoked salmon. In: Ingram M, Robers TA, editors. Botulism 1966. [unknown]: Chapman and Hall. p 66–72.

Pelroy GA, Eklund MW, Paranjpye RN, Suzuki EM, Peterson ME. 1982. Inhibition of *Clostridium botulinum* types A and E toxin formation by sodium nitrite and sodium chloride in hot-process (smoked) salmon. J Food Prot 45(9):833–41.

Segner WP, Schmidt CF, Boltz JK. 1966. Effect of sodium chloride and pH on the outgrowth of spores of type E *Clostridium botulinum* at optimal and suboptimal temperatures. Appl Microbiol 14(1):49–54.

Southcott BA, Razzell WE. 1973. *Clostridium botulinum* control in cold-smoked salmon: a review. J Fish Res Bd Can 30(5):631–41.

Sugiyama H, Bott TL, Foster EM. 1970. *Clostridium botulinum* type E in an inland bay (Green Bay of Lake Michigan). In: Herzberg M, editor. Toxic Microorganisms. Washington D.C.: U.S. Dept. of the Interior. p 287–91.

Thatcher FS, Robinson J, Erdman I. 1962. The " vacuum pack" method of packaging foods in relation to the formation of the botulinum and staphylococcal toxins. J Appl Bacteriol 25:120–4.

Woodburn MJ, Somers E, Rodriguez J, Schantz EJ. 1979. Heat inactivation rates of botulinum toxins A, B, E and F in some foods and buffers. J Food Sci 44:1658–61.


The author wishes to specially acknowledge the following individuals for their valuable suggestions and comments: Mike Peck, Ph.D., Institute of Food Research, Norwich Research Park; and Hans Heinrich Huss, Ph.D., Danish Institute of Fisheries Research.

CHAPTER IV

# Potential Hazards in Cold-Smoked Fish: Biogenic Amines

GEORGE J. FLICK, MARIA P. ORIA, AND LAURA DOUGLAS

## Scope

This chapter of the report presents research data on the production of biogenic amines by fish species that are likely to be cold-smoked. Factors that affect the production of biogenic amines, particularly histamine, are discussed. The effect of the cold-smoking process on biogenic amine production or growth of biogenic amine-producing microorganisms is also assessed. Finally, areas requiring research to adequately determine the hazard of biogenic production in cold-smoked fish and fishery products are listed.

## 1. Introduction

Cadaverine, putrescine, and histamine are diamines that may be produced postmortem from the decarboxylation of specific free amino acids (Table IV–1) in fish or shellfish tissue (Silla Santos 1996). The decarboxylation process can proceed through two biochemical pathways: endogenous decarboxylase enzymes naturally occurring in fish or shellfish tissue or exogenous enzymes released by the various microorganisms associated with the seafood product. Endogenous production of diamines is insignificant when compared to the exogenous pathway (Wendakoon and Sakaguchi 1992a). The nature of the microflora and the composition of the product affect the amount of decarboxylase a bacterial cell may release (Wendakoon and Sakaguchi 1992b; Suzuki and others 1990). In general, histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine, and spermidine are considered the most important biogenic amines in foods (Shalaby 1996). However, β-phenylethylamine, spermine, and spermidine are not end products of bacterial decomposition in fishery products.

Fish muscle is naturally rich in free amino acids and the content may increase even further postmortem. The high content of proteolytic enzymes in the intestinal tract is responsible for the rapid autolytic process (Gilberg 1978; Aksnes 1988) and the high free amino acid content in fishery products. Amino acid formation depends on the harvesting season and feeding activity prior to capture. For example, fish harvested in summer or feeding season quickly liberated large quantities of lysine and arginine (Aksnes and Brekken 1988).

The activity of amino acid decarboxylase depends on a range of factors, including fermentable sugars, pH, and redox potential (Gale 1946). The influence of environmental temperature, nature of microflora, decarboxylase activity, and intestinal tract content on biogenic amine formation may be major reasons for the discrepancies that have been reported in the literature concerning levels of biogenic amines in fresh and processed fish. Another reason for discrepancies may be poor experimental design. Regardless of the discrepancies, it is clear that a high amino acid content and bacterial activity could rapidly result in an elevated concentration of biogenic amines if the proper controls are not in place.

### 1.1. Safety aspects

Biogenic amines, particularly histamine, have been implicated as the causative agent in a number of scombroid food poisonings. There is individual susceptibility to biogenic amines. Clinical signs are more severe in people taking medications that inhibit enzymes that normally detoxify histamine in the intestine. Histamine exerts its effects by binding to receptors on cellular membranes in the respiratory, cardiovascular, gastrointestinal, and haematological/immunological systems and the skin. The symptoms of histamine poisoning generally resemble the symptoms encountered with IgE-mediated food allergies (Taylor and others 1989) and usually appear shortly after the food is ingested with a duration of up to 24 h. Symptoms may be gastrointestinal (nausea, vomiting, diarrhea), circulatory (hypotension), cutaneous (rash, urticaria, edema, localized inflammation), and neurological (headache, palpitations, tingling, flushing or burning, itching). Antihistamines can be used effectively to treat the symptoms. Despite all uncertainties reported, histamine levels above 500 to 1000 mg/kg (500 to 1000 ppm) are considered potentially dangerous to human health based on the concentrations found in food products involved in histamine poisoning (Ten Brink and others 1990). Even less is known about the toxic dose of other amines. Threshold values of 100 to 800 mg/kg (100 to 800 ppm) for tyramine and 30 mg/kg (30 ppm) for phenylethylamine have been reported (Ten Brink and others 1990). In estimating the toxic levels of biogenic amines, one should consider the amount of food consumed, the presence of other amines in the food or other dietary components, and the use of alcohol and medicine. An additional concern, especially if nitrite were to be used in cold-smoked products, is that secondary amines such as putrescine and cadaverine can react with nitrite to form carcinogens (Hildrum and others 1976; Taylor 1986; Ten Brink and others 1990; and Veciana-Nogues and others 1997).

Fish often associated with histamine poisoning are the scombroid fish belonging to the families *Scomberesocidae* and *Scombridae*. Fish included in these families are the tunas, bonito, mackerels, bluefish, and saury. Tuna and mackerel are the most common fish associated with the poisoning, but other fish are also associated with outbreaks of scombroid poisoning. Examples include mahimahi, sardines, anchovies, herrings, and marlin. The association of type of fish and biogenic amine poisoning

**Table IV-1. Biogenic amines and their chemical precursors (from Shalaby 1996)**

| Biogenic Amine | Precursor |
|---|---|
| Histamine[1] | Histidine |
| Putrescine[2] | Ornithine |
| Cadaverine[2] | Lysine |
| Tyramine[3] | Tyrosine |
| Tryptamine[1] | Tryptophan |
| β-phenylethylamine[3] | Phenylalanine |

[1]heterocyclic amine
[2]alipathic amine
[3]aromatic amine

may reflect the amount of consumption of a specific fish.

Research on the quantitative determination of histamine, cadaverine and putrescine in fishery products at FDA have resulted in the 2 only accepted Association of Official Analytical Chemists (AOAC) methods for regulatory purposes (Rogers and Staruszkiewicz 1997). The research was the basis for the establishment of the defect action levels used in FDA's regulatory programs. Recently, the Food and Drug Administration (FDA) (21CFR123) established a guidance level for histamine of 5 mg/100 g (50 ppm) for assuring the safe consumption of scombroid or scombroid-like fish and recommended the use of other data to judge fish freshness, such as the presence of other biogenic amines associated with fish decomposition (FDA 1996). A maximum average histamine content of 10 mg/100 g (100 ppm) has been established in the European Community (EC) for acceptance of tuna and other fish belonging to the *Scombridae* and *Scomberesocidae* families (Veciana-Nogues and others 1997). The EC has suggested that in the future a maximum of 300 ppm for total biogenic amines in fish and fish products may be an appropriate legal limit. It is important to note, however, that there may be a type of poisoning that does not arise from high levels of histamine. Thus a low histamine level may not be absolute assurance of a safe product. It may be more appropriate to say that the absence of decomposition in the fish renders it a safe product. As such, a safe product would have no evidence of spoilage including odors of decomposition, high histamine levels, and other amines such as cadaverine.

## 2. Toxicity

### 2.1. Histamine toxicity

Douglas (1970) reported that very large amounts of histamine could be given orally without causing adverse effects. He attributed this to the conversion of histamine to inactive N-acetylhistamine by intestinal microflora. Human subjects given up to 67.5 mg histamine orally did not produce any subjective or objective symptoms of histamine poisoning (Granerus 1968). Sjaastad (1966), however, administered 36 mg or more of histamine to subjects who subsequently developed symptoms associated with histamine toxicity. Symptoms appeared also with tuna sandwiches containing 100, 150, and 180 mg doses of histamine. Generally, high histamine levels are able to cause a toxic response, but subsequent research has indicated that other factors may also be responsible. When Clifford and others (1989) fed portions of spoiled mackerel containing 300 mg histamine and mackerel associated with an incident diagnosed as scombrotoxicosis to volunteers, there were no significant observable effects. A second study by Clifford and others (1991) was conducted on mackerel fillets associated with an outbreak of scombrotoxicosis. Statistical analysis failed to detect any differences in amine content between fillets shown to be scombrotoxic and those failing to induce nausea, vomiting, or diarrhea, and also failed to establish any significant relationships between the amine doses. It was concluded that no relationship exists between the concentrations of six amines (including histamine, cadaverine, and putrescine) and the onset of scombrotoxic symptoms. Ienistea (1973) reported the deleterious effects in relation to the amount of histamine ingested at one meal as follows:

| | |
|---|---|
| Mild poisoning | 8 to 40 mg histamine |
| Disorders of moderate intensity | 70 to 1000 mg histamine |
| Severe incidents | 1500 to 4000 mg histamine |

The role of saurine (implicated in histamine poisonings in Japan) as a compound able to act synergistically with histamine was reviewed by Arnold and Brown (1978), but it was later concluded that the compound was in fact histamine.

### 2.2 Toxicity potentiators

Histamine appears not to be the sole factor in causing toxicity since cases have also been observed from low contents of histamine (Arnold and Brown 1978; Murray and others 1982; Taylor 1986; Clifford and others 1989; Soares and Gloria 1994). Strong evidence exists that biogenic amines such as putrescine, cadaverine, spermine, and spermidine in fish tissue can potentiate the toxic effect of histamine by inhibiting intestinal histamine-metabolizing enzymes such as diamine oxidase (Hungerford and Arefyev 1992), potentiating histamine uptake, and liberating endogenous histamine in intestinal fluids (Chu and Bjeldanes 1981; Hui and Taylor 1983; Ibe and others 1991; Halasz and others 1994). It has been reported that fish implicated in a scombroid poisoning incident had high levels of inhibitors that interfere with histamine metabolism. Monoamineoxidase inhibitor drugs used for the treatment of depression, hypertension, and tuberculosis have also been observed to potentiate the toxic effect of histamine (Maga 1978; Taylor 1986).

Studies have shown that the levels of cadaverine in toxic or decomposed fish are generally several times greater than the levels of putrescine. When cadaverine was administered through stomach catheters simultaneously with histamine, peroral toxicity was observed in the guinea pigs (Bjeldanes and others 1978). Klausen and Lund (1986) reported that at 10 °C the high cadaverine contents of mackerel in comparison with herring could be responsible for mackerel often being implicated in scombroid poisoning and not herring, since histamine levels were similar in both. Cadaverine and putrescine, as well as other diamines, have been suggested to facilitate the transport of histamine through the intestinal wall and to increase its toxicity (Fernandez-Salguero and Mackie 1987b).

Arnold and Brown (1978) reported on the possibility that bacterial endotoxins, which are widespread, could result in hypersensitivity to histamine. These compounds are complex, heat-stable, lipopolysaccharide materials produced primarily by Gram-negative bacteria. They also reported that endotoxin is known to be capable of inducing histamine release in animals (sometimes called endotoxin shock) similar to that seen in anaphylaxis. J. Baronowski (personal communication), however, reported extremely low levels of endotoxin in both good tuna and tuna known to have caused illness in humans.

From these discussions, it is clear that concentration of biogenic amines producing observable toxicity may differ significantly, depending on a variety of circumstances. Also, although a variety of histamine potentiators are known, there is not a clear understanding of the level and the manner by which synergism occurs.

## 3. Prevalence in fish

The prevalence of biogenic amines in fish depends on several factors that are described in this section. In general, concentrations in newly caught fish are low. Mietz and Karmas (1978) found that cadaverine values ranged from 1.16 to 10.36 ppm in high quality rockfish, salmon steaks, and shrimp. Also, putrescine levels ranged from 1.36 to 6.30 ppm in high quality lobster tails, salmon steaks, and shrimp. A prior study by the investigators (Mietz and Karmas 1977) reported that high quality tuna had cadaverine and putrescine values ranging from 0.24 to 5.32 and 0 to 1.84 ppm, respectively. There has been some concern regarding the accuracy of the analytical methods used in these studies. Gloria and others (1999) determined biogenic amines in 102 samples of albacore tuna (*Thunnus alalunga*) harvested off the U.S. Northwest from 1994 to 1996. There were significant differences of amine levels in fish from different years. Total levels of the six amines detected (spermine, spermidine, putrescine, cadaverine, histamine, and tyramine) varied from 0.59 to 4.65

mg/100g (5.9 to 56.5 ppm). These levels were probably lower due to the fact that the samples were frozen on board or chilled on board and immediately frozen after reaching the dock and kept at –40 °C (–40 °F) until analysis. Spermine was present at higher levels, followed by spermidine, histamine, putrescine, cadaverine, and tyramine.

### 3.1 Muscle type

In the study by Gloria and others (1999), no difference was observed on amine levels of upper and lower loin light muscles, but dark muscles contained higher spermidine (Table IV–2). Intestine wall samples contained high amine levels.

Takagi and others (1969) examined the amounts of histidine and histamine in 21 aquatic species during spoilage. Their conclusions were consistent with those of other researchers in that more histamine was produced in the red muscle fish such as tuna and mackerel than in white muscle species such as rockfish. Within a given fish species, more histidine and histamine were found in white than in red muscle.

Wendakoon and others (1990) reported that most of the bacteria that convert amino acids into nonvolatile amines possess more than one decarboxylase. In contrast to results reported by Wendakoon and Sakaguchi (1992b), they also reported that in the dark muscle, the amine levels were always much higher and the amine production was more rapid than that in the white muscle.

### 3.2 Microflora

A variety of microorganisms are able to produce biogenic amines. The production of cadaverine and putrescine by microorganisms is not surprising since the covalent linking of cadaverine and putrescine to the peptidoglycan is necessary for normal microbial growth (Suzuki and others 1988). Several inoculation studies on both culture media and on fish have demonstrated that *Morganella* spp., *Proteus morganii, Proteus* spp., *Hafnia alvei*, and *Klebsiella* spp. are able to produce histamines and other biogenic amines. The majority of the studies also concurred that the potential of these microorganisms to produce toxic levels of biogenic amines is enhanced at abusive temperatures (see section 4.2 of this chapter).

The following tables summarize research on production of biogenic amines by microorganisms. Tables IV–3 and 4 list studies on production of biogenic amines by bacterial isolates inoculated on different culture media and on fish that may be cold-smoked, respectively. In addition, studies where isolates from fish have been incubated in media and histamine production monitored are listed in Table IV-5. It is noteworthy to point out that spoilage and toxin formation occur due to a variety of microorganisms, and therefore identical storage times for similar fish species may produce varying levels of scombrotoxin.

Okuzumi and others (1990) investigated the relationship between microflora on horse mackerel (*Trachurus japonicus*) and dominant spoilage bacteria. The results of their study showed that *Pseudomonas* I/II, *Pseudomonas* III/IV-NH, *Vibrio*, and *Photobacterium* were dominant when high levels of putrescine, cadaverine, and histamine were detected.

The activity of decarboxylase can be an indirect measurement of potential for biogenic amine formation. A study by Middlebrooks and others (1988) showed that 14 bacterial isolates (*Acinetobacter lowffi*, *Aeromonas hydrophila*, *Clostridium perfringens*, *Enterobacter aerogenes*, *Enterobacter* spp., *H. alvei*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Proteus* spp., *Pseudomonas fluorescens/putida*, *Pseudomonas putrefaciens*, *Pseudomonas* spp., and *Vibrio alginolyticus*) from mackerel tissue were capable of exhibiting decarboxylase activity (production of histamine, cadaverine, and putrescine) when incubated in

**Table IV-2. Levels of biogenic amines in light and dark muscles and intestine wall of albacore tuna (from Gloria and others 1999)**

| Biogenic amines[1] (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Samples** | **SPM** | **SPD** | **HIM** | **PUT** | **CAD** | **SER** | **Total** |
| light muscle upper loin | $0.68^{b}$ (0.12) | $0.26^{c}$ (0.07) | $0.00^{b}$ | $0.22^{ab}$ (0.07) | $0.13^{b}$ (0.02) | $0.00^{b}$ | $1.29^{c}$ (0.17) |
| lower loin | $1.21^{b}$ (0.26) | $0.25^{c}$ (0.05) | $0.00^{b}$ | $0.14^{b}$ (0.05) | $0.11^{b}$ (0.06) | $0.00^{b}$ | $1.77^{c}$ (0.37) |
| dark muscle | $2.50^{ab}$ (0.97) | $0.79^{b}$ (0.18) | $0.00^{b}$ | $0.06^{b}$ (0.03) | $0.07^{b}$ (0.05) | $0.00^{b}$ | $3.42^{b}$ (0.72) |
| intestine wall | $5.35^{a}$ (2.46) | $3.63^{a}$ (1.18) | $0.52^{a}$ (0.25) | $0.43^{a}$ (0.16) | $1.96^{a}$ (0.59) | $4.38^{a}$ (1.33) | $16.3^{a}$ (4.59) |

1 Mean values (standard deviation) were calculated by using 0 for not detected levels (spermine-SPM, spermidine-SPD, histamine-HIM, putrescine-PUT, cadaverine-CAD 0.08; and serotonin-SER æ 0.18 mg/100g). Mean values with the same superscript in the same column do not differ significantly ($p \leq 0.05$), Turkey test).

Spanish mackerel at 0 °C (–32 °F), 15 °C (59 °F), and 30 °C (90 °F). Other bacteria strong histidine decarboxylase activities: *Klebsiella pneumonia* (Taylor and others 1979), *Klebsiella planticola* (Taylor and Lieber 1979), *Alteromonas putrefaciens* (Frank and others 1985), *Photobacterium phosphoreum* (Morii and others 1986); *Staphylococcus xylosus* (Rodriguez-Jerez and others 1994), *Cedecea lapagei*, *Cedecea neteri*, *Plesiomonas shigelloides* (Lopez-Sabater and others 1994a), *Providencia* spp. (Ababouch and others 1991b), *Lactobacillus curvatus* LTH 975 and *Lactobacillus buchneri* LTH 1388 (Leuschner and Hammes 1999), *Serratia* spp. (Lopez-Sabater and others 1996a), and *Escherichia* spp. (Gale 1946).

Okuzumi and others (1984) studied histamine-forming bacteria in addition to N-group (psychrophilic halophilic, histamine-forming) bacteria in and on fresh fish. The histamine-forming bacteria were N-group bacteria, *P. morganii*, *P. vulgaris*, *H. alvei*, *Citrobacter* spp., *Vibrio* spp., and *Aeromonas* spp. For the summer samples, *P. morganii* was found most frequently, followed by the N-group bacteria. On the other hand, for the winter samples, only the N-group bacteria were found, and other histamine bacteria were not detected.

Changes in the concentration of tyramine, agmatine, putrescine, cadaverine, spermidine, tryptamine, spermine, histamine, and trimethylamine were studied in parallel with the development of the microbial population and sensory scores during the storage of Mediterranean gilt-head sea bream (*Sparus aurata*) at three temperatures (0 °C [32 °F)] 8 °C [(46 °F] 15 °C [59 °F]) (Koutsoumanis and others 1999). Pseudomonads and $H_2S$-producing bacteria were dominant microorganisms. *Enterobacteriaceae* and lactic acid bacteria were also present in the fish microflora. Among the biogenic amines, putrescine and cadaverine were detected when pseudomonads exceeded $10^6$ to $10^7$ cfu/g. Histamine was produced only in samples stored at 15 °C and reached > 50 ppm levels at 48 h. Putrescine and cadaverine reached high levels also at 15 °C after 120 h. Tyramine, tryptamine, agmatine, and trimethylamine were absent regardless of the storage temperature. The authors concluded that only putrescine and cadaverine could be used as an index of freshness. The role and significance of putrescine and cadaverine in food safety and biogenic amine poisoning is yet to be established. Furthermore, in prevalence studies, researchers are challenged regularly to select or obtain samples that are representative of the existing total population of fish, muscle, microorganisms, or whatever is being studied.

## 4. Effect of processing steps

### 4.1. Gutted as compared with ungutted fish

Before cold smoking, fish often will be eviscerated. Data on

**Table IV-3. Production of biogenic amine by bacteria growing on media culture**

| Histamine producers | Histamine concentration | Temperature and time | |
|---|---|---|---|
| *Morganella* spp | 4000 ppm (max) | 76 h | Aiso and others 1958 |
| *Morganella spp* | 1000 ppm<br>1000 ppm<br>0 ppm | 25 °C for 24 h<br>25 °C for 19 h followed by 5 °C for 100 h<br>5 °C for 100 h | Klausen and Huss 1987 |
| *Proteus spp* | Large | | Kimata and others 1960 |
| *Proteus morganii* | > 200 nM/ml<br>large | <br>15, 30, 37 °C for < 24 h | Taylor and others 1978<br>Behling and Taylor 1982 |
| *Enterobacter aerogenes* | > 200 nM/ml | | Taylor and others 1978 |
| *Klebiella pneumoniae* | Large | 15, 30, 37 °C for < 24 h | Behling and Taylor 1982 |
| *Hafnia alvei* | Large | 30, 37 °C for > 48 h | Behling and Taylor 1982 |
| *Citrobacter freundii* | Large | 30, 37 °C for > 48 h | Behling and Taylor 1982 |
| *Escherichia coli* | Large | 30, 37 °C for > 48 h | Behling and Taylor 1982 |
| *Lactobacillus* (3 strains) | 2.2 mg/ml | | Masson and others 1996 |

**Table IV-4. Production of biogenic amines by bacteria isolates incubated on fish**

| Bacteria | Fish | Histamine (ppm) | Other biogenic amines (ppm) | Temperature | Reference |
|---|---|---|---|---|---|
| *Proteus morganii* | Tuna | > 50<br>< 50 | | 24, 30 °C<br>15 °C | Eitenmiller and others 1981 |
| *Acinobacter* | Spanish mackerel | > 1 | | 0 °C | Middlebrooks and others 1988 |
| *Aeromonas hydrophila* | Spanish mackerel | > 1 | | 0 °C | Middlebrooks and others 1988 |
| *Clostridium perfringens* | Spanish mackerel | > 1 | | 0 °C | Middlebrooks and others 1988 |
| *Enterobacter aerogenes*<br>*Enterobacter* spp. | Spanish mackerel<br>Mackerel (*Scomber japonicus*) | <br>detectable | > 1<br>detectable | 0 °C | Middlebrooks and others 1988<br>Wendakoon and Sagakuchi 1993 |
| *Hafnei alvei* | Spanish mackerel | > 1 | | 0 °C | Middlebrooks and others 1988 |
| *Morganella morganii* | Spanish mackerel | > 1 | | 0 °C | Middlebrooks and others 1988 |
| *Proteus* spp.<br>*Proteus vulgaris*<br>*Proteus mirabilis* | Spanish mackerel | > 1 | | 0 °C | Middlebrooks and others 1988 |
| *Pseudomonas* spp. | Spanish mackerel | > 1 | | 0 °C | Middlebrooks and others 1988 |
| *Vibrio alginolyticus* | Spanish mackerel | > 1 | | 0 °C | Middlebrooks and others 1988 |

**Table IV-5. Production of biogenic amines on culture media by microorganism isolated from fish**

| Microorganism | Fish | Histamine | Temperature and Time | Reference |
|---|---|---|---|---|
| *Proteus morganii* | Skipjack *(Euthynnus pelamis)* | Detected | | Kimata and others 1960 |
| | Jack mackerel *(Trachurus symmetricus)* | Detected | | Kimata and others 1960 |
| | Sardine | > 1000 ppm | 35 °C for 24 h | Ababouch and others 1991b |
| *Hafnia alvei* | Skipjack | Detected | | Kimata and others 1960 |
| | Jack mackerel | Detected | | Kimata and others 1960 |
| *Proteus* spp. | Skipjack | Detected | | Kimata and others 1960 |
| | Jack mackerel | Detected | | Kimata and others 1960 |
| | Sardine | > 1000 ppm | 35 °C for 24 h | Ababouch and others 1991b |
| *Klebsiella* | Skipjack | Detected | | Kimata and others 1960 |
| | Jack mackerel | Detected | | Kimata and others 1960 |
| *Morganella morganii* | Tuna *(Thunnus thunnus)* | > 1000 ppm | 37 °C for 18 h | Lopez-Sabater and others1994b |
| | Skipjack tuna *(Katsuwonus pelamis)* | > 1000 ppm | 7, 19, 30 °C *for 24 h* | Arnold and *others 1980* |
| | Albacore tuna | > 1000 ppm | 15, 25 °C | Kim and others 2000 |
| *Klebsiella* spp. | Tuna | > 1000 ppm | 37 °C for 18 h | Lopez-Sabater and others 1994b |
| *Enterobacter aerogenes* and *E. cloacae* | Tuna | 500 to 1000 ppm | 37 °C for 18 h | Lopez-Sabater and others 1994b |
| *Citrobacter freundii* | Tuna | < 250 ppm | 37 °C for 18 h | Lopez-Sabater and others 1994b |
| *Proteus mirabilis* | Tuna | < 250 ppm | 37 °C for 18 h | Lopez-Sabater and others 1994b |
| *Proteus vulgaris* | Tuna | < 250 ppm<br>> 1000 ppm | 37 °C for 18 h<br>7,19,30 °C, 24 h | Lopez-Sabater and others 1994b<br>Arnold and others 1980 |
| | Sardine | 100 to 2000 ppm | 35 °C for 24 h | Ababouch and others 1991b |
| *E. agglomerans* | Tuna | < 250 ppm | 37 °C for 18 h | Lopez-Sabater and others 1994b |
| *Serratia liquifaciens* | Tuna | < 250 ppm | 37 °C for 18 h | Lopez-Sabater and others 1994b |
| *Providencia stuarti* | Sardine | 150 to 1000 ppm | 35 °C for 24 h | Ababouch and others 1991b |
| *Vibrio* spp. | Sardine | 100 ppm | 35 °C for 24 h | Ababouch and others 1991b |
| *Stenotrophonas maltophilia* | Albacore tuna *(Thunnus alalunga)* | 25.8 ppm<br>> 1000 of other biogenic amines | 4 °C for 6 d<br>37 °C for 24 h | Ben-Gigirey and others 1999 |

the effect of evisceration on biogenic amine production is inconsistent. The rates of biogenic amine (cadaverine and putrescine) formation in fish can be summarized as follows: whole ungutted fish > fillets from whole ungutted fish; fillets > whole gutted fish (Haaland and others 1990). However, this general scheme could be different if fish were processed under varying sanitary conditions. Research by Fernandez-Salguero and Mackie (1987a) reported that histamine, cadaverine, and putrescine were produced more rapidly in haddock fillets than the whole gutted fish and that ungutted fish spoiled more rapidly than fillets. Dawood and others (1988), however, reported that eviscerated fish contained lower concentrations of amines than whole samples of rainbow trout (*Salmo irideus,* renamed *Oncorhynchus mykiss*). When gutted and ungutted mackerel (*Scomber scombrus*) were subjected to two treatments (iced immediately after catching in contrast to those left on the vessel deck at ambient temperature 6 °C—12 °C [43 to 53 °F]) (Hardy and Smith 1976), processing did not appear to influence histamine formation and histamine con-

**Table IV-6. Levels of biogenic amines on fresh fish stored at different temperature and time combinations**

| Fish | Temperature/time | Histamine content (ppm) | Other biogenic amines | Sensory | Reference |
|---|---|---|---|---|---|
| Salmon *(Oncorhynchus gorbuscha)* | 10 °C for 14 d | Not detected | | Spoilage | Crapo and Himelbloom 1999 |
| Tuna *(Thunnus thunnus*) | 4 °C | Toxic levels | | Unacceptable | Lopez Sabater and |
| | 8 °C | Toxic levels | | Acceptable | others (1996b) |
| | 20 °C | Toxic levels | | Unacceptable | Price and others (1991) |
| Albacore tuna | iced for 33 d | 825 | | | |
| *(Thunnus* | 15 to 23 °C for 24 h | insignificant | | | |
| *alalunga)* | 15 to 23 °C for 4 d | < 50 | | | Ben-Gigirey and |
| Albacore tuna (whole fish) | 25 °C for 7 d | 1000 | | | others (1998b) |
| Rainbow trout *(Salmo irideus)* | 0 °C for 24 d | < 1 | < 1 | | Dawood and others (1988) |
| Sardines | 24 h at ambient T 8 d in ice | 2350 2350 | > 1000 > 1000 | | Ababouch and others (1991b) |
| Mahimahi | 21 °C for 2 d | 1540 | | | Baranowski and |
| *(Coryphaena* | 32 °C for 12 h | 18 | | | others (1990) |
| *hippurus)* | 32 °C for 24 h | 2920 | | | |
| Sardine, saury pike, | 5 °C for 6 to 9 d | Toxic levels | | | Yamanaka and |
| mackerel, horse | 20 °C for 2 d | Toxic levels | | | others (1984) |
| mackerel | 35 °C for 2 d | Toxic levels | | | |
| Sardine *(Sardina pilchardus)*, horse mackerel *(Trachurus trachurus)*, chub mackerel (*Scomber japonicus*), and mackerel (*Scomber scombrus*) | iced for 7 d | < 100 | | | Mendes (1999) |
| | iced for 7 d | < 100 | | | |
| | iced for 7 d | < 100 | | | |
| Mackerel | 0 °C for 25 d | < 1 | | | Fernandez Salguero |
| *(Scomber* | 2 °C for 12 d | 14 | | | and Mackie (1979) |
| *scombrus*) | 10 °C for 120 h | 1820 | | | |
| | 23 °C for 36 h | 50 | | | |
| Mackerel | 6 °C for 150 to 200 h | 500 to 700 | Unacceptable | | Kimata and Kawai (1953) |
| | 17 °C for 75 h | 3540 | Unacceptable | | |
| | 23 °C for 46 h | 8260 | Unacceptable | | |
| | 35 °C for 20 h | 140 | Unacceptable | | |
| Spanish mackerel | 24 °C for 2 d | 238 | | | Edmunds and |
| *(Scomberomorus* | 0 °C for 10 d | 0 | | | Eitenmiller (1975) |
| *maculatus)* | 10 °C for 4 d | 1000 | | | |
| Mackerel (*Scomber* | 10 °C for 2d and | 200 | | | Klausen and Huss (1987) |
| *scombrus*) | 8d at 0 °C | | | | |
| Kahawai | 15 to 23 °C for 2 d | 1500 to 3500 | | | Ben-Gigirey and others (1998b) |
| Herring, *Clupea harengus pallasi* | 10 °C for 14 d | 55 | Spoiled by 6 d | | Crapo and Himelbloom (1999) |

tents were low and increased subsequent to spoilage in both gutted and ungutted fish.

## 4.2 Effect of postharvest handling

The most important factor that contributes to the production of biogenic amines during postharvest handling is the storage time at specific temperatures. Both the postmortem formation of amino acids and their rapid decarboxylation are temperature-dependent (Haaland and others 1990). While most amino acids were present at higher levels at 2 °C (35 °F) than at 20 °C (68 °F), however, amine formation was greater at 20 °C than at 2 °C.

The effect of temperature on histamine formation has frequently been studied. Table IV-6 lists selected research studies where fish were kept either in ice, refrigerated, or at abusive temperatures for different storage times. Different studies reported that skipjack tuna that was allowed to spoil under similar conditions had 100-fold variations in histamine concentrations. Although there is great variability in the results within the same study, longer storage times and higher temperatures seem to induce histamine production.

Postharvest handling conditions have a significant effect on the presence and concentration of putrescine and cadaverine. Ababouch and others (1991b) reported that bacteria on the skin and gills of freshly harvested sardines (*Sardina pilchardus*) quickly invaded and grew within the muscle tissue, reaching $5 \times 10^8$ cfu/g and $6 \times 10^8$ cfu/g respectively after 24 h at ambient temperature and 8 d in ice. Histamine, cadaverine, and putrescine accumulated to levels of 2350 ppm, 1050 ppm, and 300 ppm respectively after 8 d of ice storage but only 24 h at ambient temperature.

Dawood and others (1988) showed that initial holding temperatures above 0 °C (32 °F) resulted in increased concentrations of nonvolatile amines in freshly caught whole and eviscerated rainbow trout (*Salmo irideus*). In another study on skipjack tuna (*Euthynnus lineatus*), Mazorra-Manzano and others (2000) concluded that endogenous and microbial deterioration processes could be controlled at 0 °C, since, even after 24 d, there was < 1 ppm of any of the biogenic amines analyzed (histamine, cadaverine, and putrescine) in hook- and line-caught fish that were immediately iced upon landing. Consistent with those results, Atlantic herring (*Clupea harhengus*) and Atlantic mackerel (*Scomber scombrus*) contained insignificant amounts (< 3 ppm)

**Table IV-7. Effect of air incubation on decomposition of mahimahi held 18 h at 32 °C (from Baranowski and others 1990)**

| Storage Condition* | Histamine (ppm) | Quality Score** |
|---|---|---|
| Seawater | 1230 | 2.8 |
| Air | 101 | 6.0 |

*Four (4) fish per treatment
**Decreasing 10-point scale where 10 to 9 = fresh, acceptable; 8 to 6 = slight decomposition; 5 to 3 = definite decomposition; and 2 to 1 = advanced decomposition

**Table IV-8. Changes in histamine (ppm) in big eye tuna and skipjack during storage at 4 °C, 10 °C, and 22 °C (from Silva and others 1998).**

| | Histamine in Skipjack | | | Histamine in Big Eye Tuna | | |
|---|---|---|---|---|---|---|
| Days | 4 °C | 10 °C | 22 °C | 4 °C | 10 °C | 22 °C |
| 0 | — | 0 | 0 | 0 | 0 | 0 |
| 1 | — | 0 | 600 | — | — | 300 |
| 2 | — | — | 3000 | — | — | 500 |
| 3 | 0 | 1000 | 3500 | 0 | 300 | 1500 |
| 6 | 1000 | 5500 | — | 250 | 1500 | — |
| 9 | 2500 | 8750 | — | 1000 | 4250 | — |
| 12 | 4000 | 7000 | — | 1200 | 1750 | — |

**Table IV-9. Histamine concentration in skipjack infusion broth (mg/100 ml histamine) (Arnold and others 1980)**

| | Temperature | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 30 °C | | | 19 °C | | | 7 °C | | |
| Microorganism | 1 | 3 | 7 | 1 | 3 | 7 | 1 | 3 | 7 |
| *Proteus morganii* | 370 | 120 | 120 | 365 | 200 | 180 | 130 | 140 | 180 |
| *Proteus vulgaris* | 340 | 220 | 180 | 345 | 235 | 200 | 130 | 192 | 180 |
| *Hafnia alvei* | 0 | 70 | 110 | 0 | 20 | 44 | 0 | 0 | 4 |

of cadaverine and putrescine after 7 and 3 d of iced (1 °C, 34 °F) storage (Ritchie and Mackie 1980). These reports are in contrast to an earlier one, where the histamine content of albacore tuna was reported as 7.5 mg/100 g (75 ppm) of fresh fish during unloading from a fishing vessel (Leitao and others 1983) and 82.5 mg/100 g (825 ppm) after 33 d of ice storage (Price and others 1991). (These high values may be indicative of mishandling on the fishing vessel and the resulting fish decomposition). Also, Shewan and Liston (1955) reported that histidine was easily decarboxylated at 0 °C (32 °F). The variability of these findings reflects the challenges of representative sampling, species differences, quality of initial raw material, and other experimental conditions. Nevertheless, control of biogenic amine production by low temperatures (for example, 0 °C, 32 °F) is a constant observation.

Klausen and Huss (1987) found no histamine formation in mackerel stored in ice, whereas a rapid increase was noted at 10 °C (50 °F). Interestingly, storage at 10 °C for 2 d with no detectable histamine formation and subsequent storage at 0 °C (32 °F) led to formation of 200 ppm histamine after 8 d. These studies indicate that although the histamine-forming bacteria do not grow at 0 °C, decarboxylase formed during growth at 10 °C may be active at 0 °C.

Temperature-abuse potentiates histamine formation in fresh mahimahi (*Coryphaena hippurus*) at 32 °C (86 °F) increasing from 1.6 ppm to 2920 ppm in 24 h (see Table IV-11) (Baranowski and others 1990). In a second study, Baranowski and others (1990) showed that heat penetration was much more rapid during incubation in seawater than in air, affecting the histamine content and quality score of the fish (Table IV-7).

The histamine formation of big eye tuna (*Thunnus obesus*) and skipjack (*Katsuwonus pelamis*) tuna during storage at 4 °C (39 °F), 10 °C (50 °F), and 22 °C (72 °F) occurred very quickly at 22 °C, exceeding 50 mg/100 g (500 ppm) in 1 d for skipjack and 2 d for big eye tuna (Silva and others 1998). The rise in histamine content was delayed at refrigerated temperatures (10 °C and 4 °C), but notable amounts were detected after 3 d at 10 °C and 6 d at 4 °C (Table IV-8).

The changes in histamine content during storage at 5 °C (44 °F), 20 °C (68 °F), and 35 °C (95 °F) were examined in the ordinary and dark meats of sardine, saury pike, mackerel, yellowtail, skipjack, big eye tuna, and horse mackerel (Yamanaka and others 1984). During storage at 20 °C and 35 °C, histamine was produced and accumulated > 500 ppm levels at 2 to 6 d of storage, depending on the species. During 5 °C storage, however, the amounts of histamine gradually increased up to those levels in 9 d in sardine, saury pike, mackerel, and horse mackerel. Histamine formation in dark meat was less than that in white meat at the same temperature.

Recently, changes in histamine, cadaverine, putrescine, and agmantine contents were examined in sardine (*Sardina pilchardus*), Atlantic horse mackerel (*Trachurus trachurus*), chub mackerel (*Scomber japonicus*), and Atlantic mackerel (*Scomber scombrus*) during ice storage (2 to 3 °C, 35 to 37 °F) and storage at room temperature (20 to 23 °C, 68 to 73 °F) (Mendes 1999). At day 0, the initially high aerobic colony counts were $10^5$ to $10^6$ cfu/g. They reached a maximum within 48 to 55 h in fish stored at 22 to 23 °C, but only after prolonged times (10 to 16 d) in fish at 2 to 3 °C. Histamine formation, as well as other amines, varied greatly with species of fish and storage conditions. The levels of histamine, cadaverine, and putrescine increased gradually in all species as decomposition progressed, regardless of storage temperatures, and reached maximum limits for human consumption after 24 h of storage at room temperature. In contrast, amine production in iced fish was considerably reduced and histamine concentration increased slowly until day 7, after which a significant rise was detected, but generally was below 100 mg/Kg. No correlation was observed for histamine or other amine levels and the degree of fish decomposition. Consequently, the belief that decomposition protects consumers from hazardous biogenic amines seems disputable. Again in contrast, a recent report (Kaneko 2000) described the development of a Hazard Analysis Critical Control Point (HACCP) approach using Vessel Standard Operating Procedures for control of histamine on Hawaiian fishing vessels. They concluded that odors of decomposition were reliable indicators of histamine risk and that sensory evaluation is an effective HACCP control measure in the Hawaiian fishery. In this study, 583 mixed pelagic fish (fresh bigeye, yellowfin, albacore tuna, striped marlin, blue marlin, and mahimahi stored in ice) were sampled at the time of delivery from commercial fishing vessels. Fish were graded for quality, by using sensory indicators of decomposition, and analyzed for histamine concentration. A total of 119 fish were rejected because of decomposition. Only 14 fish exceeded 5mg/100 mg (50 ppm) histamine defect action limit. All 14 fish were first rejected from the market because of odors of decomposition. None of the fish that passed the sensory evaluation exceeded the defect action limit. These conflicting results pose a challenge if biogenic amines are to be used as legal safety indices. Another challenge is to develop an acceptable definition for an odor of decomposition.

It is imperative to recognize that the fish species affect the production of biogenic amines and that many species are rarely, if ever, currently utilized in cold-smoked fish products. Variable and differing data are frequently reported. For instance, 34 albacore tuna (*Thunnus alalunga*) samples left on the deck (deck temperature 15.5 to 23.5 °C, 59 to 73 °F) for < 12 h contained negligible histamine (< 0.40 mg/100 g muscle [4 ppm]). Four samples

**Table IV-10. Effect of chlorine disinfectants (100 ppm) on decomposition of mahimahi incubated 18 h at 32 °C (Baranowski and others 1990)**

| Incubation medium* | Histamine (ppm) | Quality Score** |
|---|---|---|
| Seawater (SW) | 1230 | 2.8 |
| SW + sodium hypochlorite | 2340 | 3.0 |
| SW + chlorine dioxide | 2360 | 2.5 |

*Four (4) fish per treatment.
**Decreasing 10-point scale where 10 to 9 = fresh, acceptable; 8 to 6 = slight decomposition; 5 to 3 = definite decomposition; and 2 to 1 = advanced decomposition

left on the deck for up to 24 h did not contain any significant amounts of histamine (< 0.18 mg/100 g [1.8 ppm]). Among 9 fish left at up to 4 d, only 2 exhibited histamine levels higher than 5.0 mg/100 g (50 ppm), that is, 9.31 and 6.19 mg/100 g (93.1 and 61.9 ppm) (Ben-Gigiery-and others 1998b). Kahawai (*Arripis trutta*) incubated for 2 d, however, showed average histamine levels of 150 and 350 mg/100 g (1500 and 3500 ppm) in two different trials (Fletcher and others 1995). Spanish mackerel (*Scomberomorus maculatus*) fillets contained histamine of 1.8 and 23.8 mg/100 g (18 and 238 ppm) when incubated at 24 °C (75 °F) for 1 and 2 d, respectively (Edmunds and Eitenmiller 1975). Mahimahi (*Coryphaena hippurus*) developed histamine of 154 mg/100 g (1540 ppm) when incubated at 21 °C (7 °F) for 2 d (Baranowski and others 1990). The optimum temperature for histamine formation in albacore tuna (*Thunnus alalunga*) was 25 °C (77 °F) (100 mg/100 g [1000 ppm]) in whole fish stored for 7 d.

Fresh pink salmon (*Oncorhynchus gorbuscha*) fillets and whole Pacific herring (*Clupea harengus pallasi*) were stored for 2 wk at 10 °C (50 °F) to determine if significant amounts of histamine were produced prior to spoilage (Crapo and Himelbloom 1999). Spoilage odors in salmon were moderate by day 4 and intense by day 7, while herring had detectable spoilage by day 4 and became potent by day 6. Aerobic colony counts increased from $10^2$ to $10^3$cfu/g initially to $10^7$ to $10^8$ cfu/g by day 14. Histamine was not detected in salmon, while concentrations reached 55 ppm in herring at day 14. If spoilage were to be used as protection from histamine poisoning, according to this study, 10 °C would be an appropriate temperature to store salmon and herring, since toxic levels were not reached before spoilage occurred. Again caution must be taken because variability among many of the findings on decomposition-histamine relationships reflect the challenges of representative sampling, species differences, and other experimental conditions.

The effect of temperature has also been investigated through inoculation studies with histamine-producing bacteria. Biogenic amine concentrations and sensory changes in fresh and *Morganella morganii* inoculated blue fish (*Pomatomum saltatrix*) stored at 5, 10, and 15 °C (41, 50, and 59 °F) were reported by Gingerich and others (1999). Histamine content in fresh fish ranged from < 1 to 99 ppm, with an average of 39 ppm. Putrescine and cadaverine were not present. Within 5 d of storage, high concentrations of histamine occurred while the fish were judged acceptable for consumption by the sensory panel. Kim and others (2000) isolated histamine-producing bacteria from albacore tuna stored at 0, 25, 30, and 37 °C (32, 77, 90, 98 °F). The optimum temperature for growth of histamine-producing bacteria was 25 °C. The bacterium producing the highest level of histamine isolated from fish abused at 25 °C was identified as *M. morganii*. The *M. morganii* isolate was inoculated into tuna fish infusion broth medium, and the effect of temperature was determined for microbial growth and formation of histamine and other biogenic amines. The isolate produced the highest level of histamine, 5253 ppm, at 25 °C in the stationary phase. At 15 °C, histamine production was reduced to 2769 ppm. Neither microbial growth nor histamine formation was detected at 4 °C. Cadaverine, putrescine, and phenylethylamine were also detected. The optimum temperature for histamine, cadaverine, putrescine, and phenylethylamine formation was 25 °C.

Histamine production by *P. morganii*, *P. vulgaris*, and *H. alvei* cultures isolated from skipjack tuna (*Katsuwonus pelamis*) was measured at storage temperatures of 1, 7, 19, and 30 °C (4, 44, 66, 90 °F) in skipjack infusion broth (Arnold and others 1980). The highest histamine concentrations were observed at 19 and 30 °C depending on the bacterial species (Table IV-9). No histamine was formed at 1 °C, indicating that rapid cooling of tuna flesh may adequately suppress histamine formation. At 19 °C and 30 °C, the *Proteus* organisms at first formed high levels of histamine, much of which was subsequently destroyed. It appears the histamine concentration may eventually depend on an equilibrium between histamine production and destruction. The authors noted that their conclusion was similar to that of other investigators who reported that tuna flesh homogenate incubated at 25 °C was able to produce approximately 600 mg/100 g (6000 ppm) histamine on day 1 but only 350 mg/100 g (3500 ppm) remained on day 3. However, this study was performed in an infusion broth and not in fish muscle, which may have changed the results.

Postharvest antimicrobial treatments did not show much promise in inhibiting histamine formation. Fish were incubated in seawater (off the coast of Hawaii) and in seawater containing 100 ppm of sodium hypochlorite or chlorine dioxide; however, neither histamine formation nor quality loss was inhibited (Table IV–10) (Baranowski and others 1990).

### 4.3 Freezing

Albacore tuna (*Thunnus alalunga*) specimens of high quality were analyzed for their biogenic amine contents after 1, 3, 6, and 9 mo of frozen storage at –18 °C (-0.4 °F) or –25 °C (–13 °F) by Ben-Gigirey and others (1998a). Putrescine showed the greatest increase, reaching concentrations of 59 ppm (815% of the initial level) and 68 ppm (942% of the initial level) in the white muscle after 9 mo of storage at –18 °C and –25 °C, respectively. Cadaverine, histamine, and spermidine concentrations were below 3, 5, and 11 ppm respectively after 9 mo of frozen storage.

Fresh mackerel (*Scomber scombrus*) with no detectable histamine contained 3, 51, and 53 mg/kg (3, 51, and 53 ppm) when stored at –20 °C (–4 °F) for 11, 22, and 33 wk, respectively (Zotos and others 1995). This is in contrast to a report by Hardy and Smith (1976) who stored high quality mackerel (*Scomber scombrus*) at –14, –21, and –29 °C (7, 6, –20 °F) for 72 wk (1.5 yr) and reported no measurable histamine formation.

Studies on the effect of incubation at 32 °C (86 °F) after frozen storage for 24 wk showed that histamine levels were greatly reduced (Table IV–11). Furthermore, fish frozen for 40 wk had almost no histamine formation during incubation suggesting that its microflora had undergone a greater reduction that occurred during the 24-wk storage period (Baranowski and others 1990).

The findings of Baranowski and others (1990) may be explained by the data of Fujii and others (1994) and Mendes and others (1999). The specific activity of histidine-decarboxylase of halophilic histamine-forming bacteria, *Photobacterium phosphoreum* and *Photobacterium histaminum*, remained at 27 to 53% of the initial value after 7 d of storage at –20 °C (–4 °F) (Fujii and others 1994). During this time the viable cells decreased by more than 6 log cycles of the initial counts. Similarly, Mendes and others (1999) reported that freezing sardines (*Sardina pilchardus*) reduced the numbers of bacteria capable of forming biogenic amines; however, even when the postfreezing viable count of histamine-forming bacteria is low, earlier reports (Yamanaka and

**Table IV-11. Effect of prior frozen storage at –20 °C on histamine formation and decomposition during incubation at 32 °C**

| | H at 32 °C | | | |
|---|---|---|---|---|
| Frozen storage* (wk) | 0 | 6 | 12 | 24 |
| | Histamine (ppm) | | | |
| 0 | 1.6 | 1.5 | 18.0 | 2920 |
| 24 | 0.5 | 0.8 | 0.5 | 850 |
| 40 | 0.7 | 0.7 | 1.2 | 12 |
| | Quality Score** | | | |
| 0 | 9.6 | 7.0 | 4.7 | 1.4 |
| 24 | 8.9 | 6.7 | 4.8 | 1.6 |
| 40 | 8.7 | 7.4 | 5.9 | 2.0 |

*Four (4) fish per treatment
**Decreasing 10-point scale where 10 to 9 = fresh, acceptable; 8 to 6 = slight decomposition; 5 to 3 = definite decomposition; and 2 to 1 = advanced decomposition
From Baranowski and others 1990

**Table IV-12. Bacterial growth characteristics during storage of sardines**

| | Storage at ambient temperature 24 to 28 °C | | | | Storage in ice | | | |
|---|---|---|---|---|---|---|---|---|
| | 0% NaCl | | 8% NaCl | | 0% NaCl | | 8% NaCl | |
| | Lag phase (h) | Generation time (h) | Lag phase (h) | Generation time (h) | Lag phase (h) | Generation time (h) | Lag phase (h) | Generation time (h) |
| TBC | 0 | 2.0 | 10 | 2.3 | 95 | 8.8 | 71 | 8.5 |
| Hist | 0 | 1.9 | 0 | 7.0 | 24 | 13.6 | 77 | 13.2 |
| Prod | | | | | | | | |
| Bac | | | | | | | | |

Ababouch and others 1991a

others 1982; Karolus and others 1985; Yamanaka and others 1987b) suggested the possibility that if the fish had been temperature-abused before freezing, histamines may still be present in toxic amounts. Therefore, it is extremely important to know the temperature history of the frozen fish, since outbreaks of scombroid fish poisoning can be caused by the ingestion of frozen-thawed fish containing degradation products if the fish were previously temperature-abused.

### 4.4 Salting

Taylor and Speckhard (1984) reported that NaCl at levels up to 2% were ineffective in preventing *M. morganii* and *Klebsiella pneumonia* growth and histamine production in TSBH medium. Henry Chin and Kohler (1986) indicated that high levels of salt concentration (3.5% to 5.5% NaCl) could inhibit the histamine production by histamine-forming bacteria. Ababouch and others (1991a), however, reported that sprinkling salt on sardines at a level of 8% (w/w) increased lag phase for total bacteria at room temperature but not in ice. Generation time of histamine producers and lag phase increased at room temperature and ice storage, respectively (Table IV-12). Salt seems to have an inhibiting effect on histamine producers at either temperature.

A subsequent study by Leroi and others (2000) showed that the inhibition of bacteria in cold-smoked salmon stored for 5 wk at 5 °C (41 °F) and salt (5% wt/wt) and smoke was linearly proportional to the salt and smoke content (the higher the concentration, the greater the inhibition). No synergistic inhibition effect was observed between the two factors.

### 4.5 Smoked product

Gessner and others (1996) reported a scombrotoxicosis-like illness occurring in an individual within 10 min after eating a 25 g strip of home-smoked sockeye salmon. The meat came from 1 of 8 salmon caught and stored in a cooler for up to 12 h. Strips cut from the fish bellies had been placed in saltwater brine for 7 min, cooled with a fan for 6 h, and smoked for 2 d at a maximum temperature of 38 to 44 °C (100 to 111 °F) using untreated alder chips. A random sample of 6 strips was tested by the FDA and showed a mean histamine level of 0.19 mg/100 g; a mean putrescine level of 0.67 mg/100 g; and a mean cadaverine level of 0.19 mg/100 g. Two fish strips had water phase salt concentrations of 2.7% and 2.1%. The patient ate an estimated 0.0006 mg of histamine/kg of body weight, well below the estimated 1 mg of histamine/kg of body weight reported to cause illness. The authors did not give a reason for this apparent high sensitivity to such a low concentration of histamine. This reported illness of a single individual is contrary to other published information on histamine toxicity. Also, the samples tested may not have represented the product consumed by the subject of the illness.

The production of biogenic amines during chill storage (5 °C, 41 °F) of cold-smoked salmon (*Salmo salar*) from 3 smoke houses over a 2-year period (1997 and 1998) was studied by Jorgensen and others (2000). Results of the study showed the production of biogenic amines is unlikely to result in histamine poisoning in humans as indicated by epidemiological data (Table IV–13). Some samples exceeded the defect action level of 50 ppm established by the FDA for *Scombridae* and 100 to 200 ppm by E.U. regulations for *Scombridae* and *Clupeidae,* but no samples reached toxic levels of 500 ppm, a value at which one would expect illness and that the FDA would use in legal proceedings (EEC 1991; FDA 1998).

Although the temperatures used for a hot-smoking process may inhibit histamine producers, cold smoking does not expose the fish to temperatures high enough to inhibit the latter bacteria. The effect of hot-smoking previously frozen mackerel (*Scomber scombrus*) on histamine formation was reported by Zotos and others (1995) (Table IV-14). Smoking was done for a total of 7 h, at sequential temperatures of 30, 40 and 70 °C. From Table IV–14 it can be observed that a significant ($p > 0.05$) increase in histamine formation in fresh, frozen (11 or 33 wk) mackerel was solely due to the smoking process. The histamine increase appeared to be independent of frozen storage time prior to smoking. Although this is a hot-smoking process example, it demonstrates the importance of controlling the temperature and time of the smoking process.

In many situations the production of biogenic amines is highly variable and difficult to predict. For example, cold-smoked, fermented rainbow trout (*Oncorhynchus mykiss*) were prepared with 3 different lactic acid bacteria (LAB) inocula plus staphylococci, with the control group being prepared without inoculum (Petaja and others 2000). The fish were cured by injecting brine (20% NaCl, 18% glucose, 0.5% ascorbic acid, and 0.625% $KNO_3$) at amounts corresponding to 5% of the weight of the fish fillet. The lactic acid bacteria inoculum was at $10^7$ cfu/g and staphylococci at $5 \times 10^6$ cfu/g. The products were acceptable by sensory analysis, the LAB inoculum grew to $> 10^8$ cfu/g, the pH reduced to 5.0 to 5.3, and $a_w$ to 0.927 and the pseudomonads, the predominate flora, disappeared. The fish raw material and products contained low amounts of biogenic amines with one exception: cadaverine, histamine, and tyramine increased in all product groups except in one experimental series (II) out of three (Table IV–15). This broad variability was again evident in this report.

Microbiological, chemical, and sensory changes in cold-smoked salmon were studied during 5 wk of vacuum storage at 5 °C (41 °F) (Leroi and others 1998). Total aerobic colony counts reached $3 \times 10^6$ after 6 d; however, the shelf life was judged by a

**Table IV–13. Processing, product, and spoilage characteristics of sliced vacuum-packed cold-smoked salmon stored at 5 °C**

| Smokehouse | A | | | | B | | | | C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| lot no. | 97–1 | 97–2 | 98–1 | 98–2 | 97–3 | 97–4 | 98–3 | 98–4 | 97–5 | 97–6 | 98–5 | 98–6 |
| ***Process*** | | | | | | | | | | | | |
| Salting | brine injection | | | | brine injection | | | | dry salting | | | |
| Ingredients | NaCl | | | | NaCl, sucrose | | | | NaCl, nitrite, sucrose | | | |
| Drying | 3 to 4 h, 26 °C, no humidity control | | | | no separate drying process | | | | 6 to 12 h, 27 °C, 50% relative humidity | | | |
| Smoking | 4 h, 26 °C, no humidity control | | | | 4 to 7 h, 21 to 22 °C, no humidity control | | | | 6 to 12 h, 27 °C, 65% relative humidity | | | |
| ***Product*** | | | | | | | | | | | | |
| initial pH | 6.09 ± 0.02[a] | 6.09 ± 0.01 | 6.14 ± 0.04 | 6.01 ± 0.02 | 6.11 ± 0.01 | 6.07 ± 0.04 | 6.13 ± 0.07 | 6.00 ± 0.04 | 6.08 ± 0.02 | 6.11 ± 0.05 | 6.16 ± 0.03 | 6.11 ± 0.03 |
| NaCl (% WPS) | 5.4 ± 0.4 | 5.0 ± 0.5 | 4.9 ± 0.2 | 4.1 ± 0.5 | 7.5 ± 0.6 | 5.9 ± 0.4 | 4.2 ± 0.3 | 4.2 ± 0.6 | 7.9 ± 1.3 | 5.6 ± 0.5 | 3.9 ± 0.5 | 4.9 ± 0.7 |
| $NaNO_2$ (ppm) | < 0.6 | < 0.6 | < 0.6 | < 0.6 | < 0.6 | < 0.6 | < 0.6 | < 0.6 | 16 ± 7 | 22 ± 10 | 8 ± 5 | 12 ± 8 |
| ***Shelf life (wks)*** | 4 to 5 | 4.5 to 5 | 4 to 5 | 4.5 to 5.5 | 8.5 to 9 | 7 to 8 | 3 to 4 | 5.5 to 6.5 | 5 to 6 | 4.5 to 5 | 4 to 4.5 | 5.5 to 6.5 |
| ***Characteristics at time of spoilage*** | | | | | | | | | | | | |
| Ph | 6.10 ± 0.01 | 6.23 ± 0.05 | 6.06 ± 0.04 | 5.98 ± 0.03 | 5.95 ± 0.08 | 5.64 ± 0.2 | 5.70 ± 0.18 | 5.63 ± 0.03 | 6.06 ± 0.08 | 6.18 ± 0.02 | 5.90 ± 0.06 | 5.99 ± 0.19 |
| ***biogenic amines (ppm)*** | | | | | | | | | | | | |
| Agmatine | 234 ± 107 | 220 ± 118 | 29 ± 26 | 121 ± 25 | 18 ± 7 | 2 ± 1 | 88 ± 24 | 32 ± 30 | 142 ± 179 | 270 ± 90 | 25 ± 13 | 2 ± 1 |
| Cadaverine | 265 ± 72 | 251 ± 63 | 135 ± 69 | 345 ± 95 | 36 ± 11 | 101 ± 27 | 152 ± 70 | 131 ± 135 | 168 ± 170 | 277 ± 33 | 178 ± 66 | 303 ± 140 |
| Histamine | 135 ± 73 | 190 ± 130 | 3 ± 3 | 96 ± 20 | 19 ± 27 | 4 ± 2 | 102 ± 15 | 50 ± 41 | 108 ± 118 | 240 ± 64 | 10 ± 6 | 16 ± 10 |
| Putrescine | 11 ± 4 | 3 ± 1 | 11 ± 9 | 28 ± 16 | 31 ± 16 | 8 ± 6 | 7 ± 3 | 40 ± 34 | 33 ± 32 | 32 ± 18 | 190 ± 64 | 383 ± 32 |
| Tyramine | 137 ± 63 | 228 ± 23 | 180 ± 10 | 235 ± 15 | 202 ± 21 | 128 ± 42 | 82 ± 29 | 158 ± 74 | 108 ± 102 | 235 ± 40 | 223 ± 33 | 335 ± 31 |
| PBA[b] | I | I | II | I | IV | II | I | II | I | I | III | III |
| ***sensory attributes*** | | | | | | | | | | | | |
| off-flavors | *sour,*[c] *bitter, fishy, rancid* | *sour, faecal, rancid* | *sour, faecal* | *sour, faecal* | *rancid, sour* | *sour, chemical* | *sour* | *sour* | *sour, faecal* | *sour, faecal* | *sour, faecal* | *sour, faecal* |
| Texture | *pasty, sticky* | *soft, sticky* | *soft, sticky* | *soft, sticky* | *soft* | *soft* | *soft* | *soft* | *soft* | *soft* | *soft* | *soft* |
| ***microflora, $log_{10}$ (CFU/g)*** | | | | | | | | | | | | |
| TPC | 6.9 ± 0.1 | 7.6 ± 0.2 | 7.3 ± 0.3 | 7.6 ± 0.2 | 8.2 ± 0.4 | 8.7 ± 0.3 | 7.8 ± 0.4 | 8.5 ± 0.1 | 6.9 ± 0.3 | 7.2 ± 0.2 | 8.3 ± 01 | 8.5 ± 0.2 |
| LAB | 6.8 ± 0.2 | 7.5 ± 0.1 | 7.6 ± 0.1 | 7.8 ± 0.2 | 8.1 ± 0.4 | 8.5 ± 0.3 | 8.6 ± 0.1 | 8.7 ± 0.2 | 6.8 ± 0.3 | 7.1 ± 0.2 | 8.4 ± 0.1 | 8.4 ± 0.3 |
| Enterobacteriaceae | 4.1 ± 1.0 | 6.7 ± 0.6 | 6.1 ± 0.2 | 6.1 ± 0.8 | < 3.0 | 6.5 ± 0.5 | 3.4 ± 1.0 | 6.1 ± 1.5 | < 3.0 | 4.2 ± 1.6 | 5.9 ± 1.4 | 6.5 ± 0.8 |

[a]Average ± standard deviation of 3 or 4 individual packs, lots 97–1 to 97–6 and 98–1 to 98–6, respectively
[b]Profile of biogenic amines (PBA).
[c]Attributes responsible for spoilage are indicated in italics.
Jorgensen and others 2000

**Table IV-14. Effect of smoking previously frozen mackerel on histamine formation (mean ± SD) (Zotos and others 1995)**

| Sample | Thawed[a] (mg histamine $kg^{-1}$) | Smoked[a] (mg histamine $kg^{-1}$) |
|---|---|---|
| Fresh | 0.0 | 42.0 ± 0.45 |
| Frozen 11 wk | 3.0 ± 0.05[a] | 44.0 ± 0.16 |
| Frozen 22 wk | 51.0 ± 0.56 | 63.0 ± 0.67 |
| Frozen 33 wk | 53.0 ± 0.34 | 94.0 ± 0 |

[a]Dry, salt-free sample (sic)

sensory panel to be acceptable for 2 to 3 wk. During the first 2 wk, Gram-negative bacteria were dominant, mainly represented by *Swanella putrefaciens* immediately after the smoking process and then *Photobacterium phosphoreum*. *Aeromonas* spp. were present throughout the storage but in smaller amounts. Gram-negative bacteria then progressively decreased while Gram-positive bacteria increased, dominated by LAB. A diversification was observed at the end of storage, with the appearance of *Lactobacillus farciminis*, *Lactobacillus sake*, and *Lactobacillus alimentarius*.

## 4.6 Packaging

Three bacterial suspensions (final concentrations for *Klebsiella oxytoca* $T_2$, $5.6 \times 10^6$/ml; *M. morganii* JM, $1.3 \times 10^6$/ml; *H. alvei* $T_8$, $1.2 \times 10^6$/ml) were used by Wei and others (1990) to inoculate yellowfin tuna (*Thunnus albacares*). Vacuum- and nonvacuum-packaged samples were stored at 2 and 10 °C (50 °F) and examined for growth and histamine formation on days 3, 6, 10, and 15. The bacteria were also placed in culture and incubated at 3, 5, 7, 120, 15, or 25 °C (37, 41, 44, 248, 59, 77 °F) for a maximum of 10 d. Spiked tuna stored at 2 °C contained < 12 mg/100 g (120 ppm) histamine while samples stored at 10 °C had high levels, > 200 mg/100 g (2000 ppm). The lowest temperature at which *K. oxytoca* $T_2$, *K. morganii* JM and *H. alvei* $T_8$ produced histamine was 7, 7, and 20 °C, respectively, and for growth was 5, 7, and 3 °C, respectively. Vacuum packaging did not show any beneficial effect in controlling histamine production and bacterial growth. Low temperature storage was more effective than vacuum packaging.

Reddy and others (1992) reported that the growth of common aerobic spoilage bacteria from genera such as *Pseudomonas*, *Flavobacterium*, *Micrococcus*, and *Moraxella* are inhibited by $CO_2$ in MA-packaged fish during refrigerated storage. Inhibition of these common spoilage psychrotrophic bacteria increases the shelf life, permitting a different type of spoilage flora (that is, the slower-growing Gram-positive bacteria, including *Lactobacillus* spp). The inhibition of the Gram-negative bacteria by modified atmosphere packaging may result in an initial reduction rate of histamine formation, thereby providing some increased control on raw material for cold-smoked fish product.

## 4.7 Other miscellaneous considerations

Taylor and Speckhard (1984) observed that potassium sorbate at a concentration of 0.5% inhibited growth and histamine production of the bacteria in the same medium at both 10 °C (50 °F) for up to 216 h and 32 °C (86 °F) for up to 120 h.

The histamine content of mackerel fillets inoculated by dipping for 30 s in a $7.5 \times 10^3$ cfu/ml suspension of *M. morganii* and

**Table IV-15. Levels of biogenic amines in experimental fish fillets after 0 and 3 d of fermenting and 35 d after preparation (7 d of fermenting +28 d of storing)**

| Amine | Fish fillet group | 0 d | | | 3 d | | | 35 d | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | I | II | III | I | II | III |
| Cadaverine | 1 Control | < 1.0 | < 1.0 | < 1.0 | 8.5 | 40 | 4.4 | 2.7 | 250 | 4.0 |
| | 2 POHK | < 1.0 | < 1.0 | < 1.0 | < 1.0 | 5.2 | 5.0 | 2.6 | 160 | 1.3 |
| | 3 MLHK | < 1.0 | < 1.0 | < 1.0 | 8.9 | 1.4 | 4.6 | 1.9 | 36 | 2.0 |
| | 4 CC–430 | < 1.0 | < 1.0 | < 1.0 | 8.2 | 9.0 | 5.1 | 2.2 | 110 | 1.9 |
| Histamine | 1 Control | 4.7 | 7.8 | 9.4 | 3.3 | 24 | 5.0 | 7.4 | 78 | 10 |
| | 2 POHK | 4.7 | 7.8 | 9.4 | 4.2 | 18 | 13 | 9.1 | 48 | 7.5 |
| | 3 MLHK | 4.7 | 7.8 | 9.4 | 4.9 | 16 | 8.8 | 6.7 | 29 | 10 |
| | 4 CC–430 | 4.7 | 7.8 | 9.4 | 2.3 | 16 | 6.0 | 5.6 | 61 | 5.7 |
| Tyramine | 1 Control | 9.3 | 7.7 | 13 | 6.1 | 9.5 | 13 | 7.2 | 58 | 7.1 |
| | 2 POHK | 9.3 | 7.7 | 13 | 6.1 | 16 | 15 | 6.5 | 45 | 5.4 |
| | 3 MLHK | 9.3 | 7.7 | 13 | 20 | 17 | 7.6 | 12 | 9.6 | 11 |
| | 4 CC–430 | 9.3 | 7.7 | 13 | 10 | 25 | 3.9 | 6.2 | 54 | 5.5 |

POHK pediococcus strain POHK and Pökelferment 77 starter; MLHK pediococcus strain MLHK and Pökelferment 77 starter; CC–430 starter.
Petaja and others 2000

stored for 8 d at 4 °C (39 °F) with combinations of NaCl, potassium sorbate and modified atmospheric packaging (MAP) were measured (Aytac and others 2000). Samples treated with 1% potassium sorbate solution contained histamine content lower than a control during 2 d of storage. MAP combined with 1% potassium sorbate also retarded the growth of *M. morganii* during 3 d, when compared to the control. After 3 d, *M. morganii* counts were $1.2 \times 10^5$/g, $5 \times 10^5$/g, and $8 \times 10^5$/g for 100% $CO_2$, 100% $CO_2$ combined with 1% potassium sorbate, and control, respectively. Although when $CO_2$ was used alone, histamine production was slower than in the control, it reached higher levels than the control after 8 d (198 mg/100 g [1980 ppm] in the $CO_2$ stored group versus 75.2 mg/100 g [752 ppm] in the control). It appears that after longer times of storage, none of the treatments was effective in controlling the formation of biogenic amines. It is apparent that the shelf-life extension of MAP fish can be extended only if sanitary conditions combined with proper temperature are maintained from harvest.

The histamine content of irradiated samples increased gradually during storage at 4 °C (39 °F) of mackerel samples inoculated for 30 s in a $7.5 \times 10^3$ cfu/ml suspension of *M. morganii* (Aytac and others 2000). Maximum histamine levels after 8 d were 202 mg/100 g (202 ppm) and 206 mg/100 g (206 ppm) for the samples irradiated with the doses of 0.5 and 2.0 kGy respectively, compared to an initial concentration of 41.2 mg/100 g (412 ppm). *M. morganella* grew approximately 2.0 and 0.7 logs in samples irradiated with 0.5 kGy and 2.0 kGy during 8 d. This is in contrast to Mutluer and others (1989), who concluded that irradiation using 1.0 and 2.0 kGy, in conjunction with refrigeration at 5 °C (41 °F), effectively retarded production of histamine for a 10-d period in mackerel fillets inoculated with *M. morganii*. Again, the variability of these findings reflects the challenges of representative sampling, species differences, quality of initial raw material, and other experimental conditions.

## 5. Conclusions

The following are conclusions about the potential for cold-smoked fish consumption to result in scombrotoxin foodborne illness:

- The majority of species that are cold-smoked have not been identified by the scientific community as causing scombrotoxin illness. Therefore, the risk of foodborne illness is limited in the majority of cold-smoked products available in the marketplace.
- Only relatively high and sometimes controversial concentrations of histamine have usually resulted in illness. The contribution of other biogenic amines to the onset of symptoms is not well understood.
- Most scombrotoxin results from extrinsic, rather than intrinsic, spoilage through the growth of certain bacteria, generally members of the family *Enterobacteriaceae*. Some bacteria are capable of producing greater quantities of decarboxylase enzymes than others.
- Certain processing operations, such as freezing, salting or smoking may be capable of inhibiting or inactivating biogenic amine-producing microorganisms. However, microorganism growth and potential toxun formation may occur after thawing and postprocessing.
- Under certain conditions addition of lactic acid-producing microorganisms suppresses the growth of biogenic amine-forming microorganisms.
- Vacuum packaging does not prevent growth of biogenic amine-forming microorganisms.
- While biogenic amine-forming microorganisms may grow at refrigeration temperatures, generally the minimal temperature for growth is lower than the minimal temperature for toxin production.
- The most effective methods of preventing biogenic amine formation are handling and processing under sanitary conditions, rapid cooling of the fish, and continued refrigeration from harvest through consumption.
- Limited research has shown that histamine production is greater in light (white) meat rather than dark (red) meat, but the histidine concentration is greater in the dark meat species of fish.
- Much of the research reported in the scientific literature on scombrotoxin utilized fish samples obtained from processing facilities and retail food stores. Only a limited number of studies followed samples from harvest through analysis. Also, sensory analyses were not always incorporated into microbiological and analytical chemical studies. There is a lack of reports describing comprehensive and integrated projects.

## 6. Research Needs

The following is a list of research areas that the panel suggests need further attention:

- Determine the influence of modified atmosphere packaging on the inhibition of biogenic amine production by Gram-negative bacteria.
- Define the minimum temperatures for growth and biogenic amine production of biogenic amine-forming microorganisms.
- Identify practical temperatures that would minimize the levels of biogenic amines in all steps of the chain production and

processing and in the final product.

- Determine the effect of salt and redox potential on the formation of biogenic amines in the final product.
- Determine the impact of interrelationship(s) among histamine, putrescine, and cadaverine, and perhaps other biogenic amine concentrations in scombrotoxin and their effects on subsequent host responses.
- Investigate the effects of various cold-smoked fish processes (water phase salt concentrations, process times and temperatures) on biogenic amine formation.
- Apply new processes, such as irradiation, modified atmospheres, or high pressure, to reduce specific groups of microorganisms to determine if control of those responsible for biogenic amine formation reduces the hazard.
- Evaluate the effects of harvesting methods and postharvest handling practices on biogenic amine formation under varying environmental conditions.
- Investigate practical methods for cold-smoked fish processors to determine the histamine/scombrotoxin risk in the raw material used for smoking.
- Identify specific methods for representative and effective sampling and for accurate and precise analysis of biogenic amines.

## References


Ababouch L, Afilal ME, Benabdeljelil H, Busta FF. 1991a. Quantitative changes in bacteria, amino acids and biogenic amines in sardine (*Sardina pilchardus*) stored at ambient temperature (25 to 28° C) and in ice. Int J Food Sci Tech 26:297–306.

Ababouch L, Afilal ME, Rhafiri S, Busta FF. 1991b. Identification of histamine-producing bacteria isolated from sardine (*Sardina pilchardus*) stored in ice and at ambient temperature (25° C). Food Microbiol 8:127–36.

Aiiso K, Toyoura H, Iida H. 1958. Distribution and activity of histidine decarboxylase in *Morganella.* Jap J Microbiol 2(2):143–7.

Aksnes A. 1988. Location of enzymes responsible for autolysis in bulk-stored capelin (*Mallotus villosus*). J Sci Food Agric 44:263–71.

Aksnes A, Brekken B. 1988. Tissue degradation, amino acid liberation and bacterial decomposition of bulk stored capelin. J Sci Food Agric 45:53–60.

Arnold SH, Brown WD. 1978. Histamine (?) toxicity from fish products. Adv Food Res 24:113–54.

Arnold SH, Price RJ, Brown WD. 1980. Histamine formation by bacteria isolated from skipjack tuna, *Katsuwonus pelamis*. Bull Jap Soc Sci Fish 46(8):991–5.

Aytac SA, Ozbas ZY, Vural H. 2000. Effects of irradiation, antimicrobial agents and modified-atmosphere packaging on histamine production by *Morganella morganii* in mackerel fillets. Archiv Fur Lebensmittelhygiene 51:28–30.

Baranowski JD, Frank HA, Brust PA, Chongsiriwatana M, Premaratne RJ. 1990. Decomposition and histamine content in mahimahi (*Coryphaena hippurus*). J Food Prot 53(3):217–22.

Behling AR, Taylor SL. 1982. Bacterial histamine production as a function of temperature and time of incubation. J Food Sci 47:1311–4, 1317.

Ben-Gigirey B, Craven C, An H. 1998b. Histamine formation in albacore muscle analyzed by AOAC and enzymatic methods. J Food Sci 63(2):210–4.

Ben-Gigirey B, Vieites Baptista de Sousa JM, Villa TG, Barros-Velazquez J. 1998a. Changes in biogenic amines and microbiological analysis in albacore (*Thunnus alalunga*) muscle during frozen storage. J Food Prot 61(5):608–15.

Ben-Gigirey B, Vietites Baptista de Sousa JM, Villa TG, Barros-Velazquez J. 1999. Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (*Thunnus alalunga*). J Food Prot 62(8):933–9.

Bjeldanes LF, Schutz DE, Mooris MM. 1978. On the aetiology of scombroid poisoning: cadaverine potentiation of histamine toxicity in the guinea-pig. Food Cosmet Toxicol 16(2):157–9.

Chu C-H, Bjeldanes LF. 1981. Effect of diamines, polyamines and tuna fish extracts on the binding of histamine to mucin in vitro. J Food Sci 47:79-?

Clifford MN, Walker R, Ijomah P, Wright J, Murray CK, Hardy R. 1991. Is there a role for amines other than histamines in the aetiology of scombrotoxicosis. Food Addit Contam 8(5):641–52.

Clifford MN, Walker R, Wright J, Hardy R, Murray CK. 1989. Studies with volunteers on the role of histamine in suspected scombrotoxicosis. J Sci Food Agric 47:365–75.

Crapo C, Himelbloom B. 1999. Spoilage and histamine in whole Pacific herring (*Clupea harengus pallasi*) and pink salmon (*Oncorhynchus gorbuscha*) fillets. J Food Safety 19:45–55.

Dawood AA, Karkalas J, Roy RN, Williams CS. 1988. The occurrence of nonvolatile amines in chilled-stored rainbow trout (*Salmo irideus).* Food Chem 27:33–45.

Douglas WW. 1970. Histamine and antihistamines; 5-Hydroxytryptamine and antagonists. In: Goodman LS, Gilman A, editors. The pharmacological basis of therapeutics. 5th ed. New York: Macmillan. p 621–62.

Edmunds WJ, Eitenmiller RR. 1975. Effect of storage time and temperature on histamine content and histidine decarboxylase activity of aquatic species. J Food Sci 40:516–9.

EEC. 1991. Council directive 91/493/EEC of 22nd July 1991 laying down the health conditions for the production and the placing on the market of fishery products. Off J Eur Comm(Nr L268):15–32.

Eitenmiller RR, Wallis JW, Orr JH, Phillips RD. 1981. Production of histidine decarboxylase and histamine by *Proteus morganii*. J Food Prot 44(11):815–20.

[FDA] Food and Drug Administration. 1996. Fish & fisheries products hazards & controls guide: first edition. Washington D.C.: FDA, Center for Food Safety and Applied Nutrition, Office of Seafood.

[FDA] Food and Drug Administration. 1998. Fish & Fisheries Products Hazards & Controls Guide. 2nd ed. Washington, D.C.: FDA, Office of Seafood. 276 p.

Fernandez-Salguero J, Mackie IM. 1979. Histidine metabolism in mackerel (*Scomber scombrus*). Studies on histidine decarboxylase activity and histamine formation during storage of flesh and liver under sterile and nonsterile conditions. J Food Technol 14:131–9.

Fernandez-Salguero J, Mackie IM. 1987a. Comparative rates of spoilage of fillets and whole fish during storage of haddock (*Melanogrammus aeglefinus*) and herring (*Clupea harengus*) as determined by the formation of nonvolatile and volatile amines. Int J Food Sci Tech 22:385–90.

Fernandez-Salguero J, Mackie IM. 1987b. Technical note: Preliminary survey of the content of histamine and other higher amines in some samples of Spanish canned fish. Int J Food Sci Tech 22:409–12.

Fletcher GC, Summers G, Winchester RV, Wong RJ. 1995. Histamine and histidine in New Zealand marine fish and shellfish species, particularly Kahawai (*Arripis trutta*). J Aquat Food Prod Technol 4(2):53–74.

Frank HA, Baranowski JD, Chongsiriwatana M, Brust PA, Premaratne RJ. 1985. Identification and decarboxylase activities of bacteria isolated from decomposed mahimahi (*Coryphaena hippurus*) after incubation at 0 and 32° C. Int J Food Microbiol 2:331–40.

Fujii T, Kurihara K, Okuzumi M. 1994. Viability and histidine decarboxylase activity of halophilic histamine-forming bacteria during frozen storage. J Food Prot 57(7):611–3.

Gale EF. 1946. The bacterial amino acid decarboxylases. Adv Enzymology and Related Subjects of Biochemistry 6:1–32.

Gessner BD, Hokama Y, Isto S. 1996. Scombrotoxicosis-like illness following the ingestion of smoked salmon that demonstrated low histamine levels and high toxicity on mouse bioassay. Clinical Infectious Diseases 23:1316–8.

Gildberg A. 1978. Proteolytic activity and the frequency of burst bellies in capelin. J Food Technol 13:409–16.

Gingerich TM, Lorca T, Flick GJ, Pierson MD, McNair HM. 1999. Biogenic amine survey and organoleptic changes in fresh, stored, and temperature-abused bluefish (*Pomatomus saltatrix).* J Food Prot 62(9):1033–7.

Gloria MBA, Daeschel MA, Craven C, Hilderbrand Jr. KS. 1999. Histamine and other biogenic amines in albacore tuna. J Aqu Food Prod Technol 8(4):54–69.

Granerus G. 1968. Effects of oral histamine, histidine, and diet on urinary excretion of histamine, methylhistamine, and 1-methyl–4-imidazoleacetic acid in man. Scand J Clin Lab Invest Suppl 10(4):49–58.

Haaland H, Arnesen E, Njaa LR. 1990. Amino acid composition of whole mackerel (*Scomber scombrus*) stored anaerobically at 20° C and at 2° C. Int J Food Sci Tech 25:82–7.

Halasz A, Barath A, Simon-Sarkadi L, Holzapfel W. 1994. Biogenic amines and their production by microorganisms in food. Trends Food Sci Technol 5:42–9.

Hardy R, Smith JGM. 1976. The storage of mackerel (*Scomber scombrus*). Development of histamine and rancidity. J Sci Food Agric 27:595–9.

Henry Chin KD, Koehler PE. 1986. Effect of salt concentration and incubation temperature on formation of histamine, phenethylamine, tryptamine and tyramine during miso fermentation. J Food Prot 49(6):423–7.

Hildrum KI, Scanlan RA, Libbey LM. 1976. Nitrosamines from the nitrosation of spermidine and spermine. In: Walker EA, Bogovski P, Griciute L, editors. Environmental N-Nitroso Compounds analysis and formation: proceedings of a working conference; 1975 Oct 1–3; Polytechnical

Institute, Tallinn, Estonian SSR. International Agency for Research on Cancer. p 205–14.

Hui JY, Taylor SL. 1983. High pressure liquid chromatographic determination of putrefactive amines in foods. J AOAC 66(4):853–7.

Hungerford JM, Arefyev AA. 1992. Flow-injection assay of enzyme inhibition in fish using immobilized diamine oxidase. Analytica Chimica Acta 261:351–9.

Ibe A, Saito K, Nakazato M, Kikuchi Y, Fujinuma K, Nishima T. 1991. Quantitative determination of amines in wine by liquid chromatography. J AOAC 74(4):695–8.

Ienistea C. 1973. Significance and detection of histamine in food. In: Hobbs BC, Christian JHB, editors. The microbiological safety of foods. New York: Academic Press. p 327–43.

Jorgensen LV, Dalgaard P, Huss HH. 2000. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. J Agric Food Chem 48:2448–53.

Kaneko J, (PacMar, Inc., Honolulu, Hawaii). 2000. Development of a HACCP-based strategy for the control of histamine for the fresh tuna industry [A report by PacMar, Inc. pursuant to National Oceanographic and Atmospheric Administration]. Honolulu (Hawaii): PacMar. 2000 Jul 31. NOAA Award Nr NA86FD0067. 48 p.

Karolus JJ, LeBlanc DH, Marsh AJ, Mshar R, Furgalack TH. 1985. Presence of histamine in the bluefish, *Pomatomus saltatrix*. J Food Prot 48(2):166–8.

Kim S-H, Ben-Gigirey B, Barros-Velazquez J, Price RJ, An H. 2000. Histamine and biogenic amine production by *Morganella morganii* isolated from temperature-abused albacore. J Food Prot 63(2):244–51.

Kimata M, Akamatsu M, Ishida Y, (Kyoto Daigaku. Shokuryo Kagaku Kenkyujo). Memoirs of the Research Institute for Food Science. Kyoto: Kyoto Univ. 1960. Report nr 20. Studies on the classification of the genus *Proteus* I. p 1–7.

Kimata M, Kawai A, (Kyoto Daigaku. Shokuryo Kagaku Kenkyujo). Bulletin of the Research Institute for Food Science. Kyoto: Kyoto Univ. 1953 Oct. Report nr 12. The production of histamine by the action of bacteria causing the spoilage of fresh fish, I. 29–33 p.

Klausen NK, Huss HH. 1987. Growth and histamine production by *Morganella morganii* under various temperature conditions. Int J Food Microbiol 5:147–56.

Klausen NK, Lund E. 1986. Formation of biogenic amines in herring and mackerel. Z Lebensm Unters Forsch 182(6):459–63.

Koutsoumanis K, Lampropoulou K, Nychas G-JE. 1999. Biogenic amines and sensory changes associated with the microbial flora of Mediterranean Gilt-head sea bream (*Sparus aurata*) stored aerobically at 0, 8, and 15° C. J Food Prot 62(4):398–402.

Leitao MFF, Baldini VLS, Sales AM. 1983. Histamina em pescado e alimentos industrializados. Col Inst Technol Alim 13:123–30.

Leroi F, Joffraud JJ, Chevalier F. 2000. Effect of salt and smoke on the microbiological quality of cold-smoked salmon during storage at 5° C as estimated by the factorial design method. J Food Prot 63(4):502–8.

Leroi F, Joffraud JJ, Chevalier F, Cardinal M. 1998. Study of the microbial ecology of cold-smoked salmon during storage at 8° C. Int J Food Microbiol 39:111–21.

Leuschner RGK, Hammes WP. 1999. Formation of biogenic amine in mayonnaise, herring and tuna fish salad by *Lactobacilli*. Int J Food Sci Nut 50:159–64.

Lopez-Sabater EI, Rodriguez-Jerez JJ, Hernandez-Herrero M, Mora-Ventura MT. 1994a. Evaluation of histidine decarboxylase activity of bacteria isolated from sardine (*Sardina pilchardus*) by an enzymic method. Lett Appl Microbiol 19:70–5.

Lopez-Sabater EI, Rodriguez-Jerez JJ, Roig-Sagues AX, Mora-Ventura MAT. 1994b. Bacteriological quality of tuna fish (*Thunnus thynnus*) destined for canning: effect of tuna handling on presence of histidine decarboxylase bacteria and histidine level. J Food Prot 57(4):318–23.

Lopez-Sabater EI, Rodriguez-Jerez JJ, Hernandez-Herrero M, Mora-Ventura MT. 1996a. Incidence of histamine-forming bacteria and histamine content in scombroid fish species

from retail markets in the Barcelona area. Int J Food Microbiol 28:411–8.
Lopez-Sabater EI, Rodriguez-Jerez JJ, Hernandez-Herrero M, Roig-Sagues AX, Mora-Ventura MT. 1996b. Sensory quality and histamine formation during controlled decomposition of tuna (*Thunnus thynnus*). J Food Prot 59(2):167–74.
Maga JA. 1978. Amines in foods. CRC Crit Rev Food Sci Nutr 10:373–403.
Masson F, Talon R, Montel MC. 1996. Histamine and tyramine production by bacteria from meat products. Int J Food Microbiol 32:199–207.
Mazorra-Manzano MA, Pacheco-Aguilar R, Diaz-Rojas EI, Lugo-Sanchez ME. 2000. Postmortem changes in black skipjack muscle during storage in ice. J Food Sci 65(5):774–9.
Mendes R. 1999. Changes in biogenic amines of major Portuguese bluefish species during storage at different temperatures. J Food Biochem 23:33–43.
Mendes R, Goncalves A, Nunes ML. 1999. Changes in free amino acids and biogenic amines during ripening of fresh and frozen sardine. J Food Biochem 23:295–306.
Middlebrooks BL, Toom PM, Douglas WL, Harrison RE, McDowell S. 1988. Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (*Scomberomorus maculatus*). J Food Sci 53(4):1024–9.
Mietz JL, Karmas E. 1977. Chemical quality index of canned tuna as determined by high-pressure liquid chromatography. J Food Sci 42:155–8.
Mietz JL, Karmas E. 1978. Polyamine and histamine content of rockfish, salmon, lobster, and shrimp as an indicator of decomposition. J AOAC 61(1):139–45.
Morii H, Cann DC, Taylor LY, Murray CK. 1986. Formation of histamine by luminous bacteria isolated from scromboid fish. Bull Japan Soc Sci Fish 52(12):2135–41.
Murray CK, Hobbs G, Gilbert RJ. 1982. Scombrotoxin and scombrotoxin-like poisoning from canned fish. J Hyg 88:215–20.
Mutluer B, Ersen S, Kaya B, Akin S, Ozta-Siran I. 1989. Einfluss von Gammastrahlen auf Histaminbildung in Makrelenfilets. Fleischwirtsch 69:112–4.
Okuzumi M, Fukumoto I, Fujii T. 1990. Changes in bacterial flora and polyamines contents during storage of horse mackerel meat. Nippon Suisan Gakkasishi 56(8):1307–12.
Okuzumi M, Yamanaka H, Kubozuka T. 1984. Occurrence of various histamine-forming bacteria on/in fresh fishes. Bull Jap Soc Sci Fish 50(1):161–7.
Petaja E, Eerola S, Petaja P. 2000. Biogenic amines in cold-smoked fish fermented with lactic acid bacteria. Zeit Leben-Unter Forch 210(4):280–5.
Price RJ, Melvin EF, Bell JW. 1991. Postmortem changes in chilled round, bled and dressed albacore. J Food Sci 56:318–21.
Reddy NR, Armstrong DJ, Rhodehamel EJ, Kautter DA. 1992. Shelf-life extension and safety concerns about fresh fishery products packaged under modified atmospheres: a review. J Food Safety 12:87–118.
Ritchie AH, Mackie IM. 1980. The formation of diamines and polyamines during storage of mackerel (*Scomber scrombrus*). In: Connell J, editor. Advances in Fish Science and Technology. Surrey (England):Fishing News (Books) Ltd. P 489–94.
Rodriquez-Jerez JJ, Mora-Ventura MT, Lopez-Sabater EI, Hernandez-Herrero M. 1994. Histidine, lysine, and ornithine decarboxylase bacteria in Spanish salted semipreserved anchovies. J Food Prot 57(9):784–7, 791.
Rogers, Staruszkiewicz. 1997. Collaborative study-GLC determination of cadaverine and putrescine in seafood; fluorometric method for histamine in tuna and mahimahi. JAOAC 80:591–602.
Shalaby AR. 1996. Significance of biogenic amines to food safety and human health. Food Res Int 29(7):675–90.
Shewan JM, Liston J. 1955. A review of food poisoning caused by fish and fishery products. J Appl Bacteriol 18:522–34.
Silla Santos MH. 1996. Biogenic amines: their importance in foods. Int J Food Microbiol 29:213–31.
Silva CCG, Da Ponte DJB, Enes Dapkevicius MLN. 1998. Storage temperature effect on histamine formation in big eye tuna and skipjack. J Food Sci 63(4):644–7.
Sjaastad O. 1966. Fate of histamine and N-Acetylhistamine administered into the human gut. Acta Pharmacol Toxicol 24:189–202.
Soares VFM, Gloria MBA. 1994. Histamine levels in canned fish available in the retail market of Belo Horizonte, Minas Gerais, Brazil. J Food Comp Anal 7:102–9.
Suzuki S, Matsui Y, Takama K. 1988. Profiles of polyamine composition in putrefactive *Pseudomonas* type III/IV. Microbios Letters 38:105–9.
Suzuki S, Noda J, Takama K. 1990. Growth and polyamine production of *Alteromonas* spp. in fish meat extracts under modified atmosphere. Bull Fac Fish, Hokkaido Univ 41(4):213–20.
Takagi M, Iida A, Murayama H, Soma S. 1969. On the formation of histamine during loss of freshness and putrefaction of various marine products. Bull Fac Fish, Hokkaido Univ 20:227–34.
Taylor SL. 1986. Histamine food poisoning: toxicology and clinical aspects. CRC Crit Rev Toxicol 17(2):91–128.
Taylor SL, Guthertz LS, Leatherwood M, Lieber ER. 1979. Histamine production by *Klebsiella pneumoniae* and an incident of scombroid fish poisoning. Appl Environ Microbiol 37:274–8.
Taylor SL, Guthertz LS, Leatherwood M, Tillman F, Lieber ER. 1978. Histamine production by foodborne bacterial species. J Food Safety 1:173–87.
Taylor SL, Lieber ER. 1979. In vitro inhibition of rat intestinal histamine-metabolizing enzymes. Food Cosmet Toxicol 17:237–40.
Taylor SL, Speckhard MW. 1984. Inhibition of bacterial histamine production by sorbate and other antimicrobial agents. J Food Prot 47(7):508–11.
Taylor SL, Stratton JE, Nordlee JA. 1989. Histamine poisoning (scombroid fish poisoning): an allergy-like intoxication. Clin Toxicol 27(4&5):225–40.
ten Brink B, Damink C, Joosten HMLJ, Huis in 't Veld JHJ. 1990. Occurrence and formation of biologically active amines in foods. Int J Food Microbiol 11:73–84.
Veciana-Nogues MT, Marine-Font A, Vidal-Carou MC. 1997. Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts, ATP-related compounds, volatile amines and organoleptic changes. J Agric Food Chem 45:2036–41.
Wei CI, Chen C-M, Koburger JA, Otwell WS, Marshall MR. 1990. Bacterial growth and histamine production on vacuum packaged tuna. J Food Sci 55(1):59–63.
Wendakoon CN, Murata M, Sakaguchi M. 1990. Comparison of nonvolatile amine formation between the dark and white muscles of mackerel during storage. Nippon Suisan Gakkaishi 56(5):809–18.
Wendakoon CN, Sakaguchi M. 1992a. Effects of spices on growth of and biogenic amine formation by bacteria in fish muscle. In: Huss HH, Jakobsen M, Liston J, editors. Quality Assurance in the Fish Industry: Proceedings of an Intl. Conference; 1991 Aug 26–30; Copenhagen (DK). Amsterdam: Elsevier; 1992. p 305–13 (Development in Food Sci series; 30).
Wendakoon CN, Sakaguchi M. 1992b. Nonvolatile amine production in mackerel muscle during growth of different bacterial species. J Food Hyg Soc Jap 33(1):39–45.
Wendakoon CN, Sakaguchi M. 1993. Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. J Food Prot 56(5):410–3.
Yamanaka H, Shimakura K, Shiomi K, Kikuchi T, Okuzumi M. 1987b. Occurrence of allergy-like food poisoning caused by "mirin"-seasoned meat of dorado (*Coryphaena hippurus*). J Food Hyg Soc Japan 28(5):354–85.
Yamanaka H, Shiomi K, Kikuchi T, Okuzumi M. 1982. A pungent compound produced in the meat of frozen yellowfin tuna and marlin. Bull Jap Soc Sci Fish 48(5):685–9.
Yamanaka H, Shiomi K, Kikuchi T, Okuzumi M. 1984. Changes in histamine contents in red meat fish during storage at different temperatures. Bull Jap Soc Sci Fish 50(4):695–701.
Zotos A, Hole M, Smith G. 1995. The effect of frozen storage of mackerel (*Scomber scombrus*) on its quality when hot-smoked. J Sci Food Agric 67(1):43–8.

CHAPTER V

# Potential Hazards in Cold-Smoked Fish: Parasites

GLEYN E. BELDSOE AND MARIA P. ORIA

## Scope

This section reviews methods of control of parasites that may be of concern in cold-smoked fish. Evidence suggests some fish parasites that are currently not considered human pathogens may become a human health concern in the future, but a description and the control of such parasites are beyond the scope of this report. Consequently, this report will discuss only those parasites that have been known to cause disease in humans.

## 1. Introduction

A variety of parasites have been identified in raw fish. Most of the scientific literature describes methods to control the most significant parasites of concern in the western world, such as anisakid parasites. Although trematode diseases are endemic to countries other than the United States, interest in their control is increasing, given the number of diseases caused by these parasites. A number of parasites also are emerging as possible hazards in the future. For example, evidence shows that anisakids *Contracaecum multipapillatum* and *Hysterothylacium* type MB can infect primates and mammals, respectively. In addition, it is well known that recent climatological changes and expanded human activities will accelerate the global transport and dissemination of species and will accelerate host-shifts in a manner difficult to predict (Harvell and others 1999). The growing number of marine mammals, particularly seals and sea lions in the northern Pacific and Atlantic oceans, is increasing the occurrence of parasites in fish. This is expected to continue. Other phenomena that may result in an increase in marine infections are the global distribution and increasing popularity of undercooked seafood products (Overstreet 1999). The following descriptions of the life cycles of the parasites of concern have been summarized from Bier (1992), Hayunga (1997), Goldsmid and Speare (1997), Reilly and Kaferstein (1997), and Adams and Rausch (1997).

Anisakiasis is a disease that includes infections by all ascaroid nematodes having larval stages in aquatic hosts. The main nematodes known to have caused disease in humans are *Anisakis simplex* and *Pseudoterranova decipiens*. These nematodes reach sexual maturity in the intestinal tract of marine mammals. Eggs are expelled into the intestinal tract and then are expelled in the feces. In the water the eggs embryonate and undergo at least one moult. The larvae that hatch may infect a small crustacean that may in turn be ingested by a fish (that is, rockfish, herring, mackerel, and salmon). When an infected fish is consumed by another fish, the larvae may penetrate the viscera and infect the new fish host. Marine mammals (such as dolphins, seals, and so on) or humans may become infected from eating the infected intermediate host. In humans, these nematodes do not normally mature, but the worms can migrate from the gastrointestinal tract, becoming embedded in the gastrointestinal mucosa and causing tissue reaction and discomfort (that is, gastric pain, diarrhea, vomiting).

Cestodes are tapeworms and the species of major concern associated with consumption of fish are in the genus *Diphyllobothrium*. This tapeworm reaches sexual maturity in the intestinal tract of mammals. Eggs are excreted with the feces and develop in water into larvae that hatch into coracidium and may be eaten by a crustacean. In the crustacean body cavity it develops into the next stage, the procercoid. The larvae may then become infective to fish that ingest the crustacean. These larvae then develop into the plerocercoid. Plerocercoids may infect other fish and cease development or infect mammals when they reach sexual maturity. Humans are one of the definitive hosts. Salmon is the most common fish that transmits diphyllobothriasis, although it may also be transmitted by whitefish, trout, and pike. Because the disease is not contagious (and, in the United States, the Center for Disease Control does not require reporting the disease) often it is not reported to health authorities. Some of the symptoms include nausea, abdominal pain, diarrhea, and weakness.

Trematode (or flukes) species of concern have very similar life cycles. Depending on the species, trematodes transmitted by the ingestion of seafood may reach sexual maturity in the liver (for example, *Clonorchis, Opisthorchis*), intestine (for example, *Nanophyetus*, *Heterophyes*), or lungs (for example, *Paragonimus*) of humans and other mammals. Eggs pass out to the environment in feces and infect mollusks after being ingested. The larvae penetrate the tissue through morphologically distinct stages that asexually produce free swimming larvae. In general, they have a snail intermediate host and use various aquatic animals to harbor metacercaria, the infective stage. The clinical effects of intestinal flukes are generally not serious, although *Clonorchis sinensis* and *Opisthorchis viverrini* can cause serious liver damage to humans, and have been associated with carcinoma of the liver.

## 2. Prevalence of parasites in raw, frozen, and smoked fish

Human pathogenic parasites occur in several species of fish that may be cold-smoked, including gadoids, salmonids, sole or flounder, grouper, halibut (*Hippoglossus spp* and *Paralichthys sp.*), herring, mackerel, mullet, sablefish, sprat, small tunas, and turbot. Parasites are also identified as a potential hazard in some invertebrates that may be cold-smoked or cold-smoked and dried, including octopus, squid, snails, and crabs/crayfish. Several species of salmonid parasites, such as *Anisakis* spp. (a nematode or roundworm), *Diphyllobothrium* spp. (a cestode or tapeworm) and *Nanophyetus salmincola* are of public health concern (Turner and others 1981; Eastburn and others 1987).

All wild-caught Pacific salmon (*Oncorhynchus spp.)* are considered to have *A. simplex* larvae present. Prevalence reached more than 75% in fresh U.S. commercial sockeye salmon (*O. nerka*), chum salmon (*O. keta*), coho salmon (*O. kisutch*), and king salmon (*O. tschawyscha*) (Myers 1979; Deardoff and Overstreet 1991). High incidence was also reported from U.S. supermarkets (Rosset and others 1982). There seems to be a high prevalence of larvae in Atlantic salmon, particularly in the muscle (39%) and in-

testinal cavity (64%) (Beverley-Burton and Pippy 1978; Bristow and Berland 1991). *Anisakis simplex* larvae were detected at a high incidence (78 to 97%) in herring (*Clupea harengus*) during 1981 to 86 in the Mancha Channel (Declerck 1988) and in smoked herring from a French supermarket (Lagoin 1980). Pacini and others (1993) tested commercial samples of fresh, frozen and smoked fish on the Italian market for presence of anisakid larvae. They observed that 54% of fresh, 28% of frozen, and 75% of smoked fish samples contained nematodes belonging to Anisakidae; all larvae detected in frozen fish products were dead.

In contrast to wild-caught salmon, farmed salmon, particularly Atlantic salmon (*Salmo salar*), are not considered to be hosts of *Anisakis* spp. when fed normal pelleted feed. When 2832 Norwegian-farmed Atlantic salmon and 876 Scottish-farmed Atlantic smoked salmon fillets were analyzed for anisakid larvae infestation, none were detected (Angot and Brasseur 1993). This result is in agreement with results from previous studies that indicated that farmed salmon (Atlantic, coho, and chinook species) are virtually free from anisakid larvae (Bristow and Berland 1991; Deardoff and Kent 1989). Nevertheless, it should be emphasized that aquacultured fish can become hosts of anisakids if fed moist feeds (that is, raw fish).

Other nematodes, including *Gnathostoma* spp., *Eustrongylides* spp., and *Pseudoterranova* spp., may cause disease in humans. *Diphyllobothrium* spp. has been reported in salmonids and has caused human illness. These cestodes should be considered a possible hazard in all environments and cannot be ruled out from aquaculture systems. The majority of trematode infections occurs endemically in some countries of Eastern Asia, South America, Eastern Europe, and West Africa and derive mostly from wild-caught fish; however, with an increasing global fish trade, trematodes or flukes, including *Heterophyes* spp., *Metagonimus* spp., *Opisthorchis* spp., *Clonorchis sinensis, Echinostoma* spp, and *N. salmincola*, could become a public health concern.

## 3. Incidence

While normally not fatal, parasitic worms can cause intestinal discomfort and other more serious symptoms (Turner and others 1981). Although few cases of anisakiasis have been documented in the United States, many cases have been reported in Japan (Oshima 1972), principally resulting from the consumption of cold-smoked or raw salmon. A recent study on the occurrence of anisakiasis in 27 countries revealed 33747 cases of anisakiasis, estimated from Japanese and other databases from 1968 to 1998. In Japan, eight of those cases were from *Pseudoterranova decipiens* (Ishikura and others 1998).

At least two known outbreaks of diphyllobothriasis associated with salmon consumption have been documented in the United States (Turner and others 1981). Interestingly, the disease is thought to be more prevalent than anisakiasis, but it is not usually reported. It has been estimated that there are 13 million carriers globally (Crompton and Joyner 1980), with greater prevalence in Eastern Europe.

Fish trematode infections, particularly chlonorchiasis, opistorchiasis, and paragonimiasis, may also be derived from the consumption of raw or underprocessed fish. Trematode infections are a public health issue mainly in Eastern and Southern Asia. Although the source was not identified, it has been estimated that 50 million people are affected throughout the world (Lima dos Santos 1997). For example, between 1974 and 1985, 8 out of 10 patients in Oregon reported either gastrointestinal complaints or unexplained peripheral blood eosinophilia and had eggs typical for *N. salmincola* recovered from their stools. They also recalled eating fish prior to the onset of symptoms and had a history of ingestion of raw, incompletely cooked, or smoked salmon (Onchorhynhcus sp.), steelhead trout (Salmon gairdneri), or steelhead eggs. The authors point out that this problem exists West of the Cascade mountain range from Northern California to the Olympic Peninsula in Washington State, United States (Eastburn and others 1987).

## 4. Effects of processing steps and their use in controlling parasites

### 4.1. Salting and cold smoking

Although *A. simplex* seems to be sensitive to salt, the high salt concentrations and times needed for its elimination make salting an inadequate method of inactivation. For example, Karl and others (1995) reported that in herring processed with the traditional German and Danish procedures, larvae were killed only after being marinated for 5 to 6 wk in 8 to 9% salt. When salt concentration was lowered to 4.3%, the time to kill all the larvae increased to 7 wk. Similarly, Fan (1998) reported that metacercariae of *Clonorchis sinensis* from fresh water fish (*Pseudorasbora parva*) were killed if kept in heavy salt. These results clearly demonstrate that the more typical water phase salt contents of 3 to 3.5% in cold-smoked fish would not be sufficient to kill the organisms. In addition, dry salting does tend to kill those parasites residing on fish surfaces, but generally does not do so for those imbedded within the tissue.

Several studies have reported temperatures and times needed to kill parasites. For example, Bier (1976) indicated that 60 °C (140 °F) for 1 min was needed to kill the anisakid larvae. These temperatures are not achieved during cold smoking of fish and therefore parasites are not eliminated by the cold-smoking process. Gardiner (1990) reported that neither cold–smoking for 12 h at 25.6 °C (78 °F) nor refrigeration for 27 d reduced the amount of larvae in salmon. This analysis indicated that fresh salmon and cold-smoked salmon had 1 to 3 and 1 to 5 *Anisakis* spp. viable larvae/200 g of fish, respectively. A similar result was found in whole Pacific herring (*Clupea harengus pallasi),* where Hauck (1977) reported that Anisakis larval viability after brining and smoking at an average temperature of 19 °C (66 °F) for 24 h was 100% and 87.5%, respectively.

### 4.2. Freezing

Unlike bacteria, molds, and viruses, most parasites are relatively easy to destroy by holding the raw material or finished product at freezing temperatures for a specified period of time; of course, this is dependent upon the internal temperature of the material. The Fish and Fishery Products Hazards and Controls Guide recommends a temperature below –4 °F (–20 °C) for 7 d or –31 °F (–35 °C) (internal) for 15 h to kill the parasites of concern (FDA 1998). Although, based on the data currently available, these recommendations may appear stringent, it is because they were developed for the parasites that are considered most resistant to freezing (G. Hoskin 2001; personal communication; unreferenced). Already in 1975 (*Food Chemical News*, October 1975) G.J. Jackson cautioned that the anisakid nematodes vary in their ability to survive at low temperatures. For instance, certain species of anisakids have been reported to survive up to 52 h at –4 °F. A number of other time and temperature regimes have been prescribed to accomplish the inactivation of parasites. Another such option prescribes holding the fish at –10 °F (–23 °C) for 60 h (Ching 1984). Alternatively, E.U. regulations require freezing at a temperature of no more than –4 °F (–20 °C) in all parts of the product for not less than 24 h in order to control parasites in fish.

Some published studies support the effectiveness in controlling parasites by freezing at –4 °F (–20 °C) in all parts of the product for not less than 24 h. Very early studies by Gustafson (1953) demonstrated that temperatures of less than –17 °C (1.4 °F) for 24 h could kill *Anisakis* larvae. Higher temperatures or shorter

times were not as effective. Studies in herring (Houwing 1969) demonstrated that at –4 °F, nematodes were killed in 24 h, but if the product temperature reached –30 °C (–22 °F) by a cryogenic method, the inactivation was immediate, and no further storage was necessary. A more recent study by Deardoff and Throm (1988) used blast freezing to freeze salmon and rockfish at –31 °F (–35 °C). Fish were stored frozen for 15 h and then at –18 °C (0 °F) for up to 48 h. Out of 3545, they found no viable larvae after 1 h of storage at –18 °C. Similar results were found in herring by Karl and Leinemann (1989). They investigated the effect of freezing and cold storage on survival of *Anisakix simplex* in herring and herring fillets at –20 °C (–4 °F) for 24 h and found no surviving parasites. Although Hauck (1977) reported no viable *Anisakis* after freezing, the conditions were not detailed. The use of freezing has also been investigated for the control of other parasites of human health concern. Although the World Health Organization (1979) indicated that freezing fish at –10 °C (14 °F) for 5 d would kill all trematodes of concern, later research data indicate that longer times may be needed. For instance, Fan (1998) reported that metacercariae of *Clonorchis sinensis* from fresh water fish (*Pseudorasbora parva*) remained viable after frozen storage at –12 °C (10 °F) for 10 to 18 d and –20 °C for 3 to 7 d. As mentioned previously, clonorchiasis is not common in Western countries. The metacercariae of *Heterophyes* are also very resistant to freezing; since they survived 30 h of storage at –10 or –20 °F (Hamed and Elias 1970).

While the parasites can be killed by freezing the finished product, it is generally considered more appropriate to freeze the raw material prior to processing. Nematodes in particular will attempt to depart the gut during processing and will then establish themselves in the muscle during salting or smoking (Hauck 1977). The result may be the presence of nematodes on the surface of the finished product, often perpendicular to the surface. Their presence becomes a quality issue resulting in an aesthetically unwholesome (although safe) product. For this reason, it is a good practice to freeze susceptible raw material, even for hot-smoked fish.

Visual inspection of the product before brining or smoking is also advised. This measure, however, is effective only to ensure that visible parasites are not present rather than to ensure inactivation of viable organisms. Similarly, inspection of fish after slicing will also assist in producing a quality product but cannot be relied upon for assurance against the presence of live parasites in product from commercial operations.

Some recent research has shown that the current regulation and production practice for fishery products does not protect the consumers against allergic hazards due to the ingestion of killed parasites. Audicana and others (1997) have reported that freezing of fish may not protect against allergenic reactions to ingested Anisakis simplex antigens in humans. This issue was discussed in an opinion paper from the Scientific Committee on Veterinary Measures Relating to Public Health (EC 1998) that identified parasite antigens (what is left of the parasite in the fish after it is frozen to kill the parasite) as a possible human health hazard.

### 4.3 Irradiation

Irradiation of fish is an effective method of eliminating metacercariae and other parasites (WHO 1995). For example, low-dose irradiation (0.15 kGy or less) was sufficient to inactivate metacercariae of *C. sinensis* and *O. viverrini* without affecting the sensory qualities of the fish (FAO/IAEA 1992). Hamster infectivity of *O. viverrini* metacercariae was prevented with 0.5 kGy (Bhaibulaya 1985). Trematodes, however, appear to be more sensitive to irradiation than other parasites. Earlier studies indicated that in order to kill *A. simplex* in salted herring, doses of as high as 6 to 10 kGy were necessary (van Mameren and Houwing 1968). Similarly, another study found *A. simplex* larvae to be highly resistant to irradiation doses of 2 kGy or 10 kGy (FAO/IAEA 1992). Data from studies on sensory characteristics of such products are inconsistent. The reason that anisakid larvae require much higher doses of irradiation than other parasites (for example, metacercariae, *Trichinella* larvae, coccidian protozoa) is that anisakiasis results from infection by the larvae. The doses of irradiation must be high enough to kill the larvae. For the other parasites just mentioned, irradiation prevents the parasitic worms from developing into adults that cause the respective diseases.

The applicability and consumer acceptability of irradiation of fish as well as any organoleptic effects should be considered and evaluated before attempting commercialization of this process and these products.

## 5. Conclusions

The following conclusions are based on a thorough analysis and evaluation of the current science on control methods of parasites that may be associated with the consumption of cold-smoked fish.

- Some of the fish species used for cold-smoked processing are either intermediate or final hosts to parasites. For this reason, assuring the harvesting of parasite-free fish in the wild is difficult.
- Some aquacultured fish are considered free of parasites (if their feeding regime has not been supplemented with raw fish) because their diet can be controlled using net-pens, closed recycled systems or their equivalent, and commercially pelleted diets. Consequently, these control measures must be carefully considered and applied. An analysis of the potential control points for parasites in aquacultured fish is beyond the scope of this report.
- Freezing raw fish prior to smoking remains the most effective way to ensure that viable parasites are not present in cold-smoked products consumed by the public. It is essential, therefore, that raw fish potentially containing viable parasites be frozen and held in that state for a period of time that assures destruction of all viable parasites in that fish species.

## 6. Research Needs

The following is a list of research areas that the panel suggests need further attention:

- Describe possible alternative freezing procedures that are or could be effective for inactivation of various fish parasites.
- Establish the kinetics and lethal effect of specific regimes of freezing on various fish parasites.
- Evaluate alternative processing procedures, such as high pressure and X-ray or e-beam irradiation for control of various fish parasites.
- Investigate the possible human health risks of allergic reactions due to parasite antigens remaining after freezing the fish to inactivate the live parasites.

## References

Adams AM, Rausch RL. 1997. Diphyllobothriasis. In: Conner DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE, editors. Volume 2, Pathology of infectious diseases. Stamford (CN): Appleton and Lange. p 1377–90.

Angot V, Brasseur P. 1993. European farmed Atlantic salmon (*Salmo salar L.*) are safe from *Anisakid* larvae. Aquaculture 118:339–44.

Audicana L, Audicanna MT, Fernandez de Corres L, Kennedy MW. 1997. Cooking and freezing may not protect against allergenic reactions to ingested *Anisakis simplex* antigens in humans. The Veterinary Record 140:235.

Beverley-Burton M, Pippy JHC. 1978. Distribution, prevalence and mean numbers of larval *Anisakis simplex* (Nematoda: *Ascaridoidea*) in Atlantic salmon, *Salmo salar L.* and their use as biological indicators of hosts stocks. Environ Biol Fish 3:211–22.

Bhaibulaya M. 1985. Effect of gamma ray on the metacercarie of liver fluke (*Opisthorchis viverrini*) infective stages of parasite caused by consumption of raw or semiprocessed fish. Fd Irradiat Newsl 9(2):8.

Bier JW. 1976. Experimental *Anisakis*: cultivation and temperature tolerance determination. J Milk Food Technol 39:132.

Bristow GA, Berland B. 1991. A report on some metazoan parasites of wild marine salmon (*Salmo salar L.*) from the west coast of Norway with comments on their interactions with farmed salmon. Aquaculture 98:311–8.
Ching HL. 1984. Fish tapeworm infections (Diphyllobothriasis) in Canada, particularly British Columbia. Can Med Assoc J 130:1125–8.
Crompton DWT, Joiner SM. 1980. Parasitic Worms. London: Wyeham Publications.
Deardorff TL, Kent ML. 1989. Prevalence of larval *Anisakis simplex* in pen-reared and wild-caught salmon (*Salmonidae*) from Puget Sound, Washington. J Wildl Dis 25:416–9.
Deardorff TL, Overstreet RM. 1990. Seafood-transmitted zoonoses in the United States: the fishes, the dishes and the worms. In: Ward DR, Hackney CR, editors. Microbiology of marine food products. New York: Van Nostrand Reinhold. p 211–65.
Deardorff TL, Throm R. 1988. Commercial blast-freezing of third-stage *Anisakis* simplex larvae encapsulated in salmon and rockfish. J Parasit 74(4):600–3.
Declerck D. 1988. Presence de larves de *Anisakis* simplex dans le hareng (*Clupea harengus L.*). Revue de l'Agriculture 41(4):971–8.
Eastburn RL, Fritsche TR, and others. 1987. Human intestinal infection with *Nanophyetus salmincola* from salmonoid fishes. Am J Tropical Medicine and Hygiene 36(3):586–91.
[EC] European Commission. 1998. Scientific committee on veterinary measures relating to public health. Allergic reactions to ingested *Anisakis simplex* antigens and evaluation of the possible risk to human health.
Fan PC. 1998. Viability of metacercariae of *Clonorchis sinensis* in frozen or salted freshwater fish. Int J Parasitol 28:603–5.
FAO/IAEA. 1992. Final FAO/IAEA research co-ordination meeting on the use of irradiation to control infectivity of foodborne parasites. Food Irradiation Newsletter 16(1):5–14.
[FDA] Food and Drug Administration. 1998. Fish & Fisheries Products Hazards & Controls Guide. 2nd ed. Washington, D.C.: FDA, Office of Seafood. 276 p.
Food Chemical News. 1975 October 27. FDA-ER cautions that *Anisakine* nematodes can survive freezing. Food Chemical News:44–5.
Gardiner MA. 1990. Survival of *Anisakis* in cold-smoked salmon. Can Inst Food Sci Technol 23(2/3):143–4.
Goldsmid JM, Speare R. 1997. The parasitology of foods. In: Hocking AD, Arnold G, Jenson I, Newton K, Sutherland P, editors. Foodborne microorganisms of public health significance. 5th ed. North Sydney, NSW: Aust Inst Food Sci and Technol Inc (NSW Branch) Food Microbiol Group. p 583–602.
Gustafson PV. 1953. The effect of freezing on encysted *Anisakis* larvae. J Parasitol 39:585–8.
Hamed MGE, Elias AN. 1970. Effect of food processing methods upon survival of the trematode *Heterophyes* sp. in flesh of mullet caught from brackish Egyptian waters. J Food Sci 35:386.
Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, Hofmann EE, Lipp EK, Osterhaus ADME, Overstreet RM and others. 1999. Emerging marine diseases—climate links and anthropogenic factors. Science 285:1505–10.
Hauck AK. 1977. Occurrence and survival of the larval nematode *Anisakis* sp. in the flesh of fresh, frozen, brined, and smoked pacific herring, *Clupea harengus pallasi*. J Parasitol 63(3):515–9.
Hayunga EG. 1997. Helminths acquired from finfish, shellfish, and other food sources. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington D.C.: ASM Press. p 463–76.
Houwing H. 1969. The inactivation of herring nematodes (*Anisakis marina*) by freezing. Bull Int Inst Refrig, Annexe 1969–6:297–302.
Ishikura H, Takahashi S, Yagi K, Nakamura K, Kon S, Matsuura A, Sato N, Kikuchi K. 1998. Epidemiology: global aspects of Anisakidosis. Chiba (Japan): International Congress of Parasitology. Aug 24–28. Report nr ICOPA IX. 379–82 p.
Karl H, Roepstorff A, Huss HH, Bloemsma B. 1995. Survival of *Anisakis* larvae in marinated herring fillets. Int J Food Sci Technol 29(6):661–70.
Karl VH, Leinemann M. 1989. Uberlebensfahigkeit von nematodenlarven (*Anisaki*s sp.) in gefrosteten Heringen. Archiv fur Lebensmittelhygiene 40(1):14–6.
Lagoin Y. 1980. Donnees actuelles sur une nematodose larvaire de l'Homme, l'anisakiase ou "maladie du ver du hareng". Bull Acad Vet de France 53:139–46.
Lima dos Santos CA. 1997. The possible use of HACCP in the prevention and control of foodborne trematode infections in aquacultured fish. In: Shahidi F, editor. Seafood safety, processing and biotechnology. Lancaster (PA): Technomic. p 53–64.
Myers BJ. 1979. Anisakine nematodes in fresh commercial fish from waters along the Washington, Oregon and California coasts. J Food Prot 42:380–4.
Oshima T. 1972. *Anisakis* and anasakiasis in Japan and adjacent area. Progressive Medical Parasitology in Japan 4.
Overstreet RM. 1999. Actual and potential human health risks associated with marine parasites [abstract]. In: AAAS-Annual Meeting and Science Innovation Exposition; 1999 Jan 21–26; Anaheim (CA).
Pacini R, Panizzi L, Galleschi G, Quagli E, Galassi R, Fatighenti P, Morganti R. 1993. Presenza di larve di anisakidi in prodotti ittici freschi e congelati del commercio. Industrie Alimentari 32:942–4.
Reilly A, Kaferstein F. 1997. Food safety hazards and the application of the principles of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. Aquaculture Research 28:735–52.
Rosset JS, McClatchey KD, Higashi GI, Knisely AS. 1982. *Anisakis* larval type I in fresh salmon. Am J Clin Pathol 78:54–7.
Shin J-W, Huang Y-H. 2000. Investigation for contamination of parasite and aerobic bacteria in frozen tilapia fillets in Taiwan. J Food Drug Analysis 8(1):51–6.
Turner JA, Sorvillo FJ, Murray RA, Chin J, Middaugh JP, Dietrich PD, Wiebenga NH, Googins JA, Allard J, Ruttenber AJ and others. 1981. Diphyllobothriasis associated with salmon-United States. MMWR 30(27):331–2, 337.
Van Mameren J, Houwing H. 1968. Effect of irradiation on *Anasakis* larvae in salted herring. Elimination of harmful organisms from food and feed by irradiation. Vienna: IAEA. p 73–80.
WHO. 1979. WHO Expert Committee on Parasitic Zoonoses. Geneva: WHO. WHO Techn Rep Ser Nr 637.
WHO. 1995. Control of foodborne trematode infections, report of a study group. Geneva: WHO. WHO Techn Rep Ser Nr 849.

CHAPTER VI

# Control of Food Safety Hazards During Cold-Smoked Fish Processing

MICHAEL L. JAHNCKE ADN DANIEL HERMAN

## Scope

This section describes a general process of cold smoking fin fish (see also Appendix A) from the fishing vessel to the consumer. It was not the intention of the panel for this section to be a Hazard Analysis Critical Control Point (HACCP) plan. This section is limited to the following significant concerns: pathogenic bacteria, production of biogenic amines, and parasites. Other chemical or physical hazards are not included. Each step of this general process is analyzed in terms of identifying potential hazards, control points, and processing parameters when scientifically substantiated. Verification of the processing parameters is included in Appendix C. It is beyond the scope of this document to capture all of the wide variety of cold-smoking methods. Therefore, differences from the cold-smoking process described here may require a different analysis and evaluation. See Appendix D for survey data received from cold-smoked processor respondents. Appendix B describes some of the most common vessel practices. This chapter addresses some recommendations by the Association of Food and Drug Officials (AFDO 1991). It is noteworthy to clarify, however, that although developed by experts and widely used, these recommendations have not necessarily been scientifically evaluated.

## 1. Receiving

### 1.1. Background and support information

Hazards posed by fish and fish products vary and depend on the fish environment, both while the fish was alive and after it was caught. For example, a wild-caught, scombrotoxin-susceptible fish that has been kept for some time in the water or on a boat before being iced or frozen can have elevated toxic levels of biogenic amines. Vessel practices would help prevent biogenic amine development in scombrotoxin-susceptible species. Waters where fish are harvested may contain bacteria or spores that may be pathogenic to humans (*Clostridium botulinum* or *Listeria monocytogenes*). Fish also may come in contact with pathogenic microorganisms during harvesting, handling on board, and offloading and transportation to a smoking facility. Rinsing wild-caught fish with potable water (not environmental surface water) may aid in reducing *L. monocytogenes* in certain areas during certain seasons. Additionally, wild-caught fish may carry parasites that could be infectious to humans.

Aquaculture provides a year-round supply of fresh fish and a significant amount of raw material for cold-smoked fish processors. In general, aquaculture has the potential to be a highly controlled and hygienic operation at the initial harvesting and handling step, particularly in the case of salmon. The microbial flora present on aquacultured fish is affected by water quality and feed composition. Water quality can range from that in raceways or tanks fed by microbe-free spring water, well water, ozone-treated water, and ultraviolet light-treated water, to that in mud ponds purposely enriched with untreated human or animal waste. Hence, regardless of whether a fish has been wild-caught or farm-raised, it may carry various types of pathogenic bacteria when it arrives at the smoking facility. On the positive side, farm-raised fish that are fed with a controlled diet, such as processed feed pellets, and reared in closed systems with purified or treated water have been shown to be free of parasites (Angot and Brasseur 1993). Due to the amount of variation in water quality and conditions for farm-raised fish, a processor must consult with the supplier and not assume that the aquaculture product is parasite free.

The quality of the water should be one of the factors that determine the stringency of harvest and postharvest treatments. Processors of cold-smoked fish should be concerned with the microbiological quality of the primary product they receive from suppliers since product from contaminated sources may not be appropriate for production of cold-smoked products. In general, good sanitation procedures should be applied throughout harvest, transportation, storage, and postharvest handling.

Transportation of the fish from harvest or processing to the cold-smoking facility is another area of concern. Fish can be transported frozen or refrigerated (typically iced and thus cooled to 0 °C (32 °F). Basic sanitation practices and temperature controls need to be in place in transportation vehicles and containers. Temperature should be monitored at all times during transportation and storage before processing, especially if the fish is being frozen to control parasites or belongs to a scombrotoxin-susceptible fish.

In the United States, direct treatment of finfish to reduce microbial load is permitted after harvest and before processing. Chlorine solution dips (Eklund and others 1993), which require intense management to avoid recontamination, have been replaced by chlorine solution rinses or sprays that are followed by a rinse with potable water. Although it has been suggested that rinsing of the fish is important to reduce numbers of pathogenic microorganisms, no data on the effect of this procedure could be found in the scientific literature.

In summary, efforts directed to reduce or minimize microbial load on fish destined for cold smoking must be initiated as early in the production cycle as practical. The most critical interventions, however, are those directed to prevent growth of specific bacteria that may eventually lead to health hazards. At all times, from harvest through receiving, Good Manufacturing Practices (GMPs), Good Hygienic Practices (GHPs), appropriate sanitation procedures (that is, Sanitation Standard Operating Procedures, [SSOPs]), and control of the product temperature should be applied. After harvest, the fish should be cooled as soon as practically possible and maintained at a temperature below 40 °F (4.4 °C) until processed. Ideally, fish is stored at 32 °F (0 °C) in ice. The use of temperature-recording devices is highly recommended.

### 1.2. Potential hazards

Incoming fish may harbor parasites. Scombrotoxin-susceptible fish may have been temperature-abused and contain unsafe levels of biogenic amines. Additionally, fish may be contaminat-

ed with *C. botulinum* or *L. monocytogenes.*

### 1.3. Control point

Receiving is a control point for aquacultured and wild-caught fish. For scombrotoxin-susceptible fish, receiving is a control point to screen for harmful, unsafe levels of biogenic amines. If the product is received frozen and there are no other freezing steps in the process, this is a control point for parasites.

### 1.4. Processing parameters

If scombrotoxin-susceptible fish are received directly from the harvest vessel, all lots should be accompanied by documentation certifying proper time and temperature handling of the fish. Refrigerated scombrotoxin-susceptible fish should be received at an internal temperature of 40 °F (4.4 °C) or less. Table VI-1 shows the estimated shelf life of scrombrotoxin-forming species at various storage temperatures. In addition to the time and temperature parameters, analytical testing for histamines should be done periodically on samples of edible fish flesh (FDA guidance limits the histamine level to 50 ppm). A recent report on Hawaiian fishery (Kaneko 2000) concluded that odors of decomposition were reliable indicators of biogenic amines risk and that sensory evaluation is an effective monitoring measure. Even though an experienced processor may be doing the evaluation, sensory analysis is a very subjective and not a quantitative monitoring method; therefore, a more objective method (analytical method or temperature record) should be in place. Practically, most companies use a sensory evaluation of incoming scombrotoxin-susceptible species with a maximum receiving temperature for refrigerated raw material received. If sensory analysis points to a high biogenic amine level, analytical testing is performed.

If product is received frozen to control for parasites, a number of time and temperature combinations have been recommended in the United States, such as holding the fish at –10 °F (–23 °C) for 60 h, or less than –4 °F (–20 °C) for 7 d, or –31 °F (–35 °C) internally for 15 h. For a more detailed explanation of freezing regimes, see Chapter V.

**Table VI–1. Approximate Safe Shelf Life of Scrombrotoxin-Forming Species at Various Storage Temperatures (FDA 1998)**

| Product temperature | Safe shelf life (d) with Rapid C | Safe shelf life (d) with delayed cooling |
|---|---|---|
| 0 °F (–17.8 °C) | No limit | No limit |
| 32 °F (0 °C) | 14 | 8 |
| 38 °F (3.3 °C) | 10 | 7 |
| 40° F (4.4° C) | 7 | 5 |
| 50 °F (10 °C) | 3 | 0 |
| 70 °F (21.1 °C) | 0 | 0 |
| 90 °F (32.2 °C) | 0 | 0 |

## 2. Fresh or frozen storage

### 2.1. Background and support information

Before smoking, fish may be stored fresh or frozen. Fresh and frozen storage areas should be maintained in a clean and sanitary manner. Refrigerated fish should be stored so that their internal temperature minimizes the production of toxic biogenic amines and the outgrowth of *L. monocytogenes*. Frozen storage may potentially be a control point for parasites, if there is no other freezing step in the process. In this case, freezing of fish to the proper internal temperature and for the proper length of time is necessary to kill parasites in the incoming product that would otherwise survive the cold-smoking process.

### 2.2. Potential hazards

Contamination of the raw material or outgrowth of pathogenic microorganisms may occur if the fish is not maintained in a sanitary facility with proper refrigeration conditions.

If the frozen storage is intended to be the step to kill parasites, and the product is not frozen at the proper temperature for the proper length of time, parasites may become a hazard in the finished product.

### 2.3. Control point

If fish are stored under refrigeration, this is a control point for biogenic amines in scombrotoxin-susceptible species. If fish are stored frozen, this could be a control point for parasites, provided a previous freezing step has not been included as the control point for parasites or there is no other freezing step later in the process.

### 2.4 Processing parameters

Refrigerated fish should be stored so that their internal temperature is less than 40 °F (4.4 °C). For freezing, a number of time and temperature combinations have been recommended, such as holding the fish at –10 °F (–23 °C) for 60 h, or less than –4 °F (–20 °C) for 7 d, or –31 °F (–35 °C) internally for 15 h. For a more detailed explanation of freezing regimes, see Chapter V.

## 3. Thawing, washing, and rinsing

### 3.1. Background and support information

Thawing frozen raw fish is necessary to process cold-smoked fish. Many cold-smoked fish processors receive frozen fish, either aquaculture-produced or wild-caught, for processing. Thawed or fresh fish also needs to be thoroughly washed and rinsed after being received into the plant and then again after being butchered and processed. For these activities, it is necessary to use cold, potable, and continuously flowing water.

### 3.2. Potential hazards

Frozen raw fish should be thawed under sanitary conditions. Although this step is short and one would not anticipate biogenic amine production or pathogen growth, AFDO guidelines state that frozen raw material should be thawed under temperature control (AFDO 1991). This step is not considered to be a control point but it should be performed following GMPs and GHPs.

## 4. Butchering and evisceration

### 4.1. Background and support information

Butchering and evisceration may occur at the smoking facility or at the supplier's processing plant before the raw material is shipped to the smoker. Many cold-smoked fish processors receive raw material in the form of fillets or other products that have already been completely or partially processed (eviscerated, headed, gutted, and skinned). Temperature records are needed and the product should be checked for cleanliness when it is received into the processing facility.

If fish are eviscerated or butchered at the smoking facility, AFDO guidelines recommend that gutting be performed "with minimal disturbance of the intestinal tract contents." The guidelines further recommend that fish should be butchered in a room or area that is separate from the rest of the smoking and processing facility. The fish, especially the body cavity, should be washed and rinsed thoroughly with potable water after butchering.

### 4.2. Potential hazards

This step does not present a significant or special potential hazard and consequently is not considered to be a control point. Nevertheless, butchering and evisceration should be done

promptly and should follow GMPs and GHPs.

### 5. Washing and rinsing

See section 3 above.

### 6. Sorting, sizing, and salting

### 6.1. Background and support information

Salting of fish can be conducted by two different methods, brining and dry-salting. Brining is the process by which the fish is soaked or steeped (Kassem 1977) in a solution consisting of water, salt, sugar, various spices and flavorings, phosphates, and, depending on the recipe and species of fish (allowed in the United States for sable, salmon, shad, chub, and tuna), sodium nitrite. Brining may also be accomplished by injecting a liquid brine solution, usually with an automated or mechanized system but occasionally by hand, into the fish. In dry salting, fish are placed directly into a salt mixture usually consisting of some variation of the previously mentioned ingredients without the water.

Brining solutions are often referred to as a percent of a totally saturated salt solution and may be measured with a salometer or other methods. A saturated brine solution is one in which no more salt will dissolve into the water (aqueous phase). A salometer measures in degrees (100 °S), which can be thought of as "percent saturation" of the brine solution or directly in % salt. Tables and guides are available for preparing salt brines from the U.S. Sea Grant Extension Service (Hilderbrand 1973).

Salting should be as uniform as possible, with the correct amount of salt or brine solution absorbed into the fish flesh of each piece. To accomplish this, the fish must be sorted by size and thickness and spread out uniformly prior to salting so they will absorb the same amount of salt. Eklund pointed out that "even under the best salting conditions, it is nearly impossible to obtain the predetermined concentration of salt in all samples even within the same lot of fish" (Eklund 1989). For example, when 50 samples from a commercial operation with a target of 3.5% (WPS) were tested, WPS concentrations of the final product ranged from 2.8 % to 6.0 %, with the majority of the samples being above 4.0 %. The inherent variability in this important process must be taken into consideration.

Proper salting is essential to cold-smoked fish processing for three reasons: 1) it usually reduces the moisture content of the fish, affecting both texture and shelf life; 2) it imparts essential flavors; and 3) it is important as a preservative and inhibitor of microbial growth (Kassem 1977). Eklund points out that with cold-smoked products, the addition of sugars would provide an advantage in that the carbohydrate source would "encourage the growth of lactic acid and other spoilage bacteria and lower of the product pH which can be inhibitory to *C. botulinum*" in the final product (Eklund 1989). Other researchers, however, noted that if the product is vacuum-packaged and chilled, very rapid growth of lactic acid bacteria (*Carnobacteria* spp.) is observed, independent of addition of carbohydrates (L. Gram 2000; personal communication; unreferenced).

### 6.2. Potential hazards

*Listeria monocytogenes* and *C. botulinum* spores present on a single fish could contaminate an entire batch within the brine solution. To minimize microbial growth and cross-contamination, temperature control of the brine solution during brining is recommended, particularly if brining is done for more than a few hours. If brine injection is used, needles can get contaminated and spread a pathogen to other fish. Fish may be underbrined if the brine solution is too diluted or if the brine soak time is too short, potentially allowing botulinum toxin formation in the final product.

This step is especially important in that WPS needs to be high enough to inhibit the outgrowth and toxin formation of *C. botulinum* in the final product. Brining should be done at a salt concentration that will provide the appropriate concentration in the final product (that is, 3.5% WPS in the final product). Acidity (pH), salt, moisture (water activity), or some combination of these act in combination to inhibit *Clostridia* outgrowth and toxin formation. The salt level and refrigerated storage of the final product along with competing microflora can prevent the growth of *C. botulinum* type E and nonproteolytic types B and F. The reader is referred to the *C. botulinum* section for a review of the scientific literature on this subject.

### 6.3. Control point

Salting, including sizing and sorting of the fish, is a control point because the presence of enough salt in the fish is essential to inhibit the outgrowth of *Clostridia* species and to prevent the formation of toxins, particularly in vacuum-packaged finished products. The sizing and sorting step is an integral part of proper salting in cold-smoked fish processing. Batches of fish should be checked going into or being removed from the brine. Portions that are too thick or too large should be removed and cut to the proper size.

### 6.4. Processing parameters

A number of parameters need to be considered when salting. These include: 1) a minimum salt concentration of the brine solution, usually measured at the beginning of salting; 2) a minimum ratio of brine solution or dry salt mix to fish so that each fish is adequately exposed to the brine; 3) a minimum salting time to allow the salt to adequately and uniformly absorb into the fish; and 4) a maximum temperature of the salting. A further requirement is that the fish or fish portions be of a uniform thickness and size so that in the given amount of time for the batch, all of the portions absorb sufficient salt. As mentioned above, extra thick or large pieces in a batch would not be sufficiently salted, meaning they would not have a high enough salt concentration in the final product to inhibit *Clostridia* growth. Conversely, small or thin pieces would be oversalted at the end of this step, compromising the sensory characteristics. The AFDO guidelines recommend that the temperature of the brine not exceed 60 °F (15.5 °C) at the beginning of salting. If the salting is longer than 4 h, it is recommended that the salting be done under refrigeration.

The amount of salt, volume of the brine, weight of the fish, and duration and temperature of the process should be calculated empirically by the processor. All these parameters need to be established with the objective of obtaining a final product with at least 3.5% salt concentration in the water phase, if the product is to be vacuum-packaged (see Packaging, section 11). No scientific data specifically address the question of the salt concentration needed to inhibit *C. botulinum* toxin formation on air-packaged products. Although it is believed that spoilage of aerated products will act as a safeguard against botulism, not enough scientific evidence supports this idea (see Chapter III).

### 7. Rinsing, draining, and preparation

### 7.1. Background and support information

The AFDO guidelines recommend rinsing fish with fresh water after salting. This process, sometimes called "freshening," involves rinsing in cold, potable water for a certain amount of time. After the rinse, the fish may be laid on a rack to begin the drying and smoking stage.

### 7.2. Potential hazards

Cross-contamination with pathogens that may be present on the fish is unlikely during freshening due to rinsing with potable

water. Consequently, the freshening step is not considered to be a control point.

## 8. Drying and cold smoking

### 8.1. Background and support information

A number of cold-smoking procedures involve a drying stage with no smoke added to the product. This can be considered a separate step or an initial part of the cold-smoking step. The product is held at a specific temperature for a specific amount of time before the smoke is introduced. The duration and temperature of this initial drying step constitute the "art" of smoking fish. The parameters that are considered for this initial drying component include the type or species of fish, its fat content, and humidity. If the humidity is high, the fish will tend to be wetter longer and the drying step must be adjusted accordingly. Smoke should be applied before a pellicle (dried skin-like layer) forms on the outside of the fish fillets. If a pellicle forms first, the effectiveness of the smoke is markedly reduced. The goal is to have the surface of the fish portions a little "tacky" so the smoke can be absorbed (R. Martin 2000; personal communication; unreferenced).

There is a variety of literature on the smoking of fish and seafood products (Gilbert and Knowles 1975; Storey 1982). Variations include temperature, time, humidity, types of control (from a thermometer hanging in the smokehouse to sophisticated microprocessor feedback-controlled systems), types of smoke used (natural, generated, or liquid), and the design and types of smokehouses or kilns. Usually, fish are laid or arranged uniformly (that is, on racks) to prevent contact, ensuring that the portions receive a uniform exposure to the smoke and drying.

Traditional smokehouses or kilns allow natural airflow or convection, and their use requires a lot of expertise. Modern smokehouses or kilns control the airflow mechanically or electrically. Many also have refrigeration units; temperature monitors and controls; and microprocessor control systems that can regulate smoke exposure, humidity, drying, and temperature. This permits strict control of the complex smoking operation. Wood smoke can be generated by burning wood or, more commonly, by heating sawdust or small wood chips. Wood that has been treated with chemicals or preservatives should not be used for smoking. A wide variety of woods has been used for smoking, including oak, hickory, mahogany, pine, whitewood, cherry, apple, alder, mesquite, beech, birch, and maple to impart various flavors and colors. Some of the "original Scottish processes used smoldering peat or moss" (Storey 1982). According to some authorities, wood smoke is generally comprised of two components: the visible "tarry droplets" and the invisible gaseous or vaporous component (Storey 1982). Chemically, wood smoke contains hundreds of compounds, including a number of phenolic substances and volatile acids (Storey 1982; Gilbert and Knowles 1975). Liquid smoke products, which are produced by condensing the wood smoke in some fashion, are generally available. From a culinary perspective, the purpose of the smoking step in cold-smoked fish processing is to add flavor components and modify the taste and texture of the fish.

### 8.2. Potential hazards

Cold-smoked fish is defined as a product in which the fish flesh proteins show incomplete coagulation. Practitioners cite a range of temperatures as the upper-bound limit of cold smoking, but the true defining limit is to leave the fish proteins partially undenatured or uncoagulated.

Although it is natural to assume that the product being smoked gets hot from exposure to the smoke and is thus cooked, cold smoking is not a cooking step due to the low temperatures involved. In some instances the addition of smoke has been shown to have some inhibitory effect on microorganisms (Storey 1982). Research indicates that short-term cold smoking (< 24 h, as recommended by the AFDO guidelines [AFDO 1991]) causes a reduction rather than an increase in numbers of *L. monocytogenes* (see Chapter II for a review of this subject).

Contribution of smoke to the inactivation of pathogens, however, is not of significant importance in the overall process (see Chapters II and III for a review of the scientific literature). Rather, the low temperatures of smoking permit the survival of a significant portion of the natural microbiological flora on the fillets. Several studies have shown that growth of *L. monocytogenes* in smoked fish is hampered by a high background microflora (Rørvik and others 1991). It is a common belief that the natural flora will spoil the fish prior to botulinum toxin formation in cases of temperature abuse of air-packaged or unpackaged cold-smoked fish. On the contrary, vacuum packaging or modified atmosphere packaging (MAP) inhibits the growth of the natural flora so the rate of spoilage under temperature-abuse conditions is lower and toxin production may occur before spoilage. Consequently, relying on the competition from the naturally occurring background flora or on spoilage organisms to restrict *C. botulinum* is not a reliable and reproducible method of controlling toxin formation.

### 8.3. Control point

The cold-smoking step is a control point for *C. botulinum* growth and toxin production and for *L. monocytogenes* growth. Although cold smoking will not completely eliminate either the microorganisms or the spores of concern, inadequate temperature and time control could exacerbate the hazards. Consequently, it is important to control the temperature and time during smoking so that the background microflora is not eliminated.

## 9. Cooling

### 9.1. Background and support information

Cold-smoked fish products must be rapidly cooled to minimize possible growth of *L. monocytogenes* and bacteria capable of producing biogenic amines in the products.

### 9.2. Potential hazards

Formation of biogenic amines is possible if scombrotoxin-susceptible species are used in the cold-smoking process. Also, low levels of biogenic amines have been found in nonscombrotoxin-susceptible species such as cold-smoked salmon (Gram and Huss 2000). Formation of amines in cold-smoked fish products during vacuum-packed storage may be caused by lactic acid bacteria, which have the ability to form biogenic amines (Leisner and others 1994). The primary amine-producing bacteria are species of *Enterobacteriaceae*, which typically are associated with temperature-abuse before packing. Rapid cooling of the product is important to reduce the growth rate of bacteria capable of forming biogenic amines.

Other potential hazards include pathogen growth and cross contamination of the cold- smoked product with pathogens. *Listeria monocytogenes* can survive the salting and cold-smoking process and will grow at refrigerator temperatures (Ben-Embarak and Huss 1992; Hudson and Mott 1993; Rørvik and others 1991). Thus, although complete assurance that *L. monocytogenes* is absent from cold-smoked fish products is not possible (Anonymous 1995; FAO 1999; Gram and Huss 2000), its numbers on the finished product may be kept low by rapid cooling of the cold-smoked product, following strict GMPs, and implementing and adhering to appropriate SSOPs.

### 9.3. Control point

Cooling is a control point for scombrotoxin-susceptible spe-

cies. In addition, proper cooling will prevent or minimize growth of pathogenic bacteria, including *L. monocytogenes*.

### 9.4. Processing parameters

The AFDO guidelines state that all finished product must be cooled to a temperature of 50 °F (10 °C) or less within 3 h after cooking and further cooled to a temperature of 38 °F (3.3 °C) or below within 12 h after cooking. The New York State guidelines are similar to the AFDO guidelines except that the product must be cooled to a temperature of 50 °F (10 °C) or less within 5 h after cooking (Corby 1991).

It should be noted, however, that these temperature guidelines are under discussion as to their validity and applicability for the cold-smoked fish processing industry. Additional research is needed at this step to determine scientifically based temperature requirements at the cooling step.

## 10. Slicing and cutting

### 10.1. Background and support information

High priority must be given to the slicing and cutting step to help control possible recontamination or cross-contamination of the cold-smoked fish product with pathogens. Clean, sanitary food contact surfaces are essential. A processor should have well designed and comprehensive SSOPs and must follow GMPs to control contamination of the cold-smoked fish product at this step. The development and verification of effective sanitation and cleaning programs will help reduce the prevalence of *L. monocytogenes* on the slicing and cutting equipment and in the general processing environment. Such programs will help reduce contamination of the cold-smoked fish product. Recent studies have shown that one potential source of *L. monocytogenes* in cold-smoked salmon is the slicer, where specific DNA types can occupy a niche for extended periods of time (Fonnesbech Vogel, Ojeniyi, Ahrens and others 2001).

As Eklund and others (1993, 1995) suggested, in addition, to inactivating the bacterium on the incoming raw product and inhibiting growth of survivors in the final product, processors should strictly follow GMPs to prevent recontamination of *L. monocytogenes* during processing. Because the source of *L. monocytogenes* may be other than the incoming product, following GMPs is currently the most important measure to minimize *L. monocytogenes* presence in the final product.

### 10.2. Potential hazards

Potential hazards during slicing and cutting include possible cross-contamination of the cold-smoked product with pathogens such as *L. monocytogenes*.

### 10.3. Control point

The slicing and cutting step is not a control point but is an extremely important processing operation. Strict adherence to SSOPs and GMPs to control cross contamination of product with pathogens is essential; in particular, effective SSOPs can be used to minimize or prevent cross-contamination with *L. monocytogenes*.

## 11. Packaging and labeling

### 11.1. Background and support information

Reduced Oxygen Packaging (ROP) is defined as any packaging procedure that results in a reduced oxygen level in a sealed package (FDA 1999). Furthermore, the Food Code defines the following: 1) Controlled Atmosphere Packaging (CAP) is the packaging of a product in a modified atmosphere followed by maintaining subsequent control of that atmosphere; 2) Modified Atmosphere Packaging (MAP) is the packaging of a product in an atmosphere that had a one-time modification of gaseous composition so it differs from air; and 3) Vacuum Packaging (VAC) removes the air from a package and seals it so a near-perfect vacuum remains inside the package. For fatty fish species, packaging that excludes oxygen is preferred to prevent rancidity caused by oxidation of lipids.

The United States seafood industry uses a variety of films and packages to protect the product and extend the shelf life of cold-smoked fish products. Packaging protects and preserves foods, providing an additional mechanism for marketing products by improving shelf life, convenience, freshness, and quality (Lord 2000). These packaging materials include vacuum bags and flexible pouches, vacuum shrink barrier bags and films, vacuum multilayer bags, thermoformed and rollstock laminates, oxygen-permeable shrink bags, film overwraps, rigid sheeting, films for semirigid packages, and preformed trays. The types of packaging and film-permeability characteristics depend on the intended use and nature of the product. Packages can be designed to control film permeability to adjust oxygen, carbon dioxide, and moisture levels. Such information can be used to help extend product distribution shelf life without odor buildup within the package.

Other parameters being equal, gas permeability characteristics of the film will determine the microflora that survives in a packaged product. Therefore, gas permeability is an important parameter and should be taken into account when doing research and making decisions on food safety issues. Specifications for gas permeability, however, are product- and use-specific and are usually established at ambient temperatures under moderate humidity conditions (that is, 23 °C and 50% R.H.) using a variety of testing and verification methods. These various testing conditions make it difficult to compare gas transmission rates between films. In addition, film permeability rates also change when the film is stretched or when it contacts the product. Data are also limited on gas-transmission rates of films at product-chill temperatures (Robertson 1993).

#### 11.1.1. Pathogens

Temperatures used in the preparation of cold-smoked fish are inadequate to eliminate *C. botulinum* spores; thus, other controls such as temperature and NaCl must be included to ensure its safety. Numerous studies address the formation of botulism toxin under vacuum. A few of the studies are done with fresh, hot-smoked fish and cold-smoked fish.

Eklund (1992) conducted studies on growth of *C. botulinum* and subsequent toxin formation in hot-smoked fish packaged in an oxygen-permeable film (1.5 ml polyethylene; oxygen transmission 7195 cc/$m^2$/24 h at 760 mm Hg 23 ° C and 0% R.H.; $CO_2$ transmission 22858 cc/$m^2$/24 h) and under vacuum in an oxygen-impermeable film (108 cc/$m^2$/24 h; $CO_2$ transmission 526 cc/$m^2$/24 h) (Table VI-2). He reported that higher concentrations of NaCl were required to inhibit toxin formation by *C. botulinum* in $O_2$-impermeable films compared with $O_2$-permeable films.

In addition, under temperature-abuse conditions, toxin formation can occur in VAC- or MAP-products before or at the same time as spoilage (Eklund 1992). Although the studies were conducted at 25 °C (77 °F), Eklund reported that concentrations of NaCl, the combination of NaCl and nitrite, or other preservatives needed to inhibit *C. botulinum* were similar for growth and toxin formation at 25 °C (77 °F) as at 10 °C (50 °F). Toxin formation, however, was dramatically delayed at 10 °C compared with 25 °C. We should emphasize, though, that these studies were performed on hot-smoked fish, which are different microbiologically from cold-smoked fish.

Dufresne and others (2000) recently completed studies on the effect of packaging film permeability and storage temperatures on *C. botulinum* type E growth and toxin formation in cold-

**Table VI-2. Toxin production by *Clostridium botulinum* type E ($10^3$ spores/100 g) in hot-process whitefish steaks vacuum-packaged in $O_2$-permeable (7195 cc/m²/24 h), or $O_2$-impermeable (108 cc/m²/24 h) films (adapted from Eklund 1992)**

| | | Quantity of *C. botulinum* toxin formed ($MLD_{50}$/g) | |
|---|---|---|---|
| Storage time, d at 25 °C | Water phase salt (%) | Oxygen-permeable film | Oxygen-impermeable film |
| 3 | 1.8 | 50 | 2500 |
| | 2.6 | 0 | 500 |
| | 3.4 | 0 | 5 |
| 5 | 1.8 | 250 | 50000 |
| | 2.8 | 0 | 2500 |
| | 3.5 | 0 | 0 |
| 7 | 1.8 | 2500 | 50000 |
| | 2.8 | 0 | 5000 |
| | 3.5 | 0 | 50 |

**Table VI-3. Challenge studies with *Clostridium botulinum* type E spores ($10^2$/g) on cold-smoked trout fillets with 1.7% NaCl (WPS) packaged in films of different oxygen transmission rates and stored at 4 °C**

| Oxygen transmission rate cc/m²/d/atm @ 24 °C, 0% R.H. | Sensory shelf life[1] based on odor (d) | Nr of toxic samples at each sampling interval (d)[2] | | | |
|---|---|---|---|---|---|
| | | 7 | 14 | 21 | 28 |
| 12 | ~28 | 0 | 0 | 0 | 0 |
| 2950 | ~28 | 0 | 0 | 0 | 0 |
| 4920 | ~22 | 0 | 0 | 0 | 0 |
| 10040 | ~18 | 0 | 0 | 0 | 0 |

[1]Time (d) to reach a score of 3.5 on a hedonic scale of 1 to 7. (7 = Extremely desirable, 1 = Extremely undesirable)
[2]Trypsinized extract (in duplicate)
Dufresne and others 2000

**Table VI-4. Challenge studies with *Clostridium. botulinum* type E spores ($10^2$/g) on cold-smoked trout fillets with 1.7% NaCl (WPS), packaged in films of different oxygen transmission rates and stored at 8 °C**

| Oxygen transmission rate cc/m²/d/atm @ 24 °C, 0% R.H. | Sensory shelf life[1] based on odor (d) | Nr of toxic samples at each sampling interval (d)[2] | | | |
|---|---|---|---|---|---|
| | | 7 | 14 | 21 | 28 |
| 12 | ~14 | 0 | 0 | 0 | 0 |
| 2950 | ~13 | 0 | 0 | 0 | 0 |
| 4920 | ~16 | 0 | 0 | 0 | 1 |
| 10040 | ~ 6 | 0 | 0 | 0 | 1 |

[1]Time (d) to reach a score of 3.5 on a hedonic scale of 1 to 7. (7 = Extremely desirable, 1 = Extremely undesirable)
[2]Trypsinized extract (in duplicate)
Dufresne and others 2000

**Table VI-5. Challenge studies with *Clostridium botulinum* type E spores ($10^2$/g) on cold-smoked trout fillets with 1.7% NaCl (WPS), packaged in films of different oxygen transmission rates and stored at 12 °C**

| Oxygen transmission rate cc/m²/d/atm @ 24 °C, 0% R.H. | Sensory shelf life[1] based on odor (d) | Nr of toxic samples at each sampling interval (d)[2] | | | |
|---|---|---|---|---|---|
| | | 7 | 14 | 21 | 28 |
| 12 | ~11 | 0 | 1 | 2 | 1 |
| 2950 | ~12 | 0 | 1 | 2 | 2 |
| 4920 | ~11 | 0 | 1 | 2 | 2 |
| 10040 | ~ 6 | 0 | 1 | 2 | 2 |

[1]Time (d) to reach a score of 3.5 on a hedonic scale of 1 to 7. (7 = Extremely desirable, 1 = Extremely undesirable)
[2]Trypsinized extract (in duplicate)
Dufresne and others 2000

smoked and hot-smoked trout products. Summaries of her findings on cold-smoked fish are contained in Tables VI–3–5.

In Dufresne's studies, product shelf life for cold-smoked trout ranged from 18 to 28 d at storage temperatures of 4 °C (39 °F) with no toxin formed in any sample (Table VI–3). At 8 °C (46 °F) (Table VI–4), cold-smoked trout packaged in films with oxygen transmission rate (OTR) of 10040 cc/m²/24 h spoiled at 6 d of storage, and toxin formation occurred at 28 d of storage. Spoilage occurred at 16 d, and toxigenisis occurred by 28 d in cold-smoked trout packaged in films with OTRs of 4920cc/m²/24 h (Dufresne and others 2000). Spoilage of products packaged in films with OTRs of 12 and 2950 cc/m²/24 h occurred at 13 to 14 d, but there was no toxin formation by 28 d of storage. It should be emphasized that packages with high OTRs were toxic after 28 d, whereas packages with low OTRs were not toxic.

At 12 °C (54 °F) (Table VI–5), spoilage in smoked trout packaged in film with OTRs of 12, 2950, and 4920 cc/m²/24 h occurred between 11 and 12 d with toxin formation by 14 d of storage. Cold-smoked trout packaged in films with an OTR of 10040 cc/m²/24 h spoiled at 6 d of storage, and toxin formation occurred by 14 d of storage (Dufresne and others 2000). From Dufresne's data it would seem that at abuse temperatures (8 to 12 °C) spoilage may proceed toxin production, particularly when packaged in high $O_2$ permeable films. Spoilage may help prevent the consumption of toxic fish, but it cannot be relied upon solely as a control to prevent foodborne botulism.

These studies illustrate the need for additional research to identify the processing conditions and characteristics that must be present for a product to be considered air-packaged. Results from these studies suggest that film with OTRs of 7195 cc/m²/24 h or greater could be considered air-packaged. Additional research on $O_2$ and $CO_2$ film permeability characteristics is needed to better define these parameters. Nevertheless, storage of smoked trout at 4 °C or 8 °C or less resulted in no toxin formation prior to spoilage in the cold-smoked product, regardless of film OTRs (Dufresne and others 2000; Eklund 1984, 1992).

*Listeria monocytogenes* is another pathogen of concern in cold-smoked fish. Studies indicate that *L. monocytogenes* grows well on the finished cold-smoked product at refrigerated temperatures (Farber 1991). Peterson and others (1993) reported that vacuum-packaging initially suppressed growth of *L. monocytogenes* by 10 to 100 fold in samples with 3% or 5% WPS. Neither 3% nor 5% water-phase salt by itself, however, sufficed to prevent the growth of *L. monocytogenes* in vacuum-packaged or $O_2$-permeable film-packaged, cold-processed salmon during prolonged storage at 5 °C (41 °F) or 10 °C (50 °F). The authors suggested that in addition to NaCl, other inhibitors such as smoke and sodium nitrite ($NaNO_2$) could be used to inhibit *L. monocytogenes* in cold-smoked fish. Pelroy and others (1994b) reported that $NaNO_2$ enhanced the inhibitory effect of NaCl on *L. monocytogenes* at refrigeration temperatures, when the inoculum was low and the storage temperature was 5 °C. This inhibitory effect decreased as the storage temperature increased to 10 °C and the inoculum level increased. These results emphasized the importance of reducing or eliminating *L. monocytogenes* and adequate refrigeration during all stages of storage or cold-smoked fish products.

### 11.1.2. U.S. packaging and labeling requirements

The botulism outbreaks from hot-smoked fish during the 1960s were caused by a combination of inadequate processing of the products, WPS concentration in most cases less than 1%, and gross temperature-abuse during distribution (Eklund 1992). It is interesting to note that although WPS guidelines are part of state

regulations in New York, Michigan, and Minnesota, current U.S. HACCP guidelines do not recommend specific WPS concentrations for cold-smoked fish.

Current AFDO guidelines (followed by New York, Michigan, and Minnesota) recommend that cold-smoked products contain a WPS level of at least 2.5% for air-packaged fish; or a minimum WPS level of 3.5% for vacuum- or modified atmosphere-packaged fish; or a combination of at least 3% WPS and a nitrite level of 100 to 200 ppm. Each container of processed fish must contain identification indicating where the product was packaged, the year and day packed, and the period during which the product was packaged. Packing codes shall be changed with sufficient frequency to allow identification of lots during sale and distribution. The label should also state the need for refrigerated storage (AFDO 1991). As mentioned in the scope section of this chapter, although developed by experts and widely used, these recommendations have not necessarily been scientifically evaluated. Adequate refrigeration is the most important factor for the safe distribution of smoked fish products and has been recommended in multiple occasions (Eklund 1992; Eklund and others 1982; Eklund and others 1988; Pelroy and others 1982).

### 11.1.3. Canadian packaging and labeling requirements

The Canadian document "Food and Drugs Act and Regulations" states under Division 21 (B.21.025.) that no person shall sell marine and fresh water animals, or marine and fresh water animal products, that are packed in a container that has been sealed to exclude air and that are smoked or to which liquid smoke or flavour or liquid smoke flavour concentrate has been added, unless (a) the container has been heat-processed after sealing at a temperature and for a time sufficient to destroy all spores of the species *Clostridium botulinum;* (b) the contents of the container contain not less than nine percent salt, as determined by official method PO-38, Determination of Salt in Smoked Fish, dated March 15, 1985; (c) the contents of the container are customarily cooked before eating; or (d) the contents of the container are frozen and the principle display panel of the label of the container carries the statement "Keep Frozen Prior to Use" in the same size types used for the common name of the contents of the container (Health Canada 1994).

The Canadian government, however, allows refrigerated storage of smoked products if they are packaged in containers with an oxygen permeability equal to or greater than 2000-cc/$m^2$/24 h at 24 °C at 1 atm. These products must also be stored at 4 °C or less and should have a label that states shelf life must not exceed 14 d from the date of packaging. Processors and retailers should record the type of film used and its permeability characteristics (Health Canada 1994).

### 11.2. Potential hazards

Hazards that may arise from the time of final product manufacture to the time of consumption within the product packaging include growth of pathogens such as *L. monocytogenes* or *C. botulinum* and production of botulin toxin on the finished product. In addition, biogenic amine formation is possible if scombrotoxin-susceptible species are used for the cold-smoking process.

### 11.3. Control point

The packaging and labeling step is not considered a control point, since it is not possible to label safety into a product. Nevertheless, label information identifying appropriate storage temperatures and time for safety is critical to control biogenic amine formation in scombrotoxin-susceptible species, as well as *C. botulinum* growth and toxin formation in cold-smoked products, especially if packaged in a reduced-oxygen environment. This label information is also important in reducing the growth rate of *L. monocytogenes,* although temperature will not prevent its growth. A warning label for populations at high risk of developing listeriosis may be considered for cold-smoked fish and other ready-to eat foods in the risk category. Such a label could indicate that these products may constitute a health hazard to immunocompromised individuals and pregnant women.

### 11.4. Processing parameters

All finished products should be labeled to advise on refrigeration temperatures and storage time. This panel concluded that, with the appropriate salt concentrations (that is, 3.5% WPS), the product should be refrigerated at 40 °F (4.4 °C) or less for no more than 5 wk (for a detailed discussion on this subject, see the Chapter III). This would mean the final product needs to maintain that temperature during storage, distribution, retail, and home storage and that the recommended maximum time from manufacture to consumption is 5 wk. Frozen product must also be labeled to indicate that the "product shall remain frozen until it is thawed at refrigeration temperatures."

## 12. Storage and distribution

### 12.1. Background and support information

Cold-smoked fish products can be stored refrigerated or frozen. Companies may freeze and hold the product in frozen storage for 1 to 2 wk prior to distribution and shipment. This is done primarily for inventory control. Other companies simply refrigerate products and distribute and ship them as soon as possible. In addition, cold-smoked products are also distributed via overnight carriers and government postal services.

Kalish (1991) conducted audits of temperature readings at more than 50 major warehouses and distribution centers. She reported that most warehouses and distribution centers maintained refrigeration temperatures within the recommended temperature range (0 to 3.3 °C, 32 to 38 °F), although a few (number not specified) were as high as 10 °C (50 °F). The rotation of product in warehouses and distribution centers was good (Kalish 1991).

### 12.2. Potential hazards

Potential hazards during refrigerated storage and distribution include pathogens such as *L. monocytogenes* or *C. botulinum* and botulin toxin formation on the finished product. In addition, biogenic amine formation is possible if scombrotoxin forming species are used for the cold-smoking process. Frozen storage is not considered a control point, since pathogens will not grow and biogenic amine will not form during storage.

### 12.3. Control point

Both storage and distribution are control points for biogenic amine formation in both aerobic-packaged and ROP cold-smoked scombrotoxin susceptible species. Storage and distribution are also control point for *C. botulinum* growth and toxin formation in cold-smoked products. Although chill temperature during storage and distribution will also reduce the growth rate of *L. monocytogenes,* it will not prevent its growth.

### 12.4. Processing parameters

Cold-smoked fish should be stored and distributed at storage temperatures of less than 40 °F (4.4 °C) or frozen. Studies indicate that storage at 4 °C (39 °F) or less resulted in no botulin toxin formation prior to spoilage in cold-smoked products at salt levels of 1.7% WPS (Dufresne and others 2000) (for a more detailed discussion see Chapter III). Those temperatures will also reduce the growth of scombrotoxin forming species and of *L. monocytogenes*. Frozen storage and distribution is not considered a control point,

as pathogens will not grow and biogenic amines will not form during frozen storage.

## 13 Retail

### 13.1. Background and support information

The Food Code provides criteria that must be met by the HACCP plans of operators that handle ROP products (FDA 1999). (Note: the Food Code is a voluntary recommendation from the FDA and is not codified in all states). The Food Code prohibits products from being packaged in ROP at the retail level and requires maintenance of adequate refrigeration during the entire shelf life of the product. Nevertheless, temperature-abuse does occur during retail storage. Studies have shown that at the retail level, product rotation procedures were inadequate, as sales of product dictated product rotation frequency (Kalish 1991).

Approximately 2000 retail stores, including back-room storage facilities and chill cases, were checked. Kalish reported that only 37% of refrigerated foods were stored within the 32 to 38 °F (0 to 3.3 °C) range. Products were also found stacked on the floor without any refrigeration, and the temperature of many refrigerated cases was 44 °F (6.7 °C) with some as high as 56 °F (13.3 °C) (Kalish 1991).

### 13.2. Potential hazards

Potential hazards during all retail operations include pathogens such as *L. monocytogenes* or *C. botulinum* on the finished product. In addition, biogenic amine formation is possible if scombrotoxin-susceptible species are used for the cold-smoking process.

### 13.3. Control point

Temperature and time control at all retail operations are control points to control biogenic amine formation in both air-packaged and ROP cold-smoked scombrotoxin-susceptible species and to control *C. botulinum* growth and toxin formation in cold-smoked products. Low temperature will also reduce the rate of growth of *L. monocytogenes*, although it will not prevent its growth. If the product is distributed frozen to the retailer, frozen storage is not considered a control point, as pathogens will not grow and biogenic amine will not form during storage. Thawing of the product, however, should be conducted at refrigeration temperatures.

### 13.4. Processing parameters

Cold-smoked fish should be stored, handled, prepared, and displayed at temperatures < 40 °F (4.4 °C) or frozen. Studies indicate that storage at 4 °C or less resulted in no botulin toxin formation prior to spoilage in cold-smoked products at salt levels of 1.7% WPS (Dufresne and others 2000) (for a more detail discussion see Chapter III*)*. Those temperatures will also reduce the growth of scombrotoxin-forming species and *L. monocytogenes*.

If the product is received frozen, it should kept stored and displayed frozen or thawed under refrigerated temperatures and handled as indicated in the previous paragraph.

## 14. Consumer

### 14.1. Background and support information

Beard (1991) reported that consumers should be educated about the potential hazards associated with food products and provided information on proper product rotation. Scientists have recommended that better and more effective consumer education programs are needed to reduce the incidence of foodborne disease outbreaks (Garrett 1987; Sachs 1989; NMFS 1991b). Consumers should be educated about potential risks associated with home preparation aspects of food consumption (NMFS 1991a).

For each of the years from 1993 through 1997, the most commonly reported food preparation practice that contributed to foodborne disease was improper holding temperature; the second most commonly reported practice was inadequate cooking of food (MMWR 2000).

Beard (1991) reported that out of 14 home refrigerators and 11 freezers, only 7 refrigerators and 1 freezer had thermometers. Refrigerator temperatures ranged from 0 to 13 °C (32 to 55 °F). The panel recommends labeling frozen products with thawing instructions and storing the thawed product below 40 °F (4.4 ° C).

### 14.2. Potential hazards

Potential hazards at its consumer stage include pathogens such as *L. monocytogenes* or *C. botulinum,* or botulin toxin formation on the finished product. In addition, biogenic amine formation is possible if scombrotoxin susceptible species are used for the cold-smoking process.

### 14.3. Control point

The refrigerator of the consumer is a control point to control biogenic amine formation in both air-packaged and ROP cold-smoked scombrotoxin-susceptible species and to control *C. botulinum* growth and toxin formation in cold-smoked products. Low temperature will also reduce the rate of growth of *L. monocytogenes,* although it will not prevent its growth.

### 14.4. Processing parameters

Cold-smoked fish should be stored at storage temperatures of < 40 °F (4.4 °C). Studies indicate that storage at 4 °C or less resulted in no botulin toxin formation prior to spoilage in cold-smoked products at salt levels of 1.7% WPS (Dufresne and others 2000) (for a more detailed discussion see section 11 of this chapter and Chapter III*)*. Those temperatures will also reduce the growth of scombrotoxin-forming species and *L. monocytogenes*.

## References


[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. York (PA): Association of Food and Drug Officials. 7 p.

Angot V, Brasseur P. 1993. European farmed Atlantic salmon (*Salmo salar L.*) are safe from *Anisakid* larvae. Aquaculture 118:339–44.

Anonymous. 1995. International forum supports above-zero tolerance for *Listeria* in low risk foods. World Food Chem News July 26:15–7.

Beard TD. 1991. HACCP and the home: the need for consumer education. Food Technol 45(4):123–4.

Ben Embarek PK, Huss HH. 1992. Growth of *Listeria monocytogenes* in lightly preserved fish products. In: Huss HH, Jakobsen M, Liston J, editors. Quality assurance in the fish industry. Amsterdam: Elsevier. p 293–303.

Corby JJ. 1991. Circular 102 Rules and regulations relating to fish processing and smoking establishments pursuant to Article 17 of the Agriculture and Markets Law. Albany, NY: New York State Department of Agriculture and Markets, Division of Food Inspection Services. Part 262 of Title 1 of the Official Compiliation of Codes, Rules, and Regulations of the state of New York.

Dufresne I, Smith JP, Liu JN, Tarte I, Blanchfield B, Austin JW. 2000. Effect of films of different oxygen transmission rate on toxin production by *Clostridium botulinum* type E in vacuum packaged cold and hot-smoked trout fillets. J Food Saf 20:251–68.

Eklund M. 1984. Effect of CO2 modified atmospheres and vacuum packaging on *Clostridium botulinum* and spoilage organisms of fishery products. Published in: Proceedings of First National Conference on Seafood Packaging and Shipping; 1982 Nov 15–17 [Washington, DC] and 1982 Dec 7–9 [Seattle, WA]. p 298–331.

Eklund M. 1989. Comments and research data for the proposed establishment of standards for the manufacture, packaging, and labeling of processed fish including smoked fish [testimony to the New York Department of Agriculture and Markets]. [New York]: Northwest Fisheries Center, Utilization Research Division.

Eklund MW. 1992. Control in fishery products. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum*: Ecology and control in foods. New York: M Dekker. p 209–32.

Eklund MW, Pelroy GA, Paranjpye R, Peterson ME, Teeny FM. 1982. Inhibition of *Clostridium botulinum* types A and E toxin production by liquid smoke and NaCl in hot-process smoked-flavored fish. J Food Prot 45(10):935–41.

Eklund M, Pelroy G, Poysky F, Paranjpye R, Lashbrook L, Peterson M. 1993 July. Summary of interim guidelines for reduction and control of *Listeria monocytogenes* in or on smoked fish [internal report]. Seattle: Northwest Fisheries Science Center. July 1993. 14 p.

Eklund MW, Peterson ME, Paranjpye R, Pelroy GA. 1988. Feasibility of a heat-pasteurization process for the inactivation of nonproteolytic *Clostridium botulinum* types B and E in vacuum-packaged, hot-process (smoked) fish. J Food Prot 51(9):720–6.

Eklund MW, Poysky FT, Paranjpye RN, Lashbrook LC, Peterson ME, Pelroy GA. 1995. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. J Food Prot 58(5):502–8.

[FAO] Food and Agriculture Organization. 1999 May. Report of the FAO expert consultation on the trade impact of *Listeria* in fish products. Rome: FAO. FAO Fisheries Report nr 604. 34 p.

Farber JM. 1991. *Listeria monocytogenes* in fish products. J Food Prot 54(12):922–4, 934.

[FDA] Food and Drug Administration. 1998. Fish & Fisheries Products Hazards & Controls Guide. 2nd ed. Washington, D.C.: FDA, Office of Seafood. 276 p.
[FDA] Food and Drug Administration. 1999. Food Code. Washington, DC: U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration.
Fonnesbech Vogel B, Ojeniyi B, Ahrens P, Due Skov L, Huss HH, Gram L. 2001. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Appl Enviro Microbiol. Forthcoming.
Garrett ES. 1987. Testimony before the committee on Agriculture, Nutrition, and Forestry. Washington, DC: United States Senate. June 11, 1987. Report nr S1813.
Gilbert J, Knowles M. 1975. The chemistry of smoked foods: a review. J Food Technol 10:245–61.
Gram L, Huss HH. 2000. Fresh and processed fish and shellfish. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Gaithersburg (MD): Aspen. p 472–506.
Health Canada. 1994 Aug 16 [updated 1997 Mar 19]. Food and drugs act and regulations: division 21-prepared fish (B.21.025) [online publication-not the official Canada Gazette pages]. Available from: Health Canada's Food Directorate web site at http://www.hc-sc.gc.ca/food-aliment/english/publications/acts_and_regulations/food_and_drugs_acts/d-text–2.pdf
Hilderbrand K. 1973. Preparation of salt brines for the fishing industry. Corvallis: Oregon State Univ., Oregon Sea Grant Program (#OSU SG 22). Report nr NSGL# ORESU-G–73-002. Grant nr NOAA–73072505. 4 p.
Hudson JA, Mott SJ. 1993. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cold-smoked salmon under refrigeration and mild temperature abuse. Food Microbiol 10:61–8.
Kalish F. 1991. Extending the HACCP concept to product distribution. Food Technol 45(4):119–20.
Kaneko J, (PacMar, Inc., Honolulu, Hawaii). 2000. Development of a HACCP-based strategy for the control of histamine for the fresh tuna industry [A report by PacMar, Inc. pursuant to National Oceanographic and Atmospheric Administration]. Honolulu (Hawaii): PacMar; 2000 July 31. NOAA Award Nr NA86FD0067. 48 p.
Kassem CL. 1977. Smoking fish at home-a step by step guide. Blacksburg (VA): Virginia Polytechnic Institute and State Univ, Cooperative Extension Service. VPI-SG–300–2.
Leisner JJ, Millan JC, Huss HH, Larson LM. 1994. Production of histamine and tyramine by lactic acid bacteria isolated from vacuum-packed sugar-salted fish. J Appl Bacteriol 76:417–23.
Lord JB. 2000. Entering the new millennium: the food industry in transition. NFPA Feb:9–15.
[MMWR] Morbidity Mortality Weekly Report. 2000 Mar 17. Surveillance for foodborne disease outbreaks-United States, 1993–1997. MMWR 49(SS01):1–51.
[NMFS] National Marine Fisheries Service. 1991a. HACCP prototype model food service/consumer education. Model seafood surveillance project. Pascagoula (MS): NMFS, Office of Trade and Industry Services, NSIL. 1991 Dec.
[NMFS] National Marine Fisheries Service. 1991b. HACCP regulatory model smoked and cured fish. Model seafood surveillance project. Pascagoula (MS): NMFS, Office of Trade and Industry Services, NSIL. 1991 Dec.
Pelroy GA, Eklund MW, Paranjpye RN, Suzuki EM, Peterson ME. 1982. Inhibition of *Clostridium botulinum* types A and E toxin formation by sodium nitrite and sodium chloride in hot-process (smoked) salmon. J Food Prot 45(9):833–41.
Pelroy GA, Peterson ME, Paranjpye R, Almond J, Eklund M. 1994b. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium nitrite and packaging method. J Food Prot 57(2):114–9.
Peterson ME, Pelroy GA, Paranjpye RN, Poysky FT, Almond JS, Eklund MW. 1993. Parameters for control of *Listeria monocytogenes* in smoked fishery products: sodium chloride and packaging method. J Food Prot 56(11):938–43.
Robertson GL. 1993. Food Packaging: Principles and Practice. Hughes H, editor. New York: M Dekker. 676 p.
Rorvik LM, Yndestad M, Skjerve E. 1991. Growth of *Listeria monocytogenes* in vacuum-packed, smoked salmon during storage at 4° C. Int J Food Microbiol 14:111–8.
Sachs S. 1989. Forward to a margin of safety: the HACCP approach to food safety education. Washington, DC: USDA, FSIS, ILA. 1989 June.
Storey RM. 1982. Smoking. In: Aitken A, Mackie IM, Merritt JH, Windsor ML, editors. Fish handling and processing. 2nd ed. Aberdeen: Ministry of Agriculture, Fisheries and Food, Torry Research Station; Edinburgh: HMSO. p 98–114.

# Conclusions and Research Needs

## Conclusions

The following conclusions are based on a thorough analysis and evaluation of the current science on control methods of human health hazards that may be associated with the consumption of cold-smoked fish.

### *Listeria monocytogenes*

Given the ubiquitous nature of *L. monocytogenes*, the lack of listericidal steps in the cold-smoking procedure, and the ability of the organism to become established in the processing environment and recontaminate products, it is not possible to produce cold-smoked fish consistently free of *L. monocytogenes*. This is not unique to cold-smoked fish because this microorganism can be isolated from a wide range of ready-to-eat (RTE) foods.

- By adhering strictly to Good Manufacturing Practices (GMPs) and Good Hygienic Practices (GHPs) it is possible to produce cold-smoked fish with low levels of *L. monocytogenes*, preferably at < 1 cell/g at the time of production.
- Growth of *L. monocytogenes* in naturally contaminated fish products is significantly slower than predicted by models (using combinations of pH, NaCl, temperature, and lactate) and inoculation studies.
- Prevention of growth of *L. monocytogenes* in cold-smoked fish cannot be guaranteed not to occur using current combinations of NaCl and low temperature; however, growth can be prevented by freezing, by addition of certain additives (for example, nitrite), or by use of bioprotective bacterial cultures.
- If the organism cannot be eliminated and growth-inhibiting steps are not introduced, the hazard can be controlled by limiting shelf life (at 4.4° C, 40° F) to ensure that no more than 100 cells/g are present at time of consumption. Time limits may need to be established by each processor because the time limit should reflect the initial level of the organism in freshly produced product.
- Some countries, such as Australia, warn pregnant women about listeriosis and offer a list of food items to be avoided during the pregnancy. Labeling cold-smoked fish as well as other RTE foods in this risk category, indicating that these products may constitute a health hazard for immunocompromised individuals and pregnant women could be considered.
- There is no control point during the cold-smoking process that will guarantee the elimination of *L. monocytogenes* on the final product; however, the occurrence of *L. monocytogenes* on the finished cold-smoked fish products of processors can be minimized by: 1) obtaining the primary product from known sources (for example, those with a history of noncontaminated fish); 2) following strict adherence to GMPs to prevent recontamination during processing; and 3) inhibiting growth of any survivors by marketing the product frozen, or by using salt and other preservatives that can inhibit growth at refrigerated temperatures.

### *Clostridium botulinum*

Psychrotrophic *C. botulinum* occurs naturally in the aquatic environment, so its presence in low numbers on fresh fish must be anticipated. Spores may also be isolated infrequently from cold-smoked fish, although numbers, if present, are low. Given this low number, the probability of germination and toxin production is low but present.

- Experiments with naturally contaminated hot-smoked fish produced from fish with high levels of *C. botulinum* show that toxin may be formed under conditions of temperature abuse.
- Toxin production by psychrotrophic *C. botulinum* is controlled with a combination of a moderate level of NaCl (3.5% NaCl WPS) and storage at chill temperature (< 4.4°C, < 40 °F) for at least 4 wk. Based on the scientific data and because commercially produced cold-smoked fish has never been reported as a source of botulism, it is reasonable to conclude that the salt and cold keep the hazard under adequate control.
- Based on a range of model studies in broth and inoculation studies with hot- or cold-smoked fish, it can be concluded that a combination of 3.5% NaCl (as water phase salt) and chill storage (4.4 °C, 40 °F), allowing for short time periods of elevated temperatures up to 10 °C (50 °F), will prevent toxin formation in reduced oxygen packaging cold-smoked fish for several weeks beyond its sensory shelf life.
- As a general safeguard, salting to 3.5% for chilled stored cold-smoked fish is essential for reduced oxygen packaged (ROP) cold-smoked fish. In addition, the requirement for chilling with a sufficient salt concentration is an option for consideration in national or international regulations (for example, E.U. directives).
- For air-packaged products, levels of NaCl can, theoretically, be reduced; however, scientific data that support this argument do not exist and are needed before any reduction is recommended. Even when not packed under vacuum or modified atmosphere, pockets of anaerobic conditions may be created where slices of fish overlap or where aerobic spoilage bacteria consume the oxygen present.
- To control *C. botulinum* growth and toxin production in ROP products the following considerations are indicated: 1) A minimum 3.5% water phase salt concentration in the thickest part of the fillet for vacuum or modified atmosphere packaged fish, or a combination of at least 3% water phase salt and a nitrite level of 100 to 200 ppm is necessary for the control of *C botulinum* growth and toxin formation (**Note**: nitrite is not allowed in products sold in Europe, and is only allowed in the United States for sable, salmon, shad, chub, and tuna). 2) Packages containing refrigerated, cold-smoked fish should be labeled, "Keep Refrigerated at 40° F (4.4 °C) or below. 3) Packages containing frozen, cold-smoked fish should be labeled, "This product must remain frozen until thawed at refrigeration temperatures and shall not be refrozen," and 4) Products should not be packaged in reduced oxygen packaging by the retailer.

### Biogenic amines

The majority of species that are cold-smoked have not been identified by the scientific community as causing scombrotoxin illness. Therefore, the risk of foodborne illness is limited in the majority of cold-smoked products available in the marketplace.

- Only relatively high and sometimes controversial concentrations of histamine have usually resulted in illness. The contri-

bution of other biogenic amines to the onset of symptoms is not well understood.

- Most scombrotoxin results from extrinsic, rather than intrinsic, spoilage through the growth of certain bacteria, generally members of the family *Enterobacteriaceae*. Some bacteria are capable of producing greater quantities of decarboxylase enzymes than others.
- Certain processing operations, such as freezing, salting, or smoking may be capable of inhibiting or inactivating biogenic amine-producing microorganisms; however, microorganism growth with potential toxin production may occur after thawing and postprocessing.
- Under certain conditions addition of lactic acid-producing microorganisms suppresses the growth of biogenic amine-forming microorganisms.
- Vacuum packaging does not prevent growth of biogenic amine-forming microorganisms.
- While biogenic amine-forming microorganisms may grow at refrigeration temperatures, generally the minimal temperature for growth is lower than the minimal temperature for toxin production.
- The most effective methods of preventing biogenic amine formation are handling and processing under sanitary conditions, rapid cooling of the fish, and continued refrigeration from harvest through consumption.
- To minimize the level of biogenic amines in species susceptible to histamine formation, temperature control is important throughout the process, particularly during the storage and transportation before cold smoking, the cooling step, and the final product storage, distribution, retail, and consumer steps. The temperatures required for the control of *C. botulinum* may be appropriate to control production of biogenic amines.
- Much of the published scientific research on scombrotoxin utilized fish samples obtained from processing facilities and retail food stores. Only a limited number of studies followed samples from harvest through analysis. Also, sensory analyses were not always incorporated into microbiological and analytical chemical studies. There is a lack of reports describing comprehensive and integrated projects.

### Parasites

Some of the fish species used for cold-smoked processing are either intermediate or final hosts to parasites. For this reason, assuring the harvesting of parasite-free fish in the wild is difficult.

- Some aquacultured fish are considered free of parasites (if their feeding regime has not been supplemented with raw fish) because their diet can be controlled using net-pens, closed recycled systems or an equivalent system, and commercially pelleted diets; consequently, these control measures must be carefully considered and applied. An analysis of the potential control points for parasites in aquacultured fish is beyond the scope of this report.
- Freezing raw fish prior to smoking remains the most effective way to insure that viable parasites are not present in cold-smoked products consumed by the public. It is essential, therefore, that raw fish potentially containing viable parasites be frozen and held in that state for a period of time that assures destruction of all viable parasites in that fish species.

## Research Needs

The following is a list of research areas that the panel suggests need further attention:

### *Listeria monocytogenes*

- Conduct epidemiological investigations to determine if and to what extent cold-smoked fish is involved in cases of listeriosis. Despite prediction of a risk, only a limited number of cases have been associated with cold-smoked fish.
- Assess virulence potential of *L. monocytogenes* isolated from cold-smoked fish.
- Measure behavior of *L. monocytogenes* in naturally contaminated products. *Listeria monocytogenes* appears to grow more slowly and to lower numbers than anticipated based on model predictions and inoculation trials. An understanding of which factors cause these differences may be used to design appropriate control measures in the product.
- Determine the robustness and applicability of alternative growth inhibitory measures such as bioprotective cultures, bacteriocins, lactate and others.
- Determine how *L. monocytogenes* becomes established in smoke houses and processing facilities. Several studies show that particular DNA types become established in niches in the processing environments. Research is needed to evaluate what parameters determine which types reside—whether it be particular adhesion properties, or particular resistance properties, or other factors.
- Investigate the source of contamination in smoke houses and processing environments in order to introduce procedures specifically targeted at eliminating or limiting introduction of the organism.
- Identify GMP practices that would minimize the contamination and growth of *L. monocytogenes*.
- Determine the effectiveness of intervention strategies to reduce or eliminate *L. monocytogenes*, such as using chlorinated water to thaw and rinse incoming fish, and for rinsing fish following the brining operation.
- Develop cleaning and disinfection procedures targeted at adhered or established cells for removal of *L. monocytogenes* from surfaces.
- Determine if particular types of surfaces reduce numbers of adhering *L. monocytogenes* or if particular treatments (that is, spraying with lactic acid bacteria or lactate) can reduce surface contamination by minimizing adhesion and biofilm formation.
- Evaluate the robustness and sensory acceptability of the various procedures under investigation (that is, bioprotection, lactate, and so on) for the elimination of the possibility of growth in the product.
- Determine the effect of postprocessing methods such as irradiation and high pressure to eliminate *L. monocytogenes* in cold-smoked fish.

### *Clostridium botulinum*

- Evaluate growth and toxin production in naturally contaminated cold-smoked fish products to validate models and predictions for growth and toxin production.
- Determine the influence of redox potential, various concentrations of trimethylamine oxide (TMAO), and NaCl on toxin production by psychrotrophic *C. botulinum* in gadoid and nongadoid species.
- Determine the potential facilitation by TMAO on formation of nitrosamines, if nitrite is added, during cold smoking.
- Identify processing conditions and gas transmission rates of films under various time and temperature conditions for products to be considered "air packaged." Determine the Oxygen Transmission Rate (OTR) needed for a product with 2.5% salt concentration to provide equivalent safety compared with cold-smoked reduced oxygen-packaged products (ROP).
- Conduct challenge studies on air-packaged, cold-smoked fish in films with OTRs between 7000 and 10000 cc/$m^2$/24 h and compare to unpackaged cold-smoked fish.
- Establish minimum water phase salt concentrations re-

quired to inhibit growth and toxin formation by *C. botulinum* in air-packaged and unpackaged cold-smoked fish.

- Determine the shelf life of the product relative to product quality as well as safety under different packaging methods and storage temperatures.
- Determine appropriate sell-by dates and evaluate the use of time-temperature indicators to ensure a safe product.

### Biogenic amines

- Determine the influence of ROP on the inhibition of biogenic amine production by Gram-negative bacteria.
- Define the minimum temperatures for growth and biogenic amine production of biogenic amine-forming microorganisms.
- Identify practical temperatures that would minimize the levels of biogenic amines in all steps of the production chain and in the final product.
- Determine the effect of salt and redox potential on the formation of biogenic amines on the final product.
- Determine the impact of the interrelationship(s) among histamine, putrescine, cadaverine, and perhaps other biogenic amine concentrations in scombrotoxin and their effects on subsequent host responses.
- Investigate the effects of various cold-smoked fish processes (water phase salt concentrations, process times and temperatures) on biogenic amine formation.
- Develop practical methods for cold-smoked fish processors to determine the histamine/scombrotoxin risk in the raw material used for smoking.
- Apply new processes, such as irradiation, modified atmospheres, or high pressure, to reduce specific groups of microorganisms to determine if control of those responsible for biogenic amine formation reduce the hazard.
- Evaluate the effects of harvesting methods and postharvest handling practices on biogenic amine formation under varying environmental conditions.
- Identify specific methods for representative and effective sampling and for accurate and precise analysis of biogenic amines.

### Parasites

- Describe possible alternative freezing procedures that are or could be effective for inactivation of various fish parasites.
- Establish the kinetics and lethal effect of specific regimes of freezing on various fish parasites.
- Evaluate alternative processing procedures, such as high pressure and X-ray or e-beam irradiation for control of various fish parasites.
- Investigate the possible human health risks of allergic reactions due to parasite antigens remaining after freezing the fish to inactivate the live parasites.

# Glossary

**Association of Food and Drug Officials, AFDO:** Professional organization dedicated to the establishment and enforcement of uniform food and drug and related consumer protection laws.

**Amines:** Any of a class of basic compounds derived from ammonia by replacement of hydrogen by one or more univalent hydrocarbon radicals or other no acidic organic radicals.

**Bacterial endotoxins:** Any of a class of poisonous substances present in bacteria but separable only from the cell body on its disintegration (that is, typhoid fever).

**Bacteriocin:** A protein (peptide) that is produced by certain strain of bacteria and which are lethal (or inhibitory) in particular against closely related strains of bacteria.

**Biogenic amines:** Amines produced by the action of living organisms or amines that are essential to life and its maintenance.

**Botulism:** The disease (intoxication) typically caused by ingestion of botulism toxin formed by the bacterium *Clostridium botulinum*.

**Control point:** Any point, step or procedure at which biological, physical or chemical factors may be controlled.

**Dark muscles:** Red or brown pigmented muscles that lie beneath the skin in fish; dark muscle is related in some way to the activity of the fish—used for sustained activity so there is more dark muscle in very active fishes.

**Decarboxylase:** Any group of enzymes that accelerate decarboxylation, esp. of alpha-amino acids.

**Exposure assessment:** Estimate of the prevalence and levels of microbial contamination of the food product at the time of consumption and the amount of the product consumed at each meal by different categories of consumers.

**Food and Agriculture Organization of the United Nations, FAO:** Founded in October 1945 it is the largest autonomous agency within the United Nations system with 180 Member Nations plus the European Community (Member Organization) with a mandate to raise levels of nutrition and standards of living, to improve agricultural productivity, and to better the condition of rural populations. The Organization offers direct development assistance, collects, analyses and disseminates information, provides policy and planning advice to governments and acts as an international forum for debate on food and agriculture issues.

**Food MicroModel:** A software product developed by Food MicroModel Ltd used to predict the growth, death, and survival of microorganisms in foods.

**Generation time:** The average amount of time between the appearances of two successive generations (parent and offspring).

**Good Hygienic Practices, GHP:** the basic sanitary conditions and practices that must be maintained to produce safe foods. It includes also certain support activities such as raw material selection, product labeling and coding or recall procedures. Effective application of GHP provides the foundation upon which the Hazard Analysis Critical Control Point System (HACCP) is built. Major components of GHP as:

- Design and facilities (location, premises and rooms, equipment facilities)
- Control of operation (control of food hazards, key aspects of food hygiene control, incoming material requirements, packaging, water, management and supervision, documentation and records)
- Maintenance and cleaning (maintenance and cleaning, cleaning programs, pest control systems, waste management, monitoring effectiveness)
- Personal hygiene (health status, illness and injuries, personal cleanliness and behavior, visitors)
- Transportation (general, requirements, use and maintenance)
- Product information and consumer awareness (lot identification, product information, labeling, consumer education, handling/storage instructions)
- Training (awareness and responsibilities, training programs, instruction and supervision, refresher training).

**Good Manufacturing Practices, GMP:** GMPs are one of the HACCP prerequisite programs. GMPs relate to all aspects food processing operations that prevent product contamination from direct or indirect sources.

**Generally Recognized as Safe, GRAS substance:** The regulatory status of food ingredients not evaluated by the FDA prescribed testing procedure because their safety has been demonstrated through a history of safe use or scientific procedures. It also includes common food ingredients that were already in use when the 1959 Food Additives Amendment to the Food, Drug and Cosmetic Act was enacted.

**Halophylic:** Microorganism with a specific requirement for a significant level of salt (NaCl) ($a_w$ range 0.85 to 0.75).

**Halotolerant:** Microorganisms that will tolerate (survive but not grow) in an environment with moderate salt (NaCl) levels.

**Hazard:** A biological, chemical or physical agent that is reasonably likely to cause illness or injury in the absence of its control.

**Iced fish:** Fish and seafood that have been properly placed in ice for cooling and holding. "Ice is an ideal cooling medium for fresh fish. When used liberally it has several advantages over standard refrigeration methods. It rapidly removes heat from fish; holds fish at or near 32 °F (0 °C) throughout distribution; continuously flushes away bacteria, blood, and slime as it melts; and prevents dehydration."

**Lag phase:** The initial growth phase of a culture, during which cell number remains relatively constant prior to rapid growth.

**Light muscles:** The predominate muscle in most nonpelagic demersal fish; light to white in color.

**Listeriosis:** The infectious disease caused by the foodborne bacteria *Listeria monocytogenes*. The disease typically involves septicemia, meningoencephalitis, or abortion/stillbirth. Mortality rates for the septicemia or meningeal forms may be very high. Human listeriosis is rare and usually occurs only in immunosupressed individuals or in the fetus/neonates.

**Logarithmic phase:** The steepest slope of the growth curve of a culture—the phase of vigorous growth during which cell number doubles every 20 to 30 min.

**Mesophylic:** Microorganisms capable of growth within an intermediate temperature range, with optimal growth temperatures occurring between 30 and 45 C.

**Nonvolatile amines:** amines that are not readily vaporizable at relatively low temperatures.

**Oxydation-reduction potential, Eh** (redox potential): The electrical potential associated with the oxidation or reduction of a substance, such as an element or molecule. Classification of microorganisms as, aerobic, anaerobic, or facultative is based on Eh required for multiplication and metabolism. Aerobes require positive Eh values; anaerobes require negative Eh values, facultative grow in either positive or negative Eh values.

**Package:** An enclosure designed to protect, preserve, and contain a commodity for shipment or storage.

**Planktonic state:** The state in which bacteria are suspended or grow in liquid (as opposed to the biofilm state where the bacteria are adhered to a solid surface).

**Processing Parameters:** Biological, chemical, or physical parameters that must be controlled to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazard.

**Psycrotroph:** Microorganisms capable of growth at low (refrigeration –5 to 5 °C) temperatures, although the optimal growth temperature occurs between 25 and 30 °C.

**Redox Potential**: The electrical potential associated with the oxidation or reduction of a substance, such as an element or molecule.

**Reduced Oxygen Packaging:** any packaging procedure that results in a reduced oxygen level in a sealed package.

**Ribotyping:** Method to determine homologies and differences between bacteria at subspecies (strain) level. Chromosomal DNA is extracted and cut with restriction enzymes. After electrophoretic separation in an agarose gel, the DNA-bands are blotted onto a membrane and hybridized with DNA-probes.

**Risk analysis:** Widely recognized as the fundamental methodology underlying the development of food safety standards. Risk analysis is composed of three separate but integrated elements, namely risk assessment, risk management and risk communication.

**Risk assessment:** The scientific approach to determine magnitude of a risk. It involves 1) hazard identification (information about the pathogen/toxin and the food in question), 2) hazard characterization (severity and duration of disease, dose-response), 3) exposure assessment (see below) and 4) risk characterization (combines the above information to give a complete picture of the risk). Results in a risk estimate that is an indication of the level of disease (for example, number of cases per 100000 per year) resulting from a given exposure.

**Risk communication:** Interactive process of exchange of information and opinion on risk among risk assessors, risk managers, and other interested parties.

**Risk management:** The process of weighing policy alternatives in the light of the results of risk assessment and, if required, selecting and implementing appropriate control options, including regulatory measures.

**Scombroid food poisoning or scombrotoxicosis:** Disease generally caused by the improper preservation of scombroid fishes, which results in certain bacteria acting on histidine in the muscle of the fish, converting it to histamine; ingestion of this histamine by humans results an allergic like reaction, or scombrotoxicosis.

**Scombroid species:** Suborder of Percomorphi that comprises active streamlined, marine fishes having a narrow caudal peduncle, scales absent or small and sometimes spiny, a usually long dorsal fin without projecting spines but sometimes with finlets, a lunate or forked caudal fin and including numerous oily-fleshed fishes (mackerels, tunas, albacores, bonitos, and swordfishes).

**Serotype:** A serologically (antigenically) distinct variety within a bacterial species.

**Shelf life:** The time period from production of a food until it is considered in-edible by a consumer.

**Standard Sanitation Operation Procedures**: An action plan, usually written, that details procedures necessary to maintain sanitary conditions throughout a food processing facility.

**Stationary phase:** The plateau of the growth curve after log growth in a culture, during which cell number remains constant. New cells are produced at the same rate as older cells die.,

**Synergestic effect:** of or relating to synergism; cooperative action of discrete agencies (drugs, muscles) such that the total effect is greater than the sum of the two or more effects taken independently.

**Vessel Standard Operating Procedures:** Protocol or outline of steps and/or methods to be followed to ensure safe, sanitary and efficient operations. In the context of this paper it refers to producing fish and seafood products. Compare with Sanitation Standard Operating Procedures (SSOP).

**Virulence:** the capacity of a pathogenic organism to cause disease—defined broadly as the severity of symptoms in the host.

**Water activity, $a_w$.** Qualitatively, $a_w$ is a measure of unbound, free water in a system, available to support biological and chemical reactions. Water activity affects microorganisms' survival and reproduction, enzymes, and chemical reactions. The water activity of a substance is quantitatively equal to the vapor pressure of the substance divided by the vapor pressure of pure water (both measured at the same temperature).

# APPENDIX A
# Summary of Cold-Smoking Process

## Scope

This appendix provides a brief summary of the cold-smoking process as a complement to Chapter VI, Control of Food Safety Hazards During Cold-Smoked Processing, which includes a more detailed description of the process and identifies potential hazards, control points, and processing parameters. Both Chapter VI and this summary address some recommendations by the Association of Food and Drug Officials (AFDO 1991). It bears repeating that although developed by experts and widely used, these recommendations have not necessarily been scientifically evaluated. The recommendations from the Fish & Fishery Products Hazards & Controls Guide (FDA 1998) are indicated for reference.

### 1. Receiving

Cold-smoked fish processors receive fish that are either wild-caught or farm-raised. For both practices, scombrotoxin-susceptible fish should be received at an internal temperature of 40 °F (4.4 °C) or less. If fish are received directly from the harvest vessel, all lots should be accompanied by documentation certifying proper time and temperature handling of the fish. In addition to the time and temperature parameters, analytical testing for biogenic amines should be done periodically on samples of edible fish flesh (FDA guidance limits the histamine level to 50 ppm). Even though an experienced processor may be doing the evaluation, sensory analysis is a very subjective and not quantitative monitoring method; therefore, a more objective method (analytical method or temperature record) should be in place. Practically, most companies use a sensory evaluation of incoming scombrotoxin-susceptible species with a maximum receiving temperature for refrigerated raw material received. If sensory analysis points to a high biogenic amine level, analytical testing is performed.

If product is received frozen to control parasites, a number of time and temperature combinations have been recommended in the United States, such as holding the fish at –10 °F (–23 °C) for 60 h, or less than –4 °F (–20 °C) for 7 d, or –31 °F (–35 °C) internally for 15 h. For a more detailed explanation of freezing regimes, see Chapter V. Farm-raised fish fed a controlled diet such as processed feed pellets and reared in the proper environment are generally free of parasites.

### 2. Fresh or frozen storage

Raw fish stored fresh should be kept in an appropriate refrigerated or iced condition to maintain an internal temperature of 40 °F (4.4 °C) or less, especially if scombrotoxin-susceptible fish are to be cold-smoked. The reader is referred to the Fish & Fishery Products Hazards & Controls Guide (FDA 1998) for recommendations on refrigeration time and temperature critical limits for scombrotoxin-susceptible fish.

Wild-caught fish intended for cold-smoked processing should be frozen at some step during the process. Generally, fish are frozen either before brining or slicing. For quality purposes, it is recommended that freezing be performed before the salting and smoking steps. Often, the freezing step before slicing is conducted to make the fish "hard" enough to cut. Regardless of the stage at which fish are frozen, a number of time and temperature combinations have been recommended for proper storage to control parasites, such as holding the fish at –10 °F (–23 °C) for 60 h, or less than –4 °F (–20 °C) for 7 d, or –31 °F (–35 °C) internally for 15 h.

### 3. Thawing, washing, and rinsing

Thawing, washing, and rinsing of the fish should be done under sanitary conditions and temperature control. AFDO guidelines offer the following recommendations for thawing temperatures. If the fish are thawed in water, the water should be continuously flowing or spraying, with the water temperature below 60 °F (15.5 °C). The internal temperature of the fish should remain below 45 °F (7.2 °C). After thawing, the fish should be washed and rinsed thoroughly with potable water.

### 4. Butchering and evisceration

Fish should be butchered separate from the rest of the processing area. Gutting should be performed with minimal disturbance of the intestinal tract contents. After butchering and evisceration, the fish should be washed and rinsed thoroughly, especially the body cavities, with continuously flowing or spraying water.

### 5. Sorting, sizing, and salting

Brining is the process by which the fish is soaked in a solution consisting of water, salt, sugar, various spices and flavorings, phosphates, and, depending on the recipe and species of fish (sable, salmon, shad, chub, and tuna), additives such as sodium nitrite. Dry-salting involves placing fish for a certain period of time in a dry mixture of salt and other ingredients. Fish also may be brined by injecting the fish with a solution, either by hand or machine.

Salting should be as uniform as possible, with the correct amount of salt or brine solution absorbed into each piece of fish flesh.. To achieve uniformity, fish must be sorted by size and thickness; different species of fish should not be mixed in the same brining tank, and the weight-volume ratio, time, and temperature of the process should be controlled. It is extremely important that brining solutions not be reused or recycled unless treated in some way to maintain an acceptable microbiological profile. If the fish is washed after salting, washing and rinsing should be done with potable water.

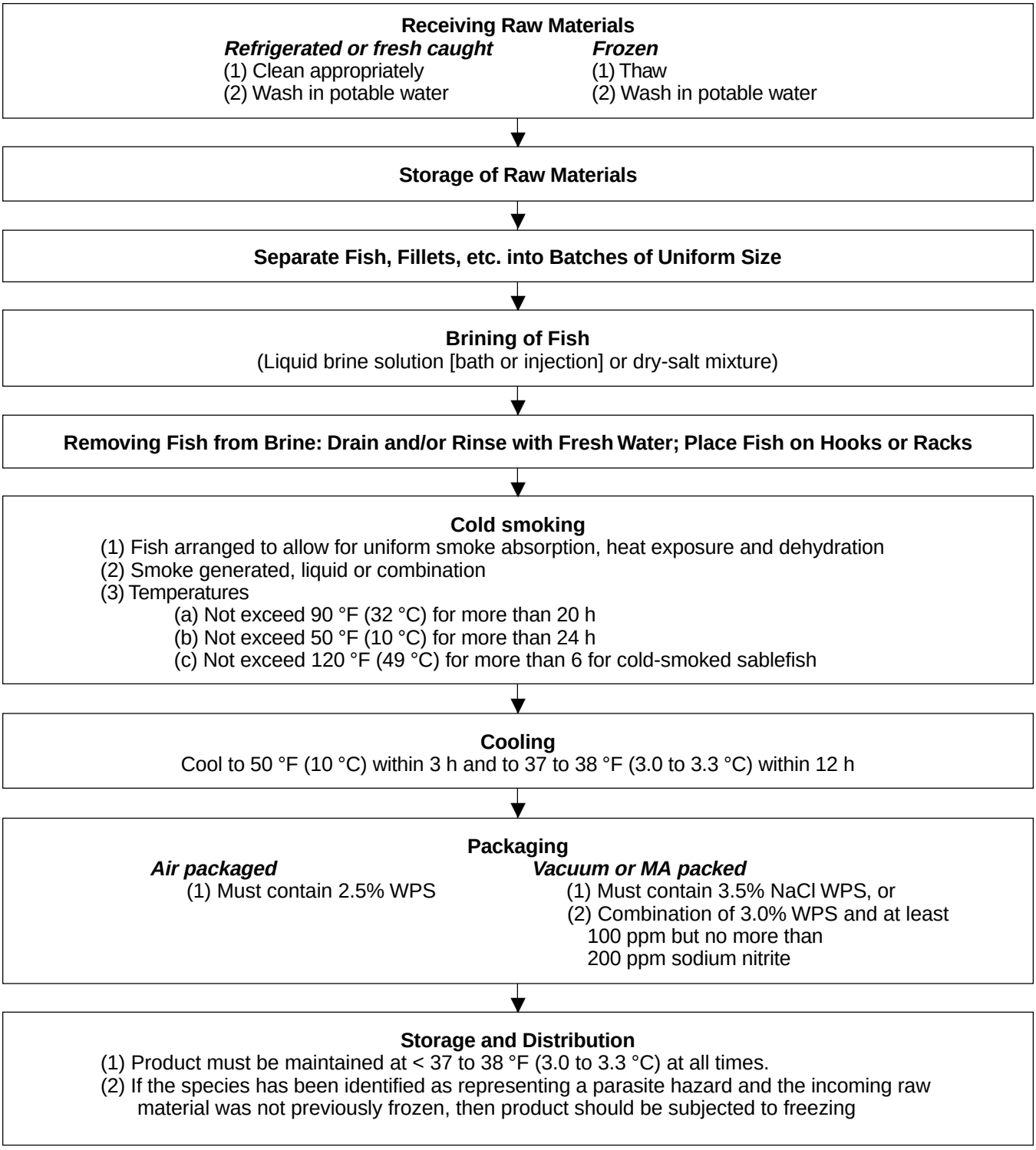
Salting times may vary from < 1 h to 24 h. The time and size of the fish pieces are empirically determined. Brining is usually a cold process, but it also may be done at room temperature (50 to 60 °F, 10 to 15.5 °C). AFDO guidelines recommend that the temperature of the brine not exceed 60 °F (15.5 °C) at the beginning of brining. If the brining or soak time is longer than 4 h, the brining must be done under refrigerated conditions. Fish processed with a dry-cure mixture should be held under refrigerated conditions in the salt mixture. If brining is done by injection, a saturated salt solution is used up to a temperature of 65 °F (18.5 °C).

### 6. Drying and cold smoking

A number of cold-smoking procedures involve a drying stage with no smoke added to the product. The product is held at a certain temperature, often refrigeration temperature, for a certain amount of time before the smoke is introduced. The parameters of this initial drying component include the type or species of fish, its fat content, and humidity. During this time, a pellicle is formed on the outside surfaces of the fish pieces.

During the cold-smoking step, the fish must be arranged to allow for uniform smoke absorption, temperature exposure, and drying. The smoke can be "natural" (generated), liquid, or a combination of the two.

In Europe, cold-smoking temperatures are below 30 °C (86 °F), based on protein coagulation temperatures. AFDO guidelines recommend that cold-smoking temperatures should not exceed 90 °F (32.2 °C) for more than 20 h; 50 °F (10 °C) for more than 24 h; or 120 °F (48.8 °C) for more than 6 h for cold-smoked sablefish. In the United States, cold smoking is seldom performed at temperatures above 100 °F. The duration is usually in the range 6 to 12 h.



### 7. Cooling

According to AFDO guidelines, after cold smoking the fish should be cooled to 50 °F (10 °C) within 3 h and to 38 °F (3.3 °C) within 12 h. These recommendations, however, are under reviewed.

### 8. Slicing and cutting

After cooling, cold-smoked fish are often sliced and cut. A processor must have well designed and comprehensive Sanitation Standard Operating Procedures (SSOPs) and must follow Good Manufacturing Practices (GMPs) during slicing and cutting.

### 9. Packaging

Cold-smoked fish is packaged using film with variable oxygen transmission rates (OTRs). Gas permeability is an important parameter and should be taken into account when doing research and making decisions on food safety issues. Specifications for gas permeability, however, are product- and use-specific and are usually established at ambient temperatures under moderate humidity conditions (for example, 23 °C and 50% R.H.) using a variety of testing and verification methods. Consequently, it is difficult to extrapolate the OTR of a package to a specific combination of package, product, and conditions.

The Fish & Fishery Products Hazards & Controls Guide (FDA 1998) recommends at least a 2.5% WPS in the loin muscle of the finished product in air-packaged fish. For vacuum-packaged or modified atmosphere-packaged fish, a salt concentration of at least 3.5% WPS in the loin muscle of the finished product or a combination of 3.0% WPS in the loin muscle and at least 100 ppm but not more than 200 ppm of sodium nitrite is recommended (allowed in the United States for sable, salmon, shad, and shub).

The final product should be kept under refrigeration. If the product has the appropriate salt content, scientific data support that the storage temperature should be maintained at a maximum of 40 °F (4.4 °C) for 4 wk for safety. The product should be labeled as to the required refrigeration temperature and storage time.

### 10. Storage and distribution

Cold-smoked fish products can be stored refrigerated or frozen. In any case, finished product must be maintained at or below 40 °F (4.4 °C) at all times throughout storage and distribution.

## References

[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. [York (PA)]: Association of Food and Drug Officials. 7 p.

[FDA] Food and Drug Administration. 1998. Fish & Fisheries Products Hazards & Controls Guide. 2nd ed. Washington, D.C.: FDA, Office of Seafood. 276 p.

# APPENDIX B
# On-Board and Aquaculture Postharvest Handling of Fish

Minimizing the microbial load on seafood products begins prior to the harvest of the product. Raw materials for cold-smoked fish products include both wild and aquaculture species. In either case, the microbial flora present will be determined by that present in the waters from which the fish are harvested and the feed materials consumed by the fish. In general, fish harvested from the open ocean will be relatively free of human pathogens (with the exception of certain parasites) while those harvested from near-shore saltwater, fresh water, and aquaculture sources are at greater risk for contamination. Sources of the microorganisms include general pollution resulting from human, animal, and agriculture waste.

Regardless of the source of the raw material, a good sanitation regimen is critical to minimize the growth of the existing microbial load and even more important to minimize the risk of introducing additional organisms. Good Manufacturing Practices (GMPs), including holding, transporting, and processing at appropriate low temperatures, are also requisite.

On a harvest vessel, a good sanitation program includes regular cleaning and sanitizing of nets, brailers, equipment (harvest and processing), holds, totes, baskets, boxes, and bins. It is also critical that water, refrigeration and freezing media, such as refrigerated seawater and brines, and ice are as free of microorganisms as possible. Thus, appropriately treated water should be used in processing, ice production, contact surface cleaning and rinsing, and in other applications whereby contamination is possible. Several publications prescribe excellent on-board procedures for both harvest and transporting vessels (Crapo and others 1986; Crapo and Elliot 1987; Crapo and Paust 1987; Rasco and others 2001).

The microbiological flora present on farmed fish is affected by water quality and feed composition. The quality of water can vary dramatically. Specific harvest and postharvest treatment, therefore, must be related to the specific level of risk associated with the water source. In general, good sanitation procedures should be applied throughout the harvest, transport, storage, and postharvest handling of the aquacultured fish.

Transportation of the fish from initial harvest or processing is another area of concern. Basic sanitation practices need to be applied to the transportation vehicles and containers, and temperature abuse should be minimized. It is recommended that temperature recording or indicating devises be used when transporting or even holding such products, particularly when the product is frozen and the period of transportation or holding is used to satisfy requirements directed toward controlling parasites.

Direct treatment of finfish for reduction of microbial load is practical after harvest and before processing. Again, a simple technique involves a chlorine solution rinse, preferably applied as a spray, to the round fish followed by a rinse with potable water. At one time it was prescribed that the fish be dipped in a chlorine solution (Eklund and others 1993); however, this is no longer recommended as the solution quickly became an inoculating broth unless intensively managed. Although it has been suggested that rinsing of the fish is important to reduce numbers of pathogenic microorganisms, no data on the effect of this pro-

cedure could be found in the scientific literature. The feasibility of using ozone and ultraviolet light to control microorganisms in water is also being evaluated.

In summary, efforts directed at reducing or minimizing the microbial load on fish destined for cold smoking must be initiated as early in the production cycle as practical. GMPs and appropriate sanitation procedures should be applied throughout the process. The fish should be cooled as soon after harvest as practical and maintained at a temperature less than 40 °F (4.4 °C) until processed. The use of temperature recording or indicating devices is highly recommended. The old adage, "keep it cold, keep it clean, and keep it moving," is quite appropriate.

## References

Crapo CA, Elliot E. 1987. Salmon quality: the effect of elevated refrigerated seawater chilling temperature. Fairbanks (AK): University of Alaska, SeaGrant College Program.
Crapo CA, Kramer DE, Doyle JP. 1986. Salmon quality: the effect of delayed chilling. Fairbanks (AK): University of Alaska, SeaGrant College Program.
Crapo CA, Paust B. 1987. Air shipment of fresh fish: a primer for shippers and cargo handlers. Fairbanks (AK): University of Alaska, SeaGrant College Program.
Eklund M, Pelroy G, Poysky F, Paranjpye R, Lashbrook L, Peterson M. 1993 July. Summary of interim guidelines for reduction and control of *Listeria monocytogenes* in or on smoked fish [internal report]. Seattle: Northwest Fisheries Science Center. July 1993. 14 p.
Rasco BA, Girard WA, Bledsoe GE. 2001. Frozen aquatic food products [chapter 22]. In: Smith JS, editor. Introduction to food chemistry. West Sacramento (CA): Science Technology Systems.

# APPENDIX C
# Verification Procedures and Corrective Actions During Cold-Smoked Processing

### 1. Receiving

For scombrotoxin-susceptible fish, if internal receiving temperatures are too high or sensory evaluations indicate a problem, the product can be rejected. Records of temperature monitoring, sensory evaluations, supplier certifications, and testing results should be available for every lot. If time and temperature documentation is unavailable, the product should be rejected, appropriate documentation must be requested from the supplier, or samples must be sent to a laboratory for histamine testing. To confirm the accept-or-reject decision, samples can be collected and sent to a laboratory for quantitative analysis of histamine levels. Sensory evaluation of the product may also be conducted.

### 2. Freezing

Fish for cold smoking need to be frozen at the proper temperature for the proper length of time at some point during the process to control parasites in the final product. If the product is received frozen, this may be a control point for parasites. Records of temperature monitoring or supplier certifications should be available for every lot. If no time and temperature documentation exist, the processor should either reject the product, request documents, or certification from the supplier, or freeze product for the required time and temperature. Detailed examination of periodic samples of product at each step will assist in insuring that the control methods are effective.

### 3. Sorting, sizing, and salting

This is a control point to prevent *Clostridium botulinum* toxin formation on the final product. During brining the following parameters should be monitored appropriately to assure that the final product has sufficient salt levels to inhibit *C. botulinum* toxin formation (that is, 3.5% water phase salt [WPS] in thickest portion of loin if vacuum-packaged):

- The salt concentration in the brine must be adequate (a minimum brine strength or concentration is necessary). The salt concentration should be measured at the beginning of each batch prior to the addition of sugar or other substances. An alternative procedure is to establish limits for the weight of each ingredient and keep records.
- The weight or volume of fish must be within the brining capacity of the brining solution (a maximum volume or weight of fish or fish portions). The volume or weight of the brine should be measured at the beginning of each batch.
- The fish or fish portions must not exceed a predetermined thickness (a maximum size is set).
- The maximum temperature of the brine solution must be set (brining solution is kept below a maximum temperature during the brining step).

Corrective actions should be introduced as appropriate. For example, if the brine concentration is too dilute, more salt should be added. If there is not enough brine solution for proper brining, more of the brine or brining solution should be added. If there is too much fish for the brine, some of the fish or fish portions should be removed.

All parameters should be appropriately recorded and records reviewed. Periodic calibration of scales and thermometers is needed. Additionally, periodic review of monitoring, corrective action, and calibration records is necessary.

### 4. Labeling

This step is not considered a control point, since it is not possible to label safety into a product. Nevertheless, label information identifying appropriate storage temperatures and time for safety is critical to control biogenic amine formation in scombrotoxin-susceptible species and *C. botulinum* growth, and toxin formation in cold-smoked products, especially if packaged in a reduced-oxygen environment. This label information is also important in reducing the growth rate of *Listeria monocytogenes*, although temperature will not prevent its growth. Visual checks of labels should be performed. Packages without proper labels specifying proper handling and storage conditions should be rejected and packages should be replaced with the proper labels.

### 5. Storage, distribution, retail, and consumer

Temperature during storage, distribution, retail, and consumer is a control point to prevent *C. botulinum* toxin formation, growth rate of *L. monocytogenes*, and biogenic amine formation in scombrotoxin-susceptible species. Temperature of the cooler should be monitored. If the product temperature exceeds 40 °F (4.4 °C), the temperature of the product should be reduced immediately and the product evaluated for safety.

# APPENDIX D
# Industry Survey

The panel developed a questionnaire and sent it to over 30 companies and processing facilities producing smoked-fish products. The questionnaire was adapted from a smoked-fish survey questionnaire originally developed by Dr. Roy Martin of the National Fisheries Institute. Six completed survey forms have been returned. Based on this response, it is proper to think of this as a sampling of procedures and techniques used by the Cold-Smoked Processing Industry.

A collation of the sampling follows. The responses were as expected. The types of fish used for cold smoking as well as the many variations in technique, brines, and smoke, are exemplified in this sampling. All of the companies responding indicated that their cold-smoked product either is or could be frozen for storage and distribution. Four of the companies had the capability to freeze their maximum daily production.

**Cold-smoked Processing Survey Tallies**

**1. Please check all of the species of fish that you cold-smoke.**

Company 1—Herring, Mackerel, Cod, Haddock, and Pollack.
Company 2—Salmon (Atlantic, dry brine), and Tuna
Company 3—Salmon, Sablefish, and Tuna
Company 4—Salmon and Sablefish
Company 5—Salmon, Sablefish, and Halibut
Company 6-Salmon

**2. Do you use dry salt, liquid brine, or an injection method with liquid brine to brine your product?**

Company 1—liquid Brine
Company 2—No response
Company 3—Dry Salt and Liquid Brine
Company 4—Liquid Brine
Company 5—Dry Salt and Liquid Brine
Company 6—Dry Salt and Liquid Brine

**3. If using liquid brine, what is your brine to fish ratio?**

Company 1—Salt content of 2%
Company 2—50 lbs product to 10 gallons brine for wet brine.
Company 3—1.8 gallons of brine to lbs of fish
Company 4—2 to 1
Company 5—1 to 1 (some types of fish we do dry curing others wet curing); 2 to 1
Company 6—2 to 1

**4. Does this ratio differ for each species you process?**

Company 1—No
Company 2—Yes
Company 3—No
Company 4—No
Company 5—Yes (for cold-smoke depending on specie)
Company 6—No

**5. If the answer to question #3 is yes, then please describe further.**

Company 1—No response
Company 2—Salmon (Nova (dry brined) and Kippered,)
Company 3 –
Company 4 –
Company 5—Salmon 1:1, Sablefish 2:1, and Halibut N/A
Company 6—No response

**6. At what temperature do you presently do the brining?**

Company 1—35 to 40 °F
Company 2—55 to 60 °F
Company 3—35 to 38 °F
Company 4—35 to 40 °F
Company 5—35 to 40 °F Tap water temperature and Room temperature
Company 6—Under 35 °F

**7. Do you have any difficulties brining at the above temperature? If so please describe.**

First 5 Companies said NO
Company 6—Yes, it slows the osmosis process down a little bit

**8. Do you agitate or circulate the fish and brining solution while liquid brining?**

Company 1—No
Companies 2, 3, 4, 5 and 6—Yes
If yes, what means do you use to agitate?
Companies 2, 3, 4, 5 and 6 all said *Manually, such as a paddle*

**9. How often is the brine solution changed when liquid brining?**

Company 1—After each 4 brinings
Companies 2, 3, 4, 5 and 6—With each batch

**10. Do you take any special precautions with the injection brining system to prevent Listeria (that is, changing injection needles regularly, not catching/recycling the brine, etc.)? Please describe**

Company 1—No
Company 2
Company 3
Company 4 –
Company 5—Needles are cleaned and sanitized before and after use
Company 6—Does not inject our Nova Products. Only our Kippered and nonperishable products.

**11. How do you adjust the brine strength of the liquid and/or injectable brine?**

Companies 1, 2, 3, 4, 5 and 6—With a Salometer
Company 2—By adding more salt
Company 6—By weight ratio, WPS testing on the finished product.

**12. How long does your brining treatment (please note if dry or liquid) take?**

Brining time (h)

Company 1—Brine for 10 to 15 min depending on fish

Company 2—8 to 10, 10 to 12 (Salmon N) depending on size of fillets, (Tuna) 2 hrs to 2 hrs and 15 min,

Company 3—4 to 7 d in liquid brine (Salmon, Sablefish, and Tuna)

Company 4—24 to 48 (Sable) and over 48 (Salmon)

Company 5—14 to 16 (Sablefish, Salmon, and Halibut)

Company 6—Salmon, Dry cure is 8 h to 48 h depending on species. Liquid cure is 18 to 24 h.

**13. What is the normal hanging/drying time before actual smoking?**

Hanging/Drying time (h)

Company 1—2 to 4

Company 2—No response

Company 3—0 to 2 (Salmon, Sablefish, Tuna)

Company 4—0 to 2 (Salmon and Sablefish)

Company 5—8 to 10

Company 6—0 to 2 h

**14. Please sketch below a rough picture of your brining tank(s) or truck(s).**

Are they made of:

Company 1—Stainless Steel

Company 2—Stainless Steel

Company 3—Plastic

Company 4—Stainless Steel

Company 5—Stainless Steel

Company 6—Plastic, 1000 # magnum totes

**15. What concentration of liquid and/or injectable brine do you start with?**

Company 1—30 to 40 ° Salometer

Company 2 to 55 ° Salometer

Company 3—30 to 40 ° Salometer

Company 4—50 to 60 ° Salometer

Company 5—30 to 40 ° Salometer

Company 6—60 to 70 ° Salometer

**16. By species, what liquid brine strengths do you use?**

Species Brine strength (°Salometer)

Company 1—All the same 30 to 40°

Company 2—55° (Tuna)

Company 3–30° (Salmon), 25° (Sablefish), Company 40 ° (Tuna)

Company 4—50 to 60° (Salmon)

Company 5—30 to 35% Salmon (and dry), 35% Sablefish, and Halibut (dry)

Company 6–60° Salmon

**17. Please describe how you prepare your brine.**

Company 1—Mix in a tank and add fish fillets

Company 2—Brine sinks (wet brine) water run to desired temp range—stoppered predetes volume salt added to filling sink—filled to desired level—agitated to mix salt to solution-decked with salometer—adjust if necessary.

Company 3—Clean and sanitize plastic tub or stainless tank; add 48 gallons water; add salt to product 30° salometer brine; add sugar to produce 35° salometer brine; add NO (for salmon and sable)

Company 4—No response

Company 5—A saturated brine solution is mixed with water until the desired amount/conc. Of salt is achieved (measured with a salometer).

Company 6—Take a clean plastic totes put it on a plate form scale weight out the amount of water and ingredients that are needed. After the proper amounts have been added and mixed we then check the salinity of the brine.

**18. Do you receive frozen fish as a raw material? If yes, what do you thaw the fish in and at what temperature?**

Company 1—Yes (Air Temperature)

Company 2—Yes (Air Temp. Cooler < 35 °F and Room 60 to 70 °F)

Company 3—Yes (Running Water (Temperature is usually under 60 °F)

Company 4—Yes (Water Temp. fill tank w/tap then refrigerate) and Running Water Fish not over 38 °F.)

Company 5—Yes (Water 39 to 40 °F)

Company 6—Yes, (Running Water: 40 to 60 °F)

**19. In general, how long does it take to thaw the fish?**

Company 1—12 to 18 h

Company 2—Cooler air < 38 °F and Room temperature 60 to 70 °F

Company 3—Running Water: Depends upon time of year, but generally overnight (12 to 15 h).

Company 4—In water 24 h in refrigerator then 4 to 6 h in running water

Company 5—In water, approximately 18 h

Company 6—In Running Water: 40 °F takes 8 h. 60 ° takes 4 h (500# to 600# per tote)

**20. Do you receive fresh fish as a raw material? If yes, how do you wash the fish? Please describe.**

Company 1—Yes, Chlorinated water

Company 2—Yes, Potable rinse

Company 3—Yes, in running 50 ppm chlorinated water

Company 4—Yes, Chlorine Dip then rinse

Company 5—No

Company 6—Yes, We do not wash the fish. We use only as kippered or nonperishable product.

**21. Are you subject to any special Local (city, state, or county) rules and regulations other than general sanitation standards? If yes, please describe.**

Company 1—Yes, Government of Canada C.F.I.A.—Q.M. P.R.

Company 2—NO

Company 3—Yes, New York State Code of Rules and Regulations Part 262 Fish Processing and Smoking Establishments (this is the same rule FDA has copied almost word for word).

Company 4—NO

Company 5—NO

Company 6—NO

**22. Do you take any specific precautions against Listeria during processing? Do you take any specific precautions against Listeria during cleaning & sanitation? If so, please describe.**

Company 1—NO

Company 2—Receiving specs for temperature must be less than or equal to 40 °F. Thawing when product reaches 38 °F, return to cooler.

Company 3—Processing: Chlorinated rinse water; chlorinated hand and knife dips; dips gloves and aprons are mandatory;

process schedules which specify time/temp maximums. Cleaning & Sanitizing: All equipment is cleaned and sanitized in between uses; monthly sanitizer rotation program with weekly titration monitoring; twice-weekly environmental swabbing for Listeria spp.

Company 4—Chlorine for processing, Quarternary Ammonia for Sanitizing

Company 5—Time/Temperature controls

Verifications of analytical results with incoming raw productWashed/Sanitized tanks before use

Knives are place in a bucket with sanitizer

Proper use of gloves and uniform

Limit of employee traffic through departments

Wash/Sanitize room: drains, utensils, tables, walls, product contact surface areas, personal aprons.

Company 6—We make sure there is absolutely no cross contamination from one department to another specific to utensils and equipment and employees. We also make sure there is no cross contamination from one product to another. Each department has its own color code.

**23. Do you use gravity ovens in your cold-smoking process? If yes, how many?**

Company 1—No
Company 2—No
Company 3—No
Company 4—Yes, 3
Company 5—No
Company 6—No response

**24. Do you use the newer electronic and/or microprocessor controlled ovens? If yes, how many?**

Company 1—No
Company 2—No
Company 3—Yes, 3 (1x3 cage/2x24 cage)
Company 4—No
Company 5—Yes, 6
Company 6—Yes, 4 each, 1 electronic oven with microprocessor control

**25. Do you attempt to control humidity in the cold-smoking operation? Please describe how you control the humidity?**

Company 1—Yes, Dampers
Company 2—No
Company 3—Yes, Dampers, Blowers/Air circulation, Wet Bulb, and with moist sawdust
Company 4—Blowers/Air Circulation, and with moist sawdust
Company 5—No
Company 6—Yes, Dampers, Wet Bulb, Use of Steam

**26. How do you measure the temperature during smoking?**

Company 1—With a regular thermometer
Company 2—With a thermocouple & recording device
Company 3—With a thermocouple & recording device
Company 4—With a regular thermometer
Company 5—With a regular thermometer and a thermocouple & recording device
Company 6—With a regular thermometer and thermocouple & recording device.

**27. Do you use cooling coils or some type of cooling device in the oven(s)? If so please describe the type.**

Company 1—No
Company 2—Yes, Refrigeration for drying air
Company 3—Yes, Cooling Coils
Company 4—No
Company 5—Yes, Automatic Oven Cooking System
Company 6—Yes, Stainless steel tubes

**28. How long does it take to reach proper temperature in the oven?**

Company 1—15 min
Company 2—Within 30 min of loading
Company 3-Minutes
Company 4—Cold smoking takes 10 to 12 min
Company 5—2 to 3 min (76 to 78 degrees Fahrenheit)
Company 6—No response

**29. What type of smoke do you use in your cold-smoking operation?**

Company 1—Sawdust Burning
Company 2—Natural hardwood and fruit wood smoke
Company 3—Kiln-dried commercial grade hardwood blend of maple, beech , and birch.
Company 4—Real hardwood smoke
Company 5—Wood Chips (hard wood, cube cut)
Company 6—Alder and Oak

**30. What internal temperature of the fish is your target and/or how long do you maintain the smoking at the target temperature?**

Company 1—Temperature 20 to 25 °C
Company 2—We don't target internal temp for cold-smoked products
Company 3—Salmon, max time 22 h at max temp 86 °F; Sablefish, max time 6 h at max temp 120 °F (only to Sable and Seabass.); Tuna, max time 22 h at 86 °F.
Company 4—Salmon, no target- not to exceed 90 °F and Sablefish, not to exceed 120 °F
Company 5—Salmon 78 °F, 7 to 14 h, Sablefish 78 °F, 7 to 11 h, and Halibut 78 °F 8 to 10 h.
Company 6—Salmon 4 h at 60 °F

**31. What temperature in the Smoking chamber of the Smokehouse (external to the fish) gives you the above targeted internal temperature?**

Company 1—Temperature 20 to 25 °C
Company 2—< 90 °F for cold-smoke product (kiln temp)
Company 3—When processing in a modern microprocessor-controlled convection oven, one need only monitor ambient air temperature inside the oven since the internal temperature of the fish cannot exceed the ambient oven temperature. This applies all species.
Company 4—Salmon, Ambient temp with smoldering wood fire, and Sablefish, oven temp slowly increased from 100 ° to 140 ° over several hours.
Company 5—Salmon 80 °F, Sablefish 120 °F, and Halibut 115 °F
Company 6—Salmon, 4 h at 70 °F

**32. Do you use the cooling schedule provided in the AFDO Model Code (Association of Food and Drug Officials Model Code, adopted in June, 1991)?**

Company 1—No
Company 2—Yes
Company 3—Yes
Company 4—Yes
Company 5—Yes
Company 6—No

**33. If no, please describe your cooling schedule & operation.**

Company 1—Chill Room at 0 to 2 °C for 12 to 14 h

Company 6—Nova products go immediately from the smoker into our finished product cooler, which is held below 38 °F.

**34. Do you follow either AFDO or the State of New York Guidelines?**

Company 1—Were not aware of/Do not follow either
Company 2—New York Guidelines
Company 3—State of New York
Company 4—AFDO
Company 5—AFDO
Company 6—Were not aware/Do not follow either

**35. Do you use in-plant chlorination of your water? If yes, please briefly describe where and how you use the in-plant chlorination.**

Company 1—Yes, we chlorinate well to 5 ppm

Company 2—Yes, we chlorinate a prebrine bath for raw fillets. Use an injection pump mixing chamber –10 ppm.

Company 3—Yes, Microprocessor-controlled metering system that injects desired ppm liquid chlorine into water used in thawing frozen whole fish.

Company 4—No
Company 5—No

Company 6—Yes, In our thawing totes for thawing frozen fish and in the wet or raw processing area of the plant as running water over cutting boards and on belts, etc.

**36. Do you routinely monitor the water-phase salt in the finished product?**

Company 1—No
Company 2—Yes
Company 3—Yes
Company 4—Yes
Company 5—Yes
Company 6—Yes

**37. Do you have the ability to freeze your maximum daily production? If no, what percentage can you freeze?**

Company 1—Yes, all of our products are frozen
Company 2—Yes
Company 3—No, freeze to 25%
Company 4—Yes
Company 5—Yes
Company 6—Yes

**38. Do you add nitrite to your fish? If so, please briefly describe method and amount.**

Company 1—No
Company 2—No

Company 3—Yes, Granular NO2 is dispersed in water and added to 48 gallons of brine prior to addition of fillets. Amount added depends upon time of year: during cold months 8 oz granular NO2 is added: warm months 4 oz granular NO 2 is added.

Company 4—Yes, Dissolved into Brine

Company 5—Yes, Necessary amount to achieve 170 ppm in finished product 11 oz. In 850 lbs fish/850 lbs brine.

Company 6—Yes, on some products we use wet brine with sodium nitrite.

**39. Do you measure nitrite residuals?**

Company 1—No
Company 2—No
Company 3—Yes
Company 4—No
Company 5—Yes
Company 6—Yes

**40. What technical analysis do you perform at the plant?**

Company 1—Salt/Salometer, Sensory/Organoleptic

Company 2—Salt/Salometer, Sensory/Organoleptic, Moisture, Water-phase salt, and Microbiology (send out to lab)

Company 3—Salt/Salometer, Sensory/Organoleptic
Company 4—Salt/Salometer, Sensory/Organoleptic
Company 5—Salt/Salometer, Sensory/Organoleptic

Company 6—Salt/Salometer, Sensory/Organoleptic, Moisture, Water-phase Salt and Microbiology.

**41. How often do you test/submit samples for Microbiological analysis and what do you test for?**

Company 1—Every 3 m

Company 2—Raw product—monthly (salmon), and Environmentals-weekly

Company 3—Minimum of twice weekly we test cold-smoked product for total aerobic plate count, coliform count, *Listeria monocytogenes*, water phase salt content. Minimum of 4 times yearly we test finished cold-smoked product (that contains nitrate) for ppm NO2.

Company 4—Approximately once per quarter, Salt, Nitrate, Water phase salt, Listeria, Salmonella.

Company 5—Once a year for each type of fish or more as neededÔ wps % nitrate, histamine, TPC, *C. botulinum*, *L. monocytogenes*, mold, and yeast.

Company 6—We are required to test, in our finished products, for WPS and residual nitrite levels 4 times a year. Any raw material that is going to be processed into a finished Nova product we do full micro screens. Also, we do full microscreens on 100% of our finished nova products and once a year on our kippered and nonperishable products.

**42. Do you use the services of an outside laboratory?**

All 6 Companies said Yes

**43. Do you slice any of your cold-smoked products? If yes, do you follow any specialized cleaning & sanitation procedures in this area or with this equipment? Please describe.**

Company 1—No
Company 2—No response

Company 3—Yes, at end of the production day equipment is first cleaned with soap and scurb brushes and then washed down with hot water. Then a 150 to 250 ppm chlorine solutions is sprayed onto all surfaces and allowed to sit for 5 min. Then a 200 ppm quat solution is foamed onto the equipment and allowed to air-dry overnight. Prior to start-up the following morning, all surfaces are briefly washed down with water to reactivate the quat. A monthly rotation program is in place including an acid-based quat. Strength of quat is determined and monitored by in-house titration.

Company 4—Yes, Machines are taken apart and cleaned and sanitized daily.

Company 5—Yes, Scheduled cleaning and sanitizing before and after use and between species of fish. Slicing is conducted in a temperature controlled room. Proper use of uniforms and good manufacturing practices.

Company 6—Yes. No, just our regular cleaning and sanitizing schedules before we start work and at employee breaks and lunch time. This is including any cleaning the equipment re-

ceives by the sanitation crew.

**44. Are your coolers, freezers, or other cold storage areas equipped with some type of temperature indicating device?**

All 6 Companies said Yes

**45. Are your coolers, freezers, or other cold storage areas equipped with some type of temperature indicating *and monitoring* device?**

Companies 1, 2, 3, 5 and 6 said Yes

Company 4 said No

**46. Do you slice any of your product after smoking? If so, do you take any special precautions against Listeria (that is, changing blades regularly, cleaning & sanitizing several times per shift, etc.)? Please describe**

Company 1—No

Company 2—No response

Company 3—Yes (see item #43)

Company 4—Yes, cleaning and sanitizing

Company 5—Yes, Equipment is inspected for proper sanitation and cleaning before use.

Company 6—Yes, (No)

**47. Do you vacuum-package any of your cold-smoked product? If yes, please describe the type of packaging.**

Company 1—Yes, Shrink-Wrap

Company 3—Pouch, Skin-Pack, and Shrink-Wrap

Companies 2, 4, 5 and 6—Yes, Pouch

**48. Please describe the kind of film you use with the vacuum-packed cold-smoked product. Is it oxygen permeable or impermeable, and any other characteristics or dynamics?**

Companies 1 & 6—Oxygen Impermeable

Company 2—Oxygen Permeable, our bag manufacturer has given us an oxygen permeability rating of approximately 605 cc/m, 2 to 24 at 20 °C, 0%.

Company 3—Oxygen Permeable

Company 4—No response

Company 5—Oxygen Permeable, 3.9cc/100, S.I./24 hrs., Thickness 3+/–0.3 Mics.

**49. How is the vacuum packed cold-smoked product distributed? Please describe including temperature limits and parameters, how is it shipped, etc.**

Company 1—Frozen at a temper of 18 °C Cooler

Company 2—Refrigerated trailers < 38 °F, Coolers < 38 °F.

Company 3—The product is distributed both frozen and refrigerated. When refrigerated the maximum storage temperature is 38 °F. We ship via our own trucks, common carrier, and air.

Company 4-Frozen

Company 5—Refrigerated < 38 °F, Frozen ~0 °F for extended shelf life.

Company 6—Frozen at–10 °F

**50. Is any of your cold-smoked product stored and distributed frozen? If yes, please describe.**

Company 1—Yes, 100% Frozen

Company 2—Yes, Portions of all cold-smoked product may be frozen

Company 3—Yes (see item #49)

Company 4—Yes

Company 5—Yes, for distributors, retailers, etc with the appropriate thawing procedures.

Company 6—Yes, All of our products, once vacuum sealed, are blast frozen at –40 °F. Then they are labeled, boxed and then held in our finished product freezer at 0 °F until they are shipped to a cold storage and held at –10 °F for final distribution on a frozen carrier.

**51. Other than vacuum packaging, how do you package your cold-smoked product? Please describe cartons, wraps, films, etc.**

Company 1—No response

Company 2—Small percentage of whole fillets is paper wrapped. All product is shipped-waxed, cardboard boxes.

Company 3—Whole sides are air-packed in bulk. Sliced product in retail sized unites are overwrapped (air-packed) styrofoam boats.

Company 4—Poly bags inside corrugated cartons

Company 5—No response

Company 6—Our products are labeled both on the back and front of the package along with all master cases.

**52. Is the smoking and packing/storing of the final product carried out in rooms separate from other processing and handling operations?**

Company 1—No

Company 2—Yes

Company 3 –Yes

Company 4—Yes, Smoking and storing are separate. Some packing is done in the same room as processing, but not at the same time due to spare restraints.

Company 5—Yes

Company 6—Yes

**53. Do you use temperature indicators and/or "Sell By" dates on your finished products? If so, please describe.**

Company 1—Start at 18 °C or colder. Product has to be "COOKED BEFORE EATING" on label.

Company 2—Sell by dates: 21 d

Company 3—Sell by dates

Company 4—"Sell By" dates- Are used at customer request otherwise product is sold frozen with no date.

Company 5—"Sell By" dates

Company 6—"Sell by" dates, All of our products have either a small white sticker or a stamp located on the back left hand side of the package that indicates the shelf life of the product. Either saying (Use By Date) or (Sell By Date).

**54. What do you consider your shelf life to be?**

Company 1—All product 18 m

Company 2—No response

Company 3—Salmon: Vacuum-packed 35 d at max 38 °F/air-packed 10 to 14 d max 38 °F. Sablefish: vacuum packed 5 to 7 d at max 38 °F/air-packed at max 38 °F/Tuna: vacuum-packed 35 d at max 38 °F/air-packed 10 to 14 d at max 38 °F.

Company 4—Salmon: 4 wk, Sablefish: 2 wk

Company 5—90 d (Salmon, Sablefish, and Tuna) vacuum packed

Company 6—Salmon, 21 d no preservatives and 60 d with preservatives

**55. What is your shelf life considered to be if the product is initially distributed frozen and thawed at retail?**

Company 1—No response

Company 2—21 d from thaw date.

Company 3—Salmon: vacuum-packed 60 d; Tuna: vacuum-packed 60 d

Company 4—Salmon: 4 wk, Sablefish: 2 wk

Company 5—90 d after thawed at retail. If distributed frozen 120 d

Company 6—Same as above

**56. Please give us an estimate of your annual production (pounds per year)**

Company 1—50 to 100000 lbs

Company 2—No response

Company 3—Over 500000 lbs.

Company 4—50 to 100000 lbs (Salmon 80000 lbs raw product, and Sable 1000 lbs raw product).

Companies 5 & 6—Over 500000 lbs

## References

[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. [York (PA)]: Association of Food and Drug Officials. 7 p.

Corby JJ. 1991. Circular 102 Rules and regulations relating to fish processing and smoking establishments pursuant to Article 17 of the Agriculture and Markets Law. Albany, NY: New York State Department of Agriculture and Markets, Division of Food Inspection Services. Part 262 of Title 1 of the Official Compiliation of Codes, Rules, and Regulations of the state of New York.

# List of References


Ababouch L, Afilal ME, Benabdeljelil H, Busta FF. 1991. Quantitative changes in bacteria, amino acids and biogenic amines in sardine (*Sardina pilchardus*) stored at ambient temperature (25–28° C) and in ice. Int J Food Sci Tech 26:297–306.

Ababouch L, Afilal ME, Rhafiri S, Busta FF. 1991. Identification of histamine-producing bacteria isolated from sardine (*Sardina pilchardus*) stored in ice and at ambient temperature (25° C). Food Microbiol 8:127–36.

Abrahamsson K, De Silva NN, Molin N. 1965. Toxin production by *Clostridium botulinum*, type E, in vacuum-packed, irradiated fresh fish in relation to the changes to the associated microflora. Can J Microbiol 11:523–9.

[ACMSF] Advisory Committee on the Microbiological Safety of Foods. 1992. Report on vacuum packaging and associated processes. London (UK): Her Majesty's Stationery Office.

Adams AM, Rausch RL. 1997. Diphyllobothriasis. In: Conner DH, Chandler FW, Schwartz DA, Manz HJ, Lack EE, editors. Volume 2, Pathology of infectious diseases. Stamford (CN): Appleton and Lange. p 1377–90.

[AFDO] Association of Food and Drug Officials. 1990. Retail guidelines for refrigerated food in reduced oxygen packages. J Assoc Food Drug Of 54(5):80–4.

[AFDO] Association of Food and Drug Officials. 1991 June. Cured, salted, and smoked fish establishments good manufacturing practices [model code]. [York (PA)]: Association of Food and Drug Officials. 7 p.

Aiiso K, Toyoura H, Iida H. 1958. Distribution and activity of histidine decarboxylase in *Morganella*. Jap J Microbiol 2(2):143–7.

Aksnes A. 1988. Location of enzymes responsible for autolysis in bulk-stored capelin (*Mallotus villosus*). J Sci Food Agric 44:263–71.

Aksnes A, Brekken B. 1988. Tissue degradation, amino acid liberation and bacterial decomposition of bulk stored capelin. J Sci Food Agric 45:53–60.

Angot V, Brasseur P. 1993. European farmed Atlantic salmon (*Salmo salar L.*) are safe from *Anisakid* larvae. Aquaculture 118:339–44.

Anonymous. 1985. Botulism associated with commercially distributed Kapchunka-New York City. MMWR 34(35):546–7.

Anonymous. 1987. International outbreak of type E botulism associated with ungutted, salted whitefish. MMWR 36(49):812–3.

Anonymous. 1995. International forum supports above-zero tolerance for *Listeria* in low risk foods. World Food Chem News July 26:15–7.

[AOAC] Association of Official Analytical Chemists. 1995. FDA Bacteriological Analytical Manual (BAM), 8th ed. Gaithersburg (MD): AOAC Int.

Arnold SH, Brown WD. 1978. Histamine (?) toxicity from fish products. Adv Food Res 24:113–54.

Arnold SH, Price RJ, Brown WD. 1980. Histamine formation by bacteria isolated from skipjack tuna, *Katsuwonus pelamis*. Bull Jap Soc Sci Fish 46(8):991–5.

Audicana L, Audicanna MT, Fernandez de Corres L, Kennedy MW. 1997. Cooking and freezing may not protect against allergenic reactions to ingested *Anisakis simplex* antigens in humans. The Veterinary Record 140:235.

Autio T, Hielm S, Miettinen M, Sjoberg A-M, Aarnisalo K, Bjorkroth J, Mattila-Sandholm T, Korkeala H. 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Appl Environ Microbiol 65(1):150–5.

Aytac SA, Ozbas ZY, Vural H. 2000. Effects of irradiation, antimicrobial agents and modified-atmosphere packaging on histamine production by *Morganella morganii* in mackerel fillets. Archiv Fur Lebensmittelhygiene 51:28–30.

Barakat RK, Harris LJ. 1999. Growth of *Listeria monocytogenes* and *Yersinia enterocolitica* on cooked modified-atmosphere-packaged poultry in the presence and absence of a naturally occurring microbiota. Appl Environ Microbiol 65(1):342–5.

Baranowski JD, Frank HA, Brust PA, Chongsiriwatana M, Premaratne RJ. 1990. Decomposition and histamine content in mahimahi (*Coryphaena hippurus*). J Food Prot 53(3):217–22.

Beard TD. 1991. HACCP and the home: the need for consumer education. Food Technol 45(4):123–4.

Behling AR, Taylor SL. 1982. Bacterial histamine production as a function of temperature and time of incubation. J Food Sci 47:1311–4, 1317.

Ben Embarek PK. 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. Int J Food Microbiol 23:17–34.

Ben Embarek PK, Huss HH. 1992. Growth of *Listeria monocytogenes* in lightly preserved fish products. In: Huss HH, Jakobsen M, Liston J, editors. Quality assurance in the fish industry. Amsterdam: Elsevier. p 293–303.

Ben Embarek PK, Huss HH. 1993. Heat resistance of *Listeria monocytogenes* in vacuum packaged pasteurized fish fillets. Int J Food Microbiol 20:85–95.

Ben-Gigirey B, Craven C, An H. 1998. Histamine formation in albacore muscle analyzed by AOAC and enzymatic methods. J Food Sci 63(2):210–4.

Ben-Gigirey B, Vieites Baptista de Sousa JM, Villa TG, Barros-Velazquez J. 1998. Changes in biogenic amines and microbiological analysis in albacore (*Thunnus alalunga*) muscle during frozen storage. J Food Prot 61(5):608–15.

Ben-Gigirey B, Vietites Baptista de Sousa JM, Villa TG, Barros-Velazquez J. 1999. Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (*Thunnus alalunga*). J Food Prot 62(8):933–9.

Beverley-Burton M, Pippy JHC. 1978. Distribution, prevalence and mean numbers of larval *Anisakis simplex* (Nematoda: *Ascaridoidea*) in Atlantic salmon, *Salmo salar L.* and their use as biological indicators of hosts stocks. Environ Biol Fish 3:211–22.

Bhaibulaya M. 1985. Effect of gamma ray on the metacercarie of liver fluke (*Opisthorchis viverrini*) infective stages of parasite caused by consumption of raw or semiprocessed fish. Fd Irradiat Newsl 9(2):8.

Bier JW. 1976. Experimental *Anisakis*: cultivation and temperature tolerance determination. J Milk Food Technol 39:132.

Bjeldanes LF, Schutz DE, Mooris MM. 1978. On the aetiology of scombroid poisoning: cadaverine potentiation of histamine toxicity in the guinea-pig. Food Cosmet Toxicol 16(2):157–9.

Bremer PJ, Osborne CM. 1998. Reducing total aerobic counts and *Listeria monocytogenes* on the surface of king salmon (*Oncorhynchus tshawytscha*). J Food Prot 61:849–54.

Brett MSY, Short P, McLauchlin J. 1998. A small outbreak of listeriosis associated with smoked mussels. Int J Food Microbiol 43:223–9.

Bristow GA, Berland B. 1991. A report on some metazoan parasites of wild marine salmon (*Salmo salar L.*) from the west coast of Norway with comments on their interactions with farmed salmon. Aquaculture 98:311–8.

Buchanan RL, Damert WG, Whiting RC, van Schothorst M. 1997. Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for *Listeria monocytogenes* levels and incidence of listeriosis. J Food Prot 60(8):918–22.

Buchanan RL, Klawitter LA. 1992. Effectiveness of *Carnobacterium piscicola* LK5 for controlling the growth of *Listeria monocytogenes* Scott A in refrigerated foods. J Food Saf 12:219–36.

[CAC] Codex Alimentarius Commission. 1979. Recommended International Code of Practice for Smoked Fish. Rome: Codex Alimentarius Commission. CAC/RCP 25–1979.

Cann DC, Taylor LY. 1979. The control of the botulism hazard in hot-smoked trout and mackerel. J Food Technol 14:123–9.

Cann DC, Taylor LY, Hobbs G. 1975. The incidence of *Clostridium botulinum* in farmed trout raised in Great Britain. J Appl Bacteriol 39:331–6.

Cann DC, Wilson BB, Shewan JM, Hobbs G. 1966. Incidence of *Clostridium botulinum* type E in fish products in the United Kingdom. In: Nature. p 205–6.

Ching HL. 1984. Fish tapeworm infections (Diphyllobothriasis) in Canada, particularly British Columbia. Can Med Assoc J 130:1125–8.

Chu C-H, Bjeldanes LF. 1981. Effect of diamines, polyamines and tuna fish extracts on the binding of histamine to mucin in vitro. J Food Sci 47:79-?

Civera T, Parisi E, Amerio GP, Giaccone V. 1995. Shelf-life of vacuum-packed smoked salmon: microbiological and chemical changes during storage. Arch Lebensmittelhyg 46:13–7.

Clifford MN, Walker R, Ijomah P, Wright J, Murray CK, Hardy R. 1991. Is there a role for amines other than histamines in the aetiology of scombrotoxicosis. Food Addit Contam 8(5):641–52.

Clifford MN, Walker R, Wright J, Hardy R, Murray CK. 1989. Studies with volunteers on the role of histamine in suspected scombrotoxicosis. J Sci Food Agric 47:365–75.

Corby JJ. 1991. Circular 102 Rules and regulations relating to fish processing and smoking establishments pursuant to Article 17 of the Agriculture and Markets Law. Albany, NY: New York State Department of Agriculture and Markets, Division of Food Inspection Services. Part 262 of Title 1 of the Official Compiliation of Codes, Rules, and Regulations of the state of New York.

Cortesi ML, Sarli T, Santoro A, Murru N, Pepe T. 1997. Distribution and behavior of *Listeria monocytogenes* in three lots of naturally-contaminated vacuum-packed smoked salmon stored at 2 and 10° C. Int J Food Microbiol 37:209–14.

Crapo C, Himelbloom B. 1999. Spoilage and histamine in whole Pacific herring (*Clupea harengus pallasi*) and pink salmon (*Oncorhynchus gorbuscha*) fillets. J Food Safety 19:45–55.

Crapo CA, Elliot E. 1987. Salmon quality: the effect of elevated refrigerated seawater chilling temperature. Fairbanks (AK): University of Alaska, SeaGrant College Program.

Crapo CA, Kramer DE, Doyle JP. 1986. Salmon quality: the effect of delayed chilling. Fairbanks (AK): University of Alaska, SeaGrant College Program.

Crapo CA, Paust B. 1987. Air shipment of fresh fish: a primer for shippers and cargo handlers. Fairbanks (AK): University of Alaska, SeaGrant College Program.

Crompton DWT, Joiner SM. 1980. Parasitic Worms. London: Wyeham Publications.

Cuppett SL, Gray JI, Pestka JJ, Booren AM, Price JF, Kutil CL. 1987. Effect of salt level and nitrite on toxin production by *Clostridium botulinum* type E spores in smoked great lakes whitefish. J Food Prot 50(3):212–7.

Dalgaard P, Jorgensen LV. 1998. Predicted and observed growth of *Listeria monocytogenes* in seafood challenge tests and in naturally contaminated cold-smoked salmon. Int J Food Microbiol 40:105–15.

Dauphin G, Ragimbeau C, Malle P. 2001. Use of PFGE typing for tracing contamination with *Listeria monocytogenes* in three cold-smoked salmon processing plants. Int J Food Microbiol 64:51–61.

Dawood AA, Karkalas J, Roy RN, Williams CS. 1988. The occurrence of nonvolatile amines in chilled-stored rainbow trout (*Salmo irideus*). Food Chem 27:33–45.

De Martinis ECP, Franco BDGM. 1998. Inhibition of *Listeria monocytogenes* in a pork product by a *Lactobacillus sake* strain [research communication]. Int J Food Microbiol 42:119–26.

Deardorff TL, Kent ML. 1989. Prevalence of larval *Anisakis simplex* in pen-reared and wild-caught salmon (Salmonidae) from Puget Sound, Washington. J Wildl Dis 25:416–9.

Deardorff TL, Overstreet RM. 1990. Seafood-transmitted zoonoses in the United States: the fishes, the dishes and the worms. In: Ward DR, Hackney CR, editors. Microbiology of marine food products. New York: Van Nostrand Reinhold. p 211–65.

Deardorff TL, Throm R. 1988. Commercial blast-freezing of third-stage *Anisakis simplex* larvae encapsulated in salmon and rockfish. J Parasit 74(4):600–3.

Declerck D. 1988. Presence de larves de *Anisakis* simplex dans le hareng (*Clupea harengus L.*). Revue de l'Agriculture 41(4):971–8.

Destro MT, Leitao MFF, Farber JM. 1996. Use of molecular typing methods to trace the dissemination of *Listeria monocytogenes* in a shrimp processing plant. Appl Environ Microbiol 62(2):705–11.

Dillon R, Patel T, Ratnam S. 1994. Occurrence of *Listeria* in hot and cold-smoked seafood products. Int J Food Microbiol 22:73–7.

Dodds KL. 1993. *Clostridium botulinum* in the environment. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum*: ecology and control in foods. New York: M Dekker. p 21–

52.
Dodds KL, Austin JW. 1997. *Clostridium botulinum*. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food Microbiology. Fundamentals and Frontiers: American Society for Microbiology. p 288–304.
Dorsa WJ, Marshall DL, Semien M. 1993. Effect of potassium sorbate and citric acid sprays on growth of *Listeria monocytogenes* on cooked crawfish (*Procambarus clarkii*) tail meat at 4° C. Lebensm Wiss u Technol 26:480–2.
Douglas WW. 1970. Histamine and antihistamines; 5-Hydroxytryptamine and antagonists. In: Goodman LS, Gilman A, editors. The pharmacological basis of therapeutics. 5th ed. New York: Macmillan. p 621–62.
Duffes F, Corre C, Leroi F, Dousset X, Boyaval P. 1999. Inhibition of *Listeria monocytogenes* by in situ produced and semipurified bacteriocins on *Carnobacterium* spp. on vacuum-packed, refrigerated cold-smoked salmon. J Food Prot 62(12):1394–1403.
Dufresne I, Smith JP, Liu JN, Tarte I, Blanchfield B, Austin JW. 2000. Effect of films of different oxygen transmission rate on toxin production by *Clostridium botulinum* type E in vacuum packaged cold and hot-smoked trout fillets. J Food Saf 20:251–68.
Eastburn RL, Fritsche TR, and others. 1987. Human intestinal infection with *Nanophyetus salmincola* from salmonoid fishes. Am J Tropical Medicine and Hygiene 36(3):586–91.
[EC] European Commission. 1998. Scientific committee on veterinary measures relating to public health. Allergic reactions to ingested *Anisakis simplex* antigens and evaluation of the possible risk to human health.
Edmunds WJ, Eitenmiller RR. 1975. Effect of storage time and temperature on histamine content and histidine decarboxylase activity of aquatic species. J Food Sci 40:516–9.
EEC. 1991. Council directive 91/493/EEC of 22nd July 1991 laying down the health conditions for the production and the placing on the market of fishery products. Off J Eur Comm(Nr L268):15–32.
Eitenmiller RR, Wallis JW, Orr JH, Phillips RD. 1981. Production of histidine decarboxylase and histamine by *Proteus morganii*. J Food Prot 44(11):815–20.
Eklund M. 1984. Effect of CO2 modified atmospheres and vacuum packaging on *Clostridium botulinum* and spoilage organisms of fishery products. Published in: Proceedings of First National Conference on Seafood Packaging and Shipping; 1982 Nov 15–17 [Washington, DC] and 1982 Dec 7–9 [Seattle, WA]. p 298–331.
Eklund M. 1989. Comments and research data for the proposed establishment of standards for the manufacture, packaging, and labeling of processed fish including smoked fish [testimony to the New York Department of Agriculture and Markets]. [New York]: Northwest Fisheries Center, Utilization Research Division.
Eklund M, Pelroy G, Poysky F, Paranjpye R, Lashbrook L, Peterson M. 1993 July. Summary of interim guidelines for reduction and control of *Listeria monocytogenes* in or on smoked fish [internal report]. Seattle: Northwest Fisheries Science Center. July 1993. 14 p.
Eklund MW. 1992. Control in fishery products. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum*: ecology and control in foods. New York: M Dekker. p 209–32.
Eklund MW, Pelroy GA, Paranjpye R, Peterson ME, Teeny FM. 1982. Inhibition of *Clostridium botulinum* types A and E toxin production by liquid smoke and NaCl in hot-process smoked-flavored fish. J Food Prot 45(10):935–41.
Eklund MW, Peterson ME, Paranjpye R, Pelroy GA. 1988. Feasibility of a heat-pasteurization process for the inactivation of nonproteolytic *Clostridium botulinum* types B and E in vacuum-packaged, hot-process (smoked) fish. J Food Prot 51(9):720–6.
Eklund MW, Poysky F. 1967. Incidence of *Cl. botulinum* type E from the pacific coast of the United States. In: Ingram M, Roberts TA, editors. Botulism 1966. [unknown]: Chapmann and Hall.
Eklund MW, Poysky FT, Paranjpye RN, Lashbrook LC, Peterson ME, Pelroy GA. 1995. Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. J Food Prot 58(5):502–8.
El-Kest SE, Yousef AE, Marth EH. 1991. Fate of *Listeria monocytogenes* during freezing and frozen storage. J Food Sci 56(4):1068–71.
Elliot EL, Kvenberg JE. 2000. Risk assessment used to evaluate the US position on *Listeria monocytogenes* in seafood. Int J Food Microbiol 62:253–60.
El-Shenawy MA, Marth EH. 1988. Inhibition and inactivation of *Listeria monocytogenes* by sorbic acid. J Food Prot 51:842–7.
Emodi AS, Lechowich RV. 1969. Low temperature growth of type E *Clostridium botulinum* spores. 1. Effects of sodium chloride, sodium nitrite and pH. J Food Sci 34:78–81.
Ericsson H, Eklow A, Danielsson-Tham ML, Loncarevic S, Mentzing LO, Persson I, Unnerstad H, Tham W. 1997. An outbreak of listeriosis suspected to have been caused by rainbow trout. J Clin Microbiology 35(11):2904–7.
Fan PC. 1998. Viability of metacercariae of *Clonorchis sinensis* in frozen or salted freshwater fish. Int J Parasitol 28:603–5.
[FAO] Food and Agriculture Organization. 1999 May. Report of the FAO expert consultation on the trade impact of *Listeria* in fish products. Rome: FAO. FAO Fisheries Report nr 604. 34 p.
FAO/IAEA. 1992. Final FAO/IAEA research co-ordination meeting on the use of irradiation to control infectivity of foodborne parasites. Food Irradiation Newsletter 16(1):5–14.
Farber JM. 1991. *Listeria monocytogenes* in fish products. J Food Prot 54(12):922–4, 934.
Farber JM. 2000. Present situation in Canada regarding *Listeria monocytogenes* and ready-to-eat seafood products. Int J Food Microbiol 62:247–51.
Farber JM, Peterkin PI. 1991. *Listeria monocytogenes*, a foodborne pathogen. Microbiol Rev 55:476–511.
Farber JM, Peterkin PI. 2000. *Listeria monocytogenes*. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of foods. Gaithersburg (MD): Aspen. p 1178–1232.
Farber JM, Ross WH, Harwig J. 1996. Health risk assessment of *Listeria monocytogenes* in Canada. Int J Food Microbiol 30:145–56.
[FDA] Food and Drug Administration. 1996. Fish & fisheries products hazards & controls guide: first edition. Washington D.C.: FDA, Center for Food Safety and Applied Nutrition, Office of Seafood.
[FDA] Food and Drug Administration. 1998. Fish & Fisheries Products Hazards & Controls Guide. 2nd ed. Washington, D.C.: FDA, Office of Seafood. 276 p.
[FDA] Food and Drug Administration. 1999. Food Code. Washington, DC: U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration.
Fenlon DR, Wilson J, Donachie W. 1996. The incidence and level of *Listeria monocytogenes* contamination of food sources at primary production and initial processing. J Appl Bacteriol 81:641–50.
Fernandez PS, George SM, Sills CC, Peck MW. 1997. Predictive model of the effect of CO2, pH, temperature and NaCl on growth of *Listeria monocytogenes*. Int J Food Microbiol 37:37–45.
Fernandez-Salguero J, Mackie IM. 1979. Histidine metabolism in mackerel (*Scomber scombrus*). Studies on histidine decarboxylase activity and histamine formation during storage of flesh and liver under sterile and nonsterile conditions. J Food Technol 14:131–9.
Fernandez-Salguero J, Mackie IM. 1987. Comparative rates of spoilage of fillets and whole fish during storage of haddock (*Melanogrammus aeglefinus*) and herring (*Clupea harengus*) as determined by the formation of nonvolatile and volatile amines. Int J Food Sci Tech 22:385–90.
Fernandez-Salguero J, Mackie IM. 1987. Technical note: Preliminary survey of the content of histamine and other higher amines in some samples of Spanish canned fish. Int J Food Sci Tech 22:409–12.
Fletcher GC, Summers G, Winchester RV, Wong RJ. 1995. Histamine and histidine in New Zealand marine fish and shellfish species, particularly Kahawai (*Arripis trutta*). J Aquat Food Prod Technol 4(2):53–74.
Fonnesbech Vogel B, Jorgensen LV, Ojeniyi B, Huss HH, Gram L. 2001. Diversity of *Listeria monocytogenes* isolates from cold-smoked salmon produced in different smoke houses as assessed by randomly amplified polymorphic DNA analyses. Int J Food Microbiol 65:83–92.
Fonnesbech Vogel B, Ojeniyi B, Ahrens P, Due Skov L, Huss HH, Gram L. 2001. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Appl Enviro Microbiol. Forthcoming.
Food Chemical News. 1975 October 27. FDA-ER cautions that *Anisakine* nematodes can survive freezing. Food Chemical News:44–5.
Frank HA, Baranowski JD, Chongsiriwatana M, Brust PA, Premaratne RJ. 1985. Identification and decarboxylase activities of bacteria isolated from decomposed mahimahi (*Coryphaena hippurus*) after incubation at 0 and 32° C. Int J Food Microbiol 2:331–40.
Fujii T, Kurihara K, Okuzumi M. 1994. Viability and histidine decarboxylase activity of halophilic histamine-forming bacteria during frozen storage. J Food Prot 57(7):611–3.
Gale EF. 1946. The bacterial amino acid decarboxylases. Adv Enzymology and Related Subjects of Biochemistry 6:1–32.
Gardiner MA. 1990. Survival of *Anisakis* in cold-smoked salmon. Can Inst Food Sci Technol 23(2/3):143–4.
Garrett ES. 1987. Testimony before the committee on Agriculture, Nutrition, and Forestry. Washington, DC: United States Senate. June 11, 1987. Report nr S1813.
Gessner BD, Hokama Y, Isto S. 1996. Scombrotoxicosis-like illness following the ingestion of smoked salmon that demonstrated low histamine levels and high toxicity on mouse bioassay. Clinical Infectious Diseases 23:1316–8.
Gilbert J, Knowles M. 1975. The chemistry of smoked foods: a review. J Food Technol 10:245–61.
Gildberg A. 1978. Proteolytic activity and the frequency of burst bellies in capelin. J Food Technol 13:409–16.
Gingerich TM, Lorca T, Flick GJ, Pierson MD, McNair HM. 1999. Biogenic amine survey and organoleptic changes in fresh, stored, and temperature-abused bluefish (*Pomatomus saltatrix*). J Food Prot 62(9):1033–7.
Giovannacci I, Ragimbeau C, Queguiner S, Salvat G, Vendeuvre J-L, Carlier V, Ermel G. 1999. *Listeria monocytogenes* in pork slaughtering and cutting plants use of RAPD, PFGE, and PCR-REA for tracing and molecular epidemiology. Int J Food Microbiol 53(1999):127–40.
Gloria MBA, Daeschel MA, Craven C, Hilderbrand Jr. KS. 1999. Histamine and other biogenic amines in albacore tuna. J Aqu Food Prod Technol 8(4):54–69.
Goldsmid JM, Speare R. 1997. The parasitology of foods. In: Hocking AD, Arnold G, Jenson I, Newton K, Sutherland P, editors. Foodborne microorganisms of public health significance. 5th ed. North Sydney, NSW: Aust Inst Food Sci and Technol Inc (NSW Branch) Food Microbiol Group. p 583–602.
Graham AF, Mason DR, Maxwell FJ, Peck MW. 1997. Effect of pH and NaCl on growth from spores of nonproteolytic *Clostridium botulinum* at chill temperature. Letts Applied Microbiol 24:95–100.
Graham AF, Mason DR, Peck MW. 1996. Inhibitory effect of combinations of heat treatment, pH, and sodium chloride on growth from spores of nonproteolytic *Clostridium botulinum* at refrigeration temperature. Appl Environ Microbiol 62(7):2664–8.
Graham AF, Mason DR, Peck MW. 1996. Predictive model of the effect of temperature, pH, and sodium chloride on growth from spores of nonproteolytic *Clostridium botulinum*. Int J Food Microbiol 31:69–85.
Gram L, Huss HH. 2000. Fresh and processed fish and shellfish. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Gaithersburg (MD): Aspen. p 472–506.
Gram L, Trolle G, Huss H. 1987. Detection of specific spoilage bacteria from fish stored at low (0° C) and high (20° C) temperature. Int J Food Microbiol 4:65–72.
Granerus G. 1968. Effects of oral histamine, histidine, and diet on urinary excretion of histamine, methylhistamine, and 1-methyl–4-imidazoleacetic acid in man. Scand J Clin Lab Invest Suppl 10(4):49–58.
Gustafson PV. 1953. The effect of freezing on encysted *Anisakis* larvae. J Parasitol 39:585–8.
Haaland H, Arnesen E, Njaa LR. 1990. Amino acid composition of whole mackerel (*Scomber scombrus*) stored anaerobically at 20° C and at 2° C. Int J Food Sci Tech 25:82–7.
Halasz A, Barath A, Simon-Sarkadi L, Holzapfel W. 1994. Biogenic amines and their production by microorganisms in food. Trends Food Sci Technol 5:42–9.
Hamed MGE, Elias AN. 1970. Effect of food processing methods upon survival of the trematode *Heterophyes* sp. in flesh of mullet caught from brackish Egyptian waters. J Food Sci 35:386.
Hardy R, Smith JGM. 1976. The storage of mackerel (*Scomber scombrus*). Development of histamine and rancidity. J Sci Food Agric 27:595–9.
Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, Hofmann EE, Lipp EK, Osterhaus ADME, Overstreet RM and others. 1999. Emerging marine diseases—climate links and anthropogenic factors. Science 285:1505–10.
Hauck AK. 1977. Occurrence and survival of the larval nematode *Anisakis* sp. in the flesh of fresh, frozen, brined, and smoked pacific herring, *Clupea harengus pallasi*. J Parasitol 63(3):515–9.
Hayunga EG. 1997. Helminths acquired from finfish, shellfish, and other food sources. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington D.C.: ASM Press. p 463–76.
Health Canada. 1994 Aug 16 [updated 1997 Mar 19]. Food and drugs act and regulations: division 21-prepared fish (B.21.025) [online publication-not the official Canada Gazette pages]. Available from: Health Canada's Food Directorate web site at http://www.hc-sc.gc.ca/food-aliment/english/publications/acts_and_regulations/food_and_drugs_acts/d-text–2.pdf
Heinitz ML, Johnson JM. 1998. The incidence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* in smoked fish and shellfish. J Food Prot 61(3):318–23.
Hendricks MT, Hotchkiss JH. 1997. Effect of carbon dioxide on the growth of *Pseudomonas fluorescens* and *Listeria monocytogenes* in areobic atmospheres. J Food Prot 60(12):1548–52.
Henry Chin KD, Koehler PE. 1986. Effect of salt concentration and incubation temperature on formation of histamine, phenethylamine, tryptamine and tyramine during miso fermentation. J Food Prot 49(6):423–7.
Hilderbrand K. 1973. Preparation of salt brines for the fishing industry. Corvallis: Oregon

State Univ., Oregon Sea Grant Program (#OSU SG 22). Report nr NSGL# ORESU-G–73-002. Grant nr NOAA–73072505. 4 p.
Hildrum KI, Scanlan RA, Libbey LM. 1976. Nitrosamines from the nitrosation of spermidine and spermine. In: Walker EA, Bogovski P, Griciute L, editors. Environmental N-Nitroso Compounds analysis and formation: proceedings of a working conference; 1975 Oct 1–3 1976; Polytechnical Institute, Tallinn, Estonian SSR. International Agency for Research on Cancer. p 205–14.
Hood SK, Zottola EA. 1997. Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int J Food Microbiol 37:145–53.
Houwing H. 1969. The inactivation of herring nematodes (*Anisakis marina*) by freezing. Bull Int Inst Refrig, Annexe 1969–6:297–302.
Hu AC, Shelef LA. 1996. Influence of fat content and preservatives on the behavior of *Listeria monocytogenes* in beaker sausage. J Food Saf 16:175–81.
Hudson JA, Mott SJ. 1993. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cold-smoked salmon under refrigeration and mild temperature abuse. Food Microbiol 10:61–8.
Hui JY, Taylor SL. 1983. High pressure liquid chromatographic determination of putrefactive amines in foods. J AOAC 66(4):853–7.
Hungerford JM, Arefyev AA. 1992. Flow-injection assay of enzyme inhibition in fish using immobilized diamine oxidase. Analytica Chimica Acta 261:351–9.
Huss HH. 1980. Distribution of *Clostridium botulinum*. Appl Environ Microbiol 39:764–9.
Huss HH. 1981. *Clostridium botulinum* type E and botulism [DSci thesis]. Lyngby (DK): Technical University, Technological Laboratory of the Ministry of Fisheries. 58 p.
Huss HH, Ben Embarek PK, From Jeppesen V. 1995. Control of biological hazards in cold-smoked salmon production. Food Control 6(6):335–40.
Huss HH, Pedersen A, Cann DC. 1974. The incidence of *Cl. botulinum* in Danish trout farms. II: Measures to reduce the contamination of the fish. J Food Technol 9:451–8.
Huss HH, Petersen ER. 1980. The stability of *Clostridium botulinum* type E toxin in salty and/or acid environment. J Food Technol 15:619–27.
Huss HH, Schaeffer I, Pedersen A, Jepsen A. 1980. Toxin production by *Clostridium botulinum* type E in smoked fish in Ireation to the measured oxidation reduction (Eh) potential, packaging method and the associated microflora. In: Connell JJ, editor. Advances in Fish Science and Technology: Fishing News Books Ltd. England. p 476–9.
Hyytia E, Hielm S, Korkeala H. 1998. Prevalence of *Clostridium botulinum* type E in Finnish fish and fishery products. Epidemiol Infect 120:245–50.
Hyytia E, Hielm S, Mokkila M, Kinnunen A, Korkeala H. 1999. Predicted and observed growth and toxigenesis by *Clostridium botulinum* type E in vacuum-packaged fishery product challenge tests. Int J Food Microbiol 47:161–9.
Ibe A, Saito K, Nakazato M, Kikuchi Y, Fujinuma K, Nishima T. 1991. Quantitative determination of amines in wine by liquid chromatography. J AOAC 74(4):695–8.
Ienistea C. 1973. Significance and detection of histamine in food. In: Hobbs BC, Christian JHB, editors. The microbiological safety of food. New York: Academic Press. p 327–43.
Iida T, Kanzaki M, Nakama A, Kokubo Y, Maruyama T, Kaneuchi C. 1998. Detection of *Listeria monocytogenes* in humans, animals, and foods. J Vet Med Sci 60(12):1341–3.
Ishikura H, Takahashi S, Yagi K, Nakamura K, Kon S, Matsuura A, Sato N, Kikuchi K. 1998. Epidemiology: global aspects of Anisakidosis. Chiba (Japan): International Congress of Parasitology. Aug 24–28. Report nr ICOPA IX. 379–82 p.
Jemmi T. 1990. Zum vorkommen von *Listeria monocytogenes* in importierten geraucherten und fermentierten fischen. Arch Lebensmittelhyg 41:107–9.
Jemmi T, Keusch A. 1992. Behavior of *Listeria monocytogenes* during processing and storage of experimentally contaminated hot-smoked trout. Int J Food Microbiol 15:339–46.
Jemmi T, Keusch A. 1994. Occurrence of *Listeria monocytogenes* in freshwater fish farms and fish-smoking plants. Food Microbiol 11:309–16.
Jeong DK, Frank JF. 1994. Growth of *Listeria monocytogenes* at 10° C in biofilms with microorganisms isolated from meat and dairy processing environments. J Food Prot 57(7):576–86.
Jeppesen V. 1988. Results in report: "Predictive microbiology-measurement and control of food quality". Lyngby: Danish Institute for Fisheries Research, Dept. of Seafood Research.
Jeppesen V, Huss HH. 1993. Antagonistic activity of two strains of lactic acid bacteria against *Listeria monocytogenes* and *Yersinia enterocolitica* in a model fish product at 5° C. Int J Food Microbiol 19:179–86.
Jin M, Kusunoki K, Ikejima N, Arai T, Irikura Y, Suzuki K, Hirata I, Kokubo Y, Maruyama T. 1994. Incidence of *Listeria moncytogenes* in smoked salmon. Jpn J Food Microbiol 11(2):107–11.
Jinneman KC, Wekell MM, Eklund MW. 1999. Incidence and behavior of *Listeria monocytogenes* in fish and seafood. In: Ryser ET, Marth ELH, editors. *Listeria*, listeriosis and food safety. New York: M Dekker. p 601–30.
Johansson T, Rantala L, Palmu L, Honkanen-Buzalski T. 1999. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. Int J Food Microbiol 47:111–9.
Jorgensen LV, Dalgaard P, Huss HH. 2000. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. J Agric Food Chem 48:2448–53.
Jorgensen LV, Huss HH. 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. Int J Food Microbiol 42:127–31.
Kalish F. 1991. Extending the HACCP concept to product distribution. Food Technol 45(4):119–20.
Kaneko J, (PacMar, Inc., Honolulu, Hawaii). 2000 July 31. Development of a HACCP-based strategy for the control of histamine for the fresh tuna industry [A report by PacMar, Inc. pursuant to National Oceanographic and Atmospheric Administration]. Honolulu (Hawaii): PacMar; 2000 July 31. NOAA Award Nr NA86FD0067. 48 p.
Karl H, Roepstorff A, Huss HH, Bloemsma B. 1995. Survival of *Anisakis* larvae in marinated herring fillets. Int J Food Sci Technol 29(6):661–70.
Karl VH, Leinemann M. 1989. Uberlebensfahigkeit von nematodenlarven (*Anisaki*s sp.) in gefrosteten Heringen. Archiv fur Lebensmittelhygiene 40(1):14–6.
Karolus JJ, LeBlanc DH, Marsh AJ, Mshar R, Furgalack TH. 1985. Presence of histamine in the bluefish, *Pomatomus saltatrix*. J Food Prot 48(2):166–8.
Kassem CL. 1977. Smoking fish at home-a step by step guide. Blacksburg (VA): Virginia Polytechnic Institute and State Univ, Cooperative Extension Service. VPI-SG–300–2.
Kautter DA. 1964. *Clostridium botulinum* type E in smoked fish. J Food Sci 29:843–9.
Kim J, Foegeding PM. 1993. Principles of Control. In: Hauschild AHW, Dodds KL, editors. *Clostridium botulinum.* Ecology and control in foods. New York: M Dekker. p 121–76.
Kim S-H, Ben-Gigirey B, Barros-Velazquez J, Price RJ, An H. 2000. Histamine and biogenic amine production by *Morganella morganii* isolated from temperature-abused albacore. J Food Prot 63(2):244–51.
Kimata M, Akamatsu M, Ishida Y, (Kyoto Daigaku. Shokuryo Kagaku Kenkyujo). Memoirs of the Research Institute for Food Science. Kyoto: Kyoto University. 1960. Report nr 20. Studies on the classification of the genus *Proteus* I. p 1–7.
Kimata M, Kawai A, (Kyoto Daigaku. Shokuryo Kagaku Kenkyujo). Bulletin of the Research Institute for Food Science. Kyoto: Kyoto University. 1953 Oct. Report nr 12. The production of histamine by the action of bacteria causing the spoilage of fresh fish, I. 29–33 p.
Klausen NK, Huss HH. 1987. Growth and histamine production by *Morganella morganii* under various temperature conditions. Int J Food Microbiol 5:147–56.
Klausen NK, Lund E. 1986. Formation of biogenic amines in herring and mackerel. Z Lebensm Unters Forsch 182(6):459–63.
Koutsoumanis K, Lampropoulou K, Nychas G-JE. 1999. Biogenic amines and sensory changes associated with the microbial flora of Mediterranean Gilt-head sea bream (*Sparus aurata*) stored aerobically at 0, 8, and 15° C. J Food Prot 62(4):398–402.
Lagoin Y. 1980. Donnees actuelles sur une nematodose larvaire de l'Homme, l'anisakiase ou "maladie du ver du hareng". Bull Acad Vet de France 53:139–46.
Lawrence LM, Gilmour A. 1995. Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Appl Environ Microbiol 61(6):2139–44.
Leisner JJ, Millan JC, Huss HH, Larson LM. 1994. Production of histamine and tyramine by lactic acid bacteria isolated from vacuum-packed sugar-salted fish. J Appl Bacteriol 76:417–23.
Leitao MFF, Baldini VLS, Sales AM. 1983. Histamina em pescado e alimentos industrializados. Col Inst Technol Alim 13:123–30.
Leroi F, Joffraud JJ, Chevalier F. 2000. Effect of salt and smoke on the microbiological quality of cold-smoked salmon during storage at 5° C as estimated by the factorial design method. J Food Prot 63(4):502–8.
Leroi F, Joffraud JJ, Chevalier F, Cardinal M. 1998. Study of the microbial ecology of cold-smoked salmon during storage at 8° C. Int J Food Microbiol 39:111–21.
Leuschner RGK, Hammes WP. 1999. Formation of biogenic amine in mayonnaise, herring and tuna fish salad by *Lactobacilli*. Int J Food Sci Nut 50:159–64.
Levin RE. 1968. Detection and incidence of specific species of spoilage bacteria on fish. 1. Methodology. Appl Microbiol 16:1734–7.
Lima dos Santos CA. 1997. The possible use of HACCP in the prevention and control of foodborne trematode infections in aquacultured fish. In: Shahidi F, editor. Seafood safety, processing and biotechnology. Lancaster (PA): Technomic. p 53–64.
Lopez-Sabater EI, Rodriguez-Jerez JJ, Hernadez-Herrero M, Roig-Sagues AX, Mora-Ventura MT. 1996. Sensory quality and histamine formation during controlled decomposition of tuna (*Thunnus thynnus*). J Food Prot 59(2):167–74.
Lopez-Sabater EI, Rodriguez-Jerez JJ, Hernandez-Herrero M, Mora-Ventura MT. 1994. Evaluation of histidine decarboxylase activity of bacteria isolated from sardine (*Sardina pilchardus*) by an enzymic method. Lett Appl Microbiol 19:70–5.
Lopez-Sabater EI, Rodriguez-Jerez JJ, Hernandez-Herrero M, Mora-Ventura MT. 1996. Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. Int J Food Microbiol 28:411–8.
Lopez-Sabater EI, Rodriguez-Jerez JJ, Roig-Sagues AX, Mora-Ventura MAT. 1994. Bacteriological quality of tuna fish (*Thunnus thynnus*) destined for canning: effect of tuna handling on presence of histidine decarboxylase bacteria and histidine level. J Food Prot 57(4):318–23.
Lord JB. 2000. Entering the new millennium: the food industry in transition. NFPA Feb:9–15.
Lucore LA, Shellhammer TH, Yousef AE. 2000. Inactivation of *Listeria monocytogenes* Scott A on artificially contaminated frankfurters by high-pressure processing. J Food Prot 63(5):662–4.
Lund BM, George SM, Franklin JG. 1987. Inhibition of type A and type B (proteolytic) *Clostridium botulinum* by sorbic acid. Appl Environ Microbiol 53:935-?
Lund BM, Peck MW. 2000. *Clostridium botulinum*. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of foods. Gaithersburg (MD): Aspen. p 1057-
109.
Lyver A, Smith JP, Austin J, Blanchfield B. 1998. Competitive inhibition of *Clostridium botulinum* type E by *Bacillus* species in a value-added seafood product packaged under a modified atmosphere. Food Res Int 31(4):311–9.
Maga JA. 1978. Amines in foods. CRC Crit Rev Food Sci Nutr 10:373–403.
Masson F, Talon R, Montel MC. 1996. Histamine and tyramine production by bacteria from meat products. Int J Food Microbiol 32:199–207.
Mazorra-Manzano MA, Pacheco-Aguilar R, Diaz-Rojas EI, Lugo-Sanchez ME. 2000. Postmortem changes in black skipjack muscle during storage in ice. J Food Sci 65(5):774–9.
McClure PJ, Cole MB, Smelt JPPM. 1994. Effects of water activity and pH on growth of *Clostridium botulinum*. J Appl Bacteriol Symp Suppl 76:105S–114S.
McLauchlin J. 1997. The pathogenicity of *Listeria monocytogenes*: a public health perspective. Rev Med Microbiol 8(1):1–14.
Mendes R. 1999. Changes in biogenic amines of major Portuguese bluefish species during storage at different temperatures. J Food Biochem 23:33–43.
Mendes R, Goncalves A, Nunes ML. 1999. Changes in free amino acids and biogenic amines during ripening of fresh and frozen sardine. J Food Biochem 23:295–306.
Meng J, Genigeorgis CA. 1993. Modeling lag phase of nonproteolytic *Clostridium botulinum* toxigenesis in cooked turkey and chicken breast as affected by temperature, sodium lactate, sodium chloride and spore inoculum. Int J Food Microbiol 19:109–22.
Middlebrooks BL, Toom PM, Douglas WL, Harrison RE, McDowell S. 1988. Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (*Scomberomorus maculatus*). J Food Sci 53(4):1024–9.
Miettinen MK, Bjorkroth KJ, Korkeala HJ. 1999. Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. Int J Food Microbiol 46:187–92.
Miettinen MK, Siitonen A, Heiskanen P, Haajanen H, Bjorkroth KJ, Korkeala HJ. 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. J Clin Microbiol 37(7):2358–60.
Mietz JL, Karmas E. 1977. Chemical quality index of canned tuna as determined by high-pressure liquid chromatography. J Food Sci 42:155–8.
Mietz JL, Karmas E. 1978. Polyamine and histamine content of rockfish, salmon, lobster, and shrimp as an indicator of decomposition. J AOAC 61(1):139–45.
[MMWR] Morbidity Mortality Weekly Report. 2000 Mar 17. Surveillance for foodborne disease outbreaks-United States, 1993–1997. MMWR 49(SS01):1–51.
Morii H, Cann DC, Taylor LY, Murray CK. 1986. Formation of histamine by luminous bacteria isolated from scromboid fish. Bull Japan Soc Sci Fish 52(12):2135–41.
Murray CK, Hobbs G, Gilbert RJ. 1982. Scombrotoxin and scombrotoxin-like poisoning from canned fish. J Hyg 88:215–20.
Mutluer B, Ersen S, Kaya B, Akin S, Ozta-Siran I. 1989. Einfluss von Gammastrahlen auf Histaminbildung in Makrelenfilets. Fleischwirtsch 69:112–4.

Myers BJ. 1979. Anisakine nematodes in fresh commercial fish from waters along the Washington, Oregon and California coasts. J Food Prot 42:380–4.
Nesbakken T, Kapperud G, Caugant DA. 1996. Pathways of *Listeria monocytogenes* contamination in the meat processing industry. Int J Food Microbiol 31:161–71.
Nickerson JTR, Goldblith SA, DiGioia G, Bishop WW. 1967. The presence of *Cl. botulinum*, type E in fish and mud taken from the gulf of Maine. In: Ingram M, Roberts TA, editors. Botulism 1966. [unknown]: Chapman and Hall.
Nielsen SF, Pedersen HO. 1967. Studies of the occurrence and germination of *Cl. botulinum* in smoked salmon. In: Ingram M, Robers TA, editors. Botulism 1966. [unknown]: Chapman and Hall. p 66–72.
Nilsson L. 1999. Control of *Listeria monocytogenes* in cold-smoked salmon by biopreservation [DPhil thesis]. Lyngby: Technical University of Denmark, Danish Institute for Fisheries Research. 136 p.
Nilsson L, Chen Y, Chikindas ML, Huss HH, Gram L, Montville TJ. 2000. Carbon dioxide and nisin act synergistically on *Listeria monocytogenes*. Appl Environ Microbiol 66(2):769–74.
Nilsson L, Gram L, Huss HH. 1999. Growth control of *Listeria monocytogenes* on cold-smoked salmon using a competitive lactic acid bacteria flora. J Food Prot 62(4):336–42.
Nilsson L, Huss HH, Gram L. 1997. Inhibition of *Listeria monocytogenes* on cold-smoked salmon by nisin and carbon dioxide atmosphere. Int J Food Microbiol 38:217–27.
[NMFS] National Marine Fisheries Service. 1991. HACCP prototype model food service/consumer education. Model seafood surveillance project. Pascagoula (MS): NMFS, Office of Trade and Industry Services, NSIL. 1991 Dec.
[NMFS] National Marine Fisheries Service. 1991. HACCP regulatory model smoked and cured fish. Model seafood surveillance project. Pascagoula (MS): NMFS, Office of Trade and Industry Services, NSIL. 1991 Dec.
Norrung B. 2000. Microbiological criteria for *Listeria monocytogenes* in foods under special consideration of risk assessment approaches. Int J Food Microbiol 62:217–21.
Norton DM, McCamey MA, Gall KL, Scarlett JM, Boor KJ, Wiedmann M. 2000. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry and implications for control strategies. Forthcoming.
Norton DM, Sue D, Timothe J, Scarlett JM, Boor KJ, Wiedmann M (Cornell Univ, Ithaca, NY). 2000. Characterization and pathogenic potential of *L. monocytogenes* isolates from the smoked fish industry [abstract]. Abstract submitted for presentation at the 2000 Annual Meeting of the American Society of Microbiology (ASM).
Norwood DE, Gilmour A. 2000. The growth and resistance to sodium hypochlorite of *Listeria monocytogenes* in a steady-state multispecies biofilm. J Appl Microbiol 88:512–20.
Notermans S, Dufrenne J, Teunis P, Chackraborty T. 1998. Studies on the risk assessment of *Listeria monocytogenes*. J Food Prot 61(2):244–8.
Ojeniyi B, Wegener HC, Jensen NE, Bisgaard M. 1996. *Listeria monocytogenes* in poultry and poultry products: epidemiological investigations in seven Danihs abattoirs. J Appl Bacteriol 80:395–401.
Okuzumi M, Fukumoto I, Fujii T. 1990. Changes in bacterial flora and polyamines contents during storage of horse mackerel meat. Nippon Suisan Gakkasishi 56(8):1307–12.
Okuzumi M, Yamanaka H, Kubozuka T. 1984. Occurrence of various histamine-forming bacteria on/in fresh fishes. Bull Jap Soc Sci Fish 50(1):161–7.
Oshima T. 1972. *Anisakis* and anasakiasis in Japan and adjacent area. Progressive Medical Parasitology in Japan 4.
Overstreet RM. 1999. Actual and potential human health risks associated with marine parasites [abstract]. In: AAAS-Annual Meeting and Science Innovation Exposition; 1999 Jan 21–26; Anaheim (CA).
Pacini R, Panizzi L, Galleschi G, Quagli E, Galassi R, Fatighenti P, Morganti R. 1993. Presenza di larve di anisakidi in prodotti ittici freschi e congelati del commercio. Industrie Alimentari 32:942–4.
Paludan-Muller C, Dalgaard P, Huss HH, Gram L. 1998. Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum- and modified packed cold-smoked salmon stored at 5° C. Int J Food Microbiol 39:155–66.
Pelroy GA, Eklund MW, Paranjpye RN, Suzuki EM, Peterson ME. 1982. Inhibition of *Clostridium botulinum* types A and E toxin formation by sodium nitrite and sodium chloride in hot-process (smoked) salmon. J Food Prot 45(9):833–41.
Pelroy GA, Peterson ME, Paranjpye R, Almond J, Eklund M. 1994. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium nitrite and packaging method. J Food Prot 57(2):114–9.
Pelroy GA, Peterson ME, Holland PJ, Eklund MW. 1994. Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium lactate. J Food Prot 57(2):108–13.
Petaja E, Eerola S, Petaja P. 2000. Biogenic amines in cold-smoked fish fermented with lactic acid bacteria. Zeit Leben-Unter Forch 210(4):280–5.
Peterson ME, Pelroy GA, Paranjpye RN, Poysky FT, Almond JS, Eklund MW. 1993. Parameters for control of *Listeria monocytogenes* in smoked fishery products: sodium chloride and packaging method. J Food Prot 56(11):938–43.
Price RJ, Melvin EF, Bell JW. 1991. Postmortem changes in chilled round, bled and dressed albacore. J Food Sci 56:318–21.
Rasco BA, Girard WA, Bledsoe GE. 2001. Frozen aquatic food products [chapter 22]. In: Smith JS, editor. Introduction to food chemistry. West Sacramento (CA): Science Technology Systems.
Reddy NR, Armstrong DJ, Rhodehamel EJ, Kautter DA. 1992. Shelf-life extension and safety concerns about fresh fishery products packaged under modified atmospheres: a review. J Food Safety 12:87–118.
Reilly A, Kaferstein F. 1997. Food safety hazards and the application of the principles of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. Aquaculture Research 28:735–52.
Ritchie AH, Mackie IM. 1980. The formation of diamines and polyamines during storage of mackerel (*Scomber scrombrus*). In: Connell J, editor. Advances in Fish Science and Technology. Surrey (England): Fishing News (Books) Ltd. p 489–94.
Robertson GL. 1993. Food Packaging: Principles and Practice. Hughes H, editor. New York: M Dekker. 676 p.
Rocourt J, Jacquet C, Reilly A. 2000. Epidemiology of human listeriosis and seafood. Int J Food Microbiol 62:197–209.
Rodriquez-Jerez JJ, Mora-Ventura MT, Lopez-Sabater EI, Hernandez-Herrero M. 1994. Histidine, lysine, and ornithine decarboxylase bacteria in Spanish salted semipreserved anchovies. J Food Prot 57(9):784–7, 791.
Rogers, Staruszkiewicz. 1997. Collaborative study-GLC determination of cadaverine and putrescine in seafood; fluorometric method for histamine in tuna and mahimahi. JAOAC 80:591–602.
Rorvik LM. 2000. *Listeria monocytogenes* in the smoked salmon industry. Int J Food Microbiol 62:183–90.
Rorvik LM, Caugant DA, Yndestad M. 1995. Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and a smoked salmon processing plant. Int J Food Microbiol 25:19–27.
Rorvik LM, Yndestad M. 1991. *Listeria monocytogenes* in foods in Norway. Int J Food Microbiol 13:97–104.
Rorvik LM, Yndestad M, Skjerve E. 1991. Growth of *Listeria monocytogenes* in vacuum-packed, smoked salmon during storage at 4° C. Int J Food Microbiol 14:111–8.
Ross T, Todd E, Smith M. 2000. Exposure assessment of *Listeria monocytogenes* in ready-to-eat foods: preliminary report for joint FAO/WHO expert consultation on risk assessment of microbiological hazards in foods. Rome: Food and Agriculture Organization of the United Nations. 242 p.
Rosset JS, McClatchey KD, Higashi GI, Knisely AS. 1982. *Anisakis* larval type I in fresh salmon. Am J Clin Pathol 78:54–7.
Rowan NJ, MacGregor SJ, Anderson JG, Fouractre RA, McIlvaney L, Farish O. 1999. Pulsed-light inactivation of food-related microorganisms. Appl Environ Microbiol 65:1312–5.
Sachs S. 1989. Forward to a margin of safety: the HACCP approach to food safety education. Washington, DC: USDA,FSIS, ILA. 1989 June.
Seeliger HPR, Jones D. 1986. Genus Listeria Pirie 1940, 383al. In: Sneath PHA, Mair SN, Sharpe ME, Holt JG, editors. Bergey's Manual of Systematic Bacteriology. 9th ed. Baltimore (MD): Williams and Wilkins. p 1235–45.
Segner WP, Schmidt CF, Boltz JK. 1966. Effect of sodium chloride and pH on the outgrowth of spores of type E *Clostridium botulinum* at optimal and suboptimal temperatures. Appl Microbiol
14(1):49–54.
Shalaby AR. 1996. Significance of biogenic amines to food safety and human health. Food Res Int 29(7):675–90.
Shewan JM. 1977. The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. Proceedings of the conference on handling, processing, and marketing of tropical fish. London: Tropical Products Institute. p 51–60.
Shewan JM, Liston J. 1955. A review of food poisoning caused by fish and fishery products. J Appl Bacteriol 18:522–34.
Shin J-W, HuangY-H. 2000. Investigation for contamination of parasite and aerobic bacteria in frozen tilapia fillets in Taiwan. J Food Drug Analysis 8(1):51–6.
Silla Santos MH. 1996. Biogenic amines: their importance in foods. Int J Food Microbiol 29:213–31.
Silva CCG, Da Ponte DJB, Enes Dapkevicius MLN. 1998. Storage temperature effect on histamine formation in big eye tuna and skipjack. J Food Sci 63(4):644–7.
Sjaastad O. 1966. Fate of histamine and N-Acetylhistamine administered into the human gut. Acta Pharmacol Toxicol 24:189–202.
Soares VFM, Gloria MBA. 1994. Histamine levels in canned fish available in the retail market of Belo Horizonte, Minas Gerais, Brazil. J Food Comp Anal 7:102–9.
Southcott BA, Razzell WE. 1973. *Clostridium botulinum* control in cold-smoked salmon: a review. J Fish Res Bd Can 30(5):631–41.
Storey RM. 1982. Smoking. In: Aitken A, Mackie IM, Merritt JH, Windsor ML, editors. Fish handling and processing. 2nd ed. Aberdeen: Ministry of Agriculture, Fisheries and Food, Torry Research Station; Edinburgh: HMSO. p 98–114.
Sugiyama H, Bott TL, Foster EM. 1970. *Clostridium botulinum* type E in an inland bay (Green Bay of Lake Michigan). In: Herzberg M, editor. Toxic Microorganisms. Washington D.C.: U.S. Dept. of the Interior. p 287–91.
Sunen E. 1998. Minimum inhibitory concentration of smoke wood extracts against spoilage and pathogenic micro-organisms associated with foods. Letts Appl Microbiol 27:45–8.
Suzuki S, Matsui Y, Takama K. 1988. Profiles of polyamine composition in putrefactive *Pseudomonas* type III/IV. Microbios Letters 38:105–9.
Suzuki S, Noda J, Takama K. 1990. Growth and polyamine production of alteromonas spp. in fish meat extracts under modified atmosphere. Bull Fac Fish, Hokkaido Univ 41(4):213–20.
Szabo EA, Cahill ME. 1998. The combined affects of modified atmosphere, temperature, nisin and ALTA[tm] 2341 on the growth of *Listeria monocytogenes*. Int J Food Mircobiol 43:21–31.
Takagi M, Iida A, Murayama H, Soma S. 1969. On the formation of histamine during loss of freshness and putrefaction of various marine products. Bull Fac Fish, Hokkaido Univ 20:227–34.
Taylor SL. 1986. Histamine food poisoning: toxicology and clinical aspects. CRC Crit Rev Toxicol 17(2):91–128.
Taylor SL, Guthertz LS, Leatherwood M, Lieber ER. 1979. Histamine production by *Klebsiella pneumoniae* and an incident of scombroid fish poisoning. Appl Environ Microbiol 37:274–8.
Taylor SL, Guthertz LS, Leatherwood M, Tillman F, Lieber ER. 1978. Histamine production by foodborne bacterial species. J Food Safety 1:173–87.
Taylor SL, Lieber ER. 1979. In vitro inhibition of rat intestinal histamine-metabolizing enzymes. Food Cosmet Toxicol 17:237–40.
Taylor SL, Speckhard MW. 1984. Inhibition of bacterial histamine production by sorbate and other antimicrobial agents. J Food Prot 47(7):508–11.
Taylor SL, Stratton JE, Nordlee JA. 1989. Histamine poisoning (scombroid fish poisoning): an allergy-like intoxication. Clin Toxicol 27(4&5):225–40.
ten Brink B, Damink C, Joosten HMLJ, Huis in 't Veld JHJ. 1990. Occurrence and formation of biologically active amines in foods. Int J Food Microbiol 11:73–84.
Teufel P, Bendzulla C. 1993. Bundesweite Erhebung zum vorkommen von *L. monocytogenes* in Lebenmitteln. Berlin: Bundesinstitut fur gesundheitlichen Verbraucherschutz und Veterinarmedizin. NB.
Thatcher FS, Robinson J, Erdman I. 1962. The "vacuum pack" method of packaging foods in relation to the formation of the botulinum and staphylococcal toxins. J Appl Bacteriol 25:120–4.
Thayer DW, Boyd G. 1999. Irradiation and modified atmosphere packaging for the control of *Listeria monocytogenes* on turkey meat. J Food Prot 62:1136–42.
Tompkin RB, Scott VN, Bernard DT, Sveum WH, Gombas KS. 1999. Guidelines to prevent postprocessing contamination from *Listeria monocytogenes*. Dairy, Food Environ San 19(8):551–62.
Truelstrup Hansen L, Drewes Rontved S, Huss HH. 1998. Microbiological quality and shelf life of cold-smoked salmon from three different processing plants. Food Microbiol 15:137–50.
Truelstrup Hansen L, Gill T, Drewes Rontved S, Huss HH. 1996. Importance of autolysis and microbiological activity on quality of cold-smoked salmon. Food Res Int 29:181–8.
Turner JA, Sorvillo FJ, Murray RA, Chin J, Middaugh JP, Dietrich PD, Wiebenga NH, Googins JA, Allard J, Ruttenber AJ and others. 1981. Diphyllobothriasis associated with salmon-United States. MMWR 30(27):331–2, 337.
Van Mameren J, Houwing H. 1968. Effect of irradiation on *Anasakis* larvae in salted herring. Elimination of harmful organisms from food and feed by irradiation. Vienna: IAEA. p 73–80.
Veciana-Nogues MT, Marine-Font A, Vidal-Carou MC. 1997. Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts, ATP-related compounds,

volatile amines and organoleptic changes. J Agric Food Chem 45:2036–41.
Wagner M, Maderner A, Brandl E. 1999. Development of a multiple primer RAPD assay as a tool for phylogenetic analysis in *Listeria* spp. strains isolated from milkproduct associated epidemics, sporadic cases of listeriosis and dairy environments. Int J Food Microbiol 52:29–37.
Weagant SD, Sado PA, Colburn KG, Torkelson JD, Stanley FA, Krane MH, Shields SC, Thayer CF. 1988. The incidence of *Listeria* species in frozen seafood products. J Food Prot 51:655–7.
Wei CI, Chen C-M, Koburger JA, Otwell WS, Marshall MR. 1990. Bacterial growth and histamine production on vacuum packaged tuna. J Food Sci 55(1):59–63.
Wendakoon CN, Murata M, Sakaguchi M. 1990. Comparison of nonvolatile amine formation between the dark and white muscles of mackerel during storage. Nippon Suisan Gakkaishi 56(5):809–18.
Wendakoon CN, Sakaguchi M. 1992. Effects of spices on growth of and biogenic amine formation by bacteria in fish muscle. In: Huss HH, Jakobsen M, Liston J, editors. Quality Assurance in the Fish Industry: Proceedings of an International Conference; 1991 Aug 26–30; Copenhagen (DK). Amsterdam: Elsevier; 1992. p 305–13 (Development in Food Sci series; 30).
Wendakoon CN, Sakaguchi M. 1992b. nonvolatile amine production in mackerel muscle during growth of different bacterial species. J Food Hyg Soc Jap 33(1):39–45.
Wendakoon CN, Sakaguchi M. 1993. Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. J Food Prot 56(5):410–3.
Wenger JD, Swaminathan B, Hayes PS, Green SS, Pratt M, Pinner RW, Schuchat A, Broome CV. 1990. *Listeria monocytogenes* contamination of turkey franks: evaluation of a production facility. J Food Prot 53(12):1015–9.
WHO. 1979. WHO Expert Committee on Parasitic Zoonoses. Geneva: WHO. WHO Techn Rep Ser Nr 637.
WHO. 1995. Control of foodborne trematode infections, report of a study group. Geneva: WHO. WHO Techn Rep Ser Nr 849.
Wiedmann M, Bruce JL, Keating C, Johnson AE, McDonough PL, Batt CA. 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infec Immun 65(7):2707–16.
Wirtanen G, Mattila-Sandholm T. 1992. Removal of foodborne biofilms-comparison of surface and suspension tests. Part I. Lebensm Weiss Technol 25(1):43–9.
Woodburn MJ, Somers E, Rodriguez J, Schantz EJ. 1979. Heat inactivation rates of botulinum toxins A, B, E and F in some foods and buffers. J Food Sci 44:1658–61.
Yamanaka H, Shimakura K, Shiomi K, Kikuchi T, Okuzumi M. 1987. Occurrence of allergy-like food poisoning caused by "mirin"-seasoned meat of dorado (*Coryphaena hippurus*). J Food Hyg Soc Japan 28(5):354–85.
Yamanaka H, Shiomi K, Kikuchi T, Okuzumi M. 1982. A pungent compound produced in the meat of frozen yellowfin tuna and marlin. Bull Jap Soc Sci Fish 48(5):685–9.
Yamanaka H, Shiomi K, Kikuchi T, Okuzumi M. 1984. Changes in histamine contents in red meat fish during storage at different temperatures. Bull Jap Soc Sci Fish 50(4):695–701.
Zotos A, Hole M, Smith G. 1995. The effect of frozen storage of mackerel (*Scomber scombrus*) on its quality when hot-smoked. J Sci Food Agric 67(1):43–8.

# NOTES


Institute of Food Technologists
221 North LaSalle Street, Suite 300
Chicago, Illinois 60601-1291 USA

Office of Science, Communications, and Government Relations
1025 Connecticut Avenue NW, Suite 503
Washington DC, 20036-5422
www.ift.org