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Treatment Options to Eliminate or Control Growth of *Listeria monocytogenes* on Raw Material and on Finished Product for the Smoked Fish Industry

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SUMMARY

The Smoked Seafood Working Group (SSWG), a collaboration of the National Fisheries Institute, the National Food Processors Association, several smoked fish processors and universities, reviewed scientific papers that describe possible treatments to eliminate or reduce the amount of *Listeria monocytogenes* present on incoming raw material and eliminate or minimize its growth on finished product. Suggested treatment options that are approved for use on seafood, can be used by most commercial smoked fish companies, and have potential to significantly reduce *L. monocytogenes* numbers on incoming raw fish include (1) washing of raw fish with water containing chlorine and (2) treatment of raw fish with calcium hydroxide solution (pH 12). Other potential treatments approved for raw materials include washing of fish with acidified sodium chlorite solutions, ozone treatment, steam surface pasteurization, and electrochemical brine tank treatments. Treatment options to control *L. monocytogenes* on finished product include (1) freezing of finished product to stop growth; and (2) addition of approved chemical growth inhibitors. Other treatment options that have potential to eliminate *L. monocytogenes* or control its growth on finished product but that are not currently approved for use on seafood include addition of natural growth inhibitors, addition of high levels of *Carnobacterium piscicola* (~2 × 10^6 CFU/g), and irradiation. All treatment options require validation under commercial processing conditions.

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INTRODUCTION

Listeria monocytogenes is an organism found throughout the fish processing environment and on the fish itself *(18, 31)*. *L. monocytogenes* has been isolated from many types of refrigerated foods, including ready-to-eat (RTE) meat sandwiches, deli-type salads, cheeses, deli-meats, frankfurters, and cold smoked fish products *(26)*. *L. monocytogenes* is a Gram positive, foodborne pathogen that is psychrotrophic and halotolerant *(6, 44)*. Under optimal conditions, it grows in the range of 1 to 45°C and at salt concentrations between zero and 10% NaCl. It grows in many food products that have an extended shelf life *(4, 41, 42)*. Ready-to-eat products that support growth and do not receive additional heat treatment by consumers can contain high numbers of *L. monocytogenes* by the time of consumption. Ready-to-eat fish products have been linked to sporadic cases of listeriosis outside of the United States and epidemiological evidence specifically suggests that listeriosis has been caused by smoked mussels *(7)*, "gravid" trout *(21)*, and smoked trout *(36)*.

Listeria monocytogenes has been isolated from seafood products such as smoked fish, cooked and frozen seafoods, marinated fish, and surimi *(16)*. Studies have reported prevalences of 6 to 36% in RTE cold smoked salmon and cooked fishery products *(5)*, although a recent survey by the National Food Processors Association (NFPA) suggests a prevalence of about 5% in smoked fish produced in the United States *(26)*. These studies raise concern regarding the survival and growth potential of *L. monocytogenes*, including in seafoods and other RTE foods. The ingestion of high numbers of *L. monocytogenes* is a significant health threat for people in high risk groups such as the immunocompromised, the elderly, pregnant women and their fetuses, and neonates. In these groups, the mortality from listeriosis can be as high as 20–30% *(35)*. Foods implicated in past major listeriosis outbreaks were products in which *L. monocytogenes* was able to grow to large numbers prior to consumption *(27)*. In the United States, federal agencies with responsibility for public health and food protection established a zero tolerance for *L. monocytogenes* (< one organism per 25 g sample) in RTE foods *(45)*.

Farber *(22)* reported that moderate to severe temperature abuse of contaminated fish products may greatly enhance the growth of *Listeria* spp. on fish. Nevertheless, because of the low naturally occurring levels of *L. monocytogenes* and the relatively short shelf life of many fishery products, *Listeria*-contaminated fish stored at temperatures ≤4°C or lower present little risk to public health. Although the minimum infective dose for *L. monocytogenes* has not been established, there is little evidence that low numbers cause listeriosis *(10, 22)*. Saguy *(43)* predicted that, on products stored under typical retail and consumer temperature conditions, *L. monocytogenes* numbers can reach levels that could cause infections in people with immune compromised systems. Without appropriate interventions, the number of human listeriosis cases could increase during the next several decades because of the continuing increase in the proportion of the population that is highly susceptible to this disease (e.g., elderly and immuno compromised individuals) *(8, 23)*.

The Institute of Food Technologists (IFT) assembled an expert panel to review processing parameters for cold smoked fishery products with respect to pathogens, including *L. monocytogenes (31)*. The report concluded that reduction of *L. monocytogenes* in the processing plant was directly dependent on adherence to Good Hygienic Practices (GHPs) and Good Manufacturing Practices (GMPs) *(31)*. Areas in the processing plant that require particular attention include the brine, injection needles, and slicing equipment. This report also identified other methods to control *L. monocytogenes* in fishery products (e.g., frozen storage, adding nitrite, lactate, sorbate, and bacteriocins) *(31)*. This document presents recommendations on control of *L. monocytogenes* on raw materials to be used for production of smoked seafood products, and on finished product. These recommendations were developed as a consensus document by the Smoked Seafood Working Group (SSWG), a collaboration of the National Fisheries Institute, the National Food Processors Association, several smoked fish processors and universities *(24)*. This is the third in a series of four papers describing critical components of a *L. monocytogenes* control program for smoked seafood plants. The papers include (1) development of targeted GMP and sanitation procedures to prevent finished product contamination *(24)*; (2) implementation of *Listeria* testing programs (in preparation); (3) control of *L. monocytogenes* in raw materials and finished product (this manuscript); and (4) implementation of employee training programs *(28)*.

L. MONOCYTOGENES CONTROL ON RAW MATERIALS

As part of an overall *Listeria* control program, processors of RTE seafood products must decide how to reduce or minimize *Listeria* contamination of raw materials brought into a plant. As noted above, *L. monocytogenes* can be present on raw food products such as fish and shellfish. The prevalence of *L. monocytogenes* contamination on raw fish intended for smoking can vary significantly from one source to another *(20, 29, 48)*. Processors should also evaluate

the need for raw material controls based on the type of processes utilized. The hot smoking process contains a listericidal step, and the testing or treatment of raw materials may not be as important as for cold smoking, which does not include a lethal heat treatment. Regardless of the process, smoked seafood processors should consider the importance of raw material as a source of *Listeria* contamination in the plant environment, since studies have shown that environmental sites and equipment in the plant are the most likely source of finished product contamination with *L. monocytogenes (3, 32, 39)*. Testing of raw materials is one method to monitor and control products from different suppliers that may be contaminated with *Listeria*. Another control option is to use processing treatments to eliminate *L. monocytogenes* or reduce the numbers of the organism on the incoming raw material.

Raw material testing

Processors may wish to consider testing fresh or frozen fish from various suppliers to evaluate contamination levels associated with specific species, suppliers or sources. Detailed information on testing procedures for raw materials is provided in another manuscript in this series. The type and frequency of testing is likely to be influenced by the species used and products produced, previous supplier performance, and other factors. Fresh fish are more likely to have higher numbers of *Listeria* because they are stored at refrigeration temperatures where the organism can grow. Frozen fish may also be contaminated with *Listeria* but may contain lower numbers because they are stored at freezer temperatures that are too low for *Listeria* growth. However, the potential for temperature abuse of either fresh or frozen fish should be considered when evaluating raw material sources.

Raw material treatments

Processors may wish to consider options to treat raw materials to reduce *L. monocytogenes* in lieu of raw material testing and/or in addition to testing. Treatments for controlling *L. monocytogenes* can be applied to the raw materials by the primary supplier, or by the processor after the raw material is received, or immediately before use. Two methods for treating raw material (nonprioritized) discussed below are available to most large and small smoked seafood processors. They do not require large investments of capital and equipment, and they can reduce numbers of *L. monocytogenes* on incoming raw material. In addition to the first two methods, summary information is also provided on other possible treatments to control *L. monocytogenes* on raw material. The practical application of these treatments may be limited by a variety of factors, which may include (1) lack of explicit regulatory approval of a treatment that is likely to be effective; (2) evidence indicating that approved treatments may not be effective in eliminating *L. monocytogenes*; and (3) absence of scientific studies validating the effectiveness of specific treatments on fish or other seafood products.

CHLORINE

Washing raw fish in a dilute chlorine solution can reduce the amount of *Listeria* contamination on raw fish. Chlorine concentrations in excess of 10 ppm (the level considered by the FDA to be GRAS [Generally Recognized as Safe]) are not allowed by the FDA to come into contact with seafood products. Firms using higher chlorine levels risk being cited during a regulatory inspection. However, many research studies have been conducted using higher concentrations of chlorine. For example, Eklund et al., *(19)* recommended thawing frozen fish in running water containing 20–30 ppm chlorine and exposing unfrozen fish to 20–30 ppm chlorine for 1 to 2 h. However, he reported that even at these concentrations, the treatment would not ensure that the raw material is completely free of *L. monocytogenes (19)*. The use of thaw tanks instead of running water is also an option for thawing frozen fish. Bremer and Osborne *(6)* conducted studies to determine optimum industrial scale washing regimes for thawing fish. They reported that flow regime with a turnover rate of 0.75 cycles/h for 72 min with 130 ppm chlorine provided optimum *L. monocytogenes* reduction.

Control using food grade calcium hydroxide

As an alternative to chlorine, high pH treatments may be considered to reduce *L. monocytogenes* contamination on raw materials. Studies at the University of Alaska showed that food grade calcium hydroxide $CaOH_2$ (GRAS) (*14*) can be used to reduce *L. monocytogenes* contamination on headed and gutted (H&G) salmon *(53)*. Raw salmon were inoculated with *L. monocytogenes* at two different levels (~10^4 CFU/cm^2 and ~10^6 CFU/cm^2) and then held in a water solution containing calcium hydroxide (pH 12.9) for 3, 6, and 9 h. Results indicate that *L. monocytogenes* numbers at the lower inoculum (i.e., 10^4 CFU/cm^2) were reduced to 10^2 CFU/cm^2 at 3 h and to less than 10^1 CFU/cm^2 in 6–9 h. At the higher inoculum concentration (i.e., 10^6 CFU/cm^2), *L. monocytogenes* numbers decreased to approximately 10^4 CFU/cm^2 at 3–6 h, and to 10^3 CFU/cm^2 after 9 h in limed water.

Other raw material treatments

A variety of other treatments have been studied and evaluated for their effectiveness in reducing pathogens, including *L. monocytogenes*, in many

different food products. A brief summary of other potential treatments is provided below. One of the suggested treatments, chlorine dioxide, is not currently approved for use on seafood products, but anecdotal reports by industry indicate that it may have potential to reduce *Listeria* contamination on incoming raw material.

Acidified sodium chlorite

Acidified sodium chlorite (ASC) is an antimicrobial compound recognized for its disinfectant properties and ability to control harmful microorganisms. In August 1999, the FDA approved ASC for direct contact on seafood at a concentration of 40–50 ppm in water in accordance with industry standards and GMPs *(11)*. Seafoods intended to be eaten raw, and treated with ASC, must be rinsed with potable water prior to consumption *(11)*.

Acidified sodium chlorite does not appear to be highly effective for reducing numbers of Gram positive organisms on seafood products. Su and Morrissey *(46)* reported that *L. monocytogenes* levels were reduced by only 0.52 log after salmon inoculated with *L. monocytogenes* were washed with an ASC solution of 50 ppm. Slight additional reductions (0.62 log) were observed when the salmon were first washed with ASC, followed by storage in ice containing ASC. Depending on the level of contamination, such reductions may or may not be adequate.

Ozone

The FDA has approved the use of ozone in the gas or liquid form for direct contact with foods including meat, poultry, and seafood, when used according to GMPs *(13)*. Khadre et al. *(33)* reported that ozone is effective for decontaminating produce, equipment, food contact surfaces and the general processing environment. The use of ozone to decontaminate meat products may have limited efficacy due to the high ozone demand of meat proteins. Bacteria imbedded in the meat surface may also be more resistant to ozone treatments. Goche and Cox *(25)* evaluated the effects of ozone for reducing total plate count numbers on H&G salmon. They concluded that ozone was at least as effective as chlorine for reducing total plate count numbers, but tests were not conducted against *L. monocytogenes*.

Steam surface pasteurization

Bremer and Osborne *(6)* evaluated the use of a pilot steam treatment system for reducing *L. monocytogenes* contamination on exterior surfaces of king salmon. A four-log reduction in *L. monocytogenes* was achieved after an eight second steam treatment. The researchers reported that an in-plant system was subsequently shown to reduce "naturally" occurring *L. monocytogenes* while maintaining a high quality final product.

Electrochemical brine tank treatment

Ye et al. *(52)* reported that a continuous in-line electrochemical treatment system was effective in controlling *L. monocytogenes* levels in brine tanks. An average D-value of 1.61 min was achieved at 7mA/cm^3 current in fresh brine (t = 0 h). In used brine (t = 20 h), the D-value was 2.5 min at 35mA/cm^3.

Chlorine dioxide

Chlorine dioxide (ClO_2) is not specifically approved for use on seafood products, but at a concentration not to exceed 3 ppm it is approved as an antimicrobial agent in water to wash poultry, fruits and vegetables *(12)*. One advantage of ClO_2, compared with chlorine, is that it is stable in a high organic environment, and retains some sanitizing capability up to pH 10.0. However, it is more expensive than liquid chlorine, and an on-site generating system is required *(50)*. Kim et al. *(34)* evaluated the effect of three different chlorine dioxide concentrations (40, 100, and 200 ppm available ClO_2) on reduction of bacterial numbers on red grouper (*Epinephelus morio*), salmon (*Salmo salar*), shrimp (*Penaeus aztecus*) and Calico scallops (*Aequipecten gibbus*). The ability of chlorine dioxide to reduce *L. monocytogenes* levels was not evaluated. The results indicate that chlorine dioxide reduced bacterial numbers at all concentrations, but was more effective at higher concentrations. However, concentrations of 100 and 200 ppm bleached the skin of red grouper and salmon.

L. MONOCYTOGENES CONTROL ON FINISHED PRODUCT

The cold smoking process, unlike hot smoking, does not include a listericidal kill step. Recent in-plant studies using molecular subtyping techniques indicate that the processing plant environment is responsible for most incidences of finished product contamination on both hot and cold smoked products (*3, 32, 39*). Contamination from the processing plant environment during or after processing appears to be the major source of finished product contamination for other RTE foods as well (*49*). Thus, finished products may need to be treated to eliminate *L. monocytogenes* even if steps have been taken to prevent post-processing contamination of finished products with *L. monocytogenes*. Cross contamination of RTE products with *L. monocytogenes* from the plant environment can be due to poor plant sanitation practices, poor personnel hygienic practices, poor food handling practices, etc.

Finished product testing

Finished product testing is not an essential part of a *L. monocytogenes* control program. Many manufacturers conduct product testing at the request of their customers. Manufacturers may also use periodic testing of finished products as confirmation that sanitation programs and other *L. monocytogenes* control measures are effective. When considering finished product testing, the implications of current government policies as well as finding low levels of contamination should be carefully considered *(30)*. Detailed information on finished product testing options is provided in another manuscript in this series.

Finished product treatments

Processors must consider options to minimize or prevent the growth of *L. monocytogenes* on finished products from the time they are produced until they are consumed. Although the amount and frequency of contamination is likely to be low for processors with effective *Listeria* controls, the potential for growth during the product's shelf life should be considered for some products. Information about treatment options that may be available to processors is summarized below. The practical application of these treatments may be limited by a variety of factors, including those listed for raw material treatments.

Freezing product

Freezing the finished product is an effective method to prevent the growth of *L. monocytogenes*. Freezing does not eliminate *L. monocytogenes* (if present) on the finished product, but *L. monocytogenes* will not grow in frozen storage. However, prolonged refrigerated storage of the finished product, after thawing, can result in growth of *L. monoctogenes* on the finished product. Freezing and prolonged frozen storage of finished product can also adversely affect the sensory properties of the product.

Natural growth inhibitors

Natural growth inhibitors such as nisin and ALTA™ 2341 are not currently approved for use in seafood, but some data indicate that these additives have potential to help control growth of *L. monocytogenes* on finished product. For example, in one study, smoked salmon slices were inoculated with a mixture of seven *L. monocytogenes* isolates (2.5 $\log_{10}$ CFU/g), treated with nisin (400 or 1250 IU/g) or ALTA™ 2341 (0.1 or 1%), packaged under vacuum or 100% CO_2 and then stored at 4°C for 28 days or 10° C for 9 days. Untreated (i.e., no added nisin or ALTA™ 2341) inoculated salmon fillets were also packaged and stored at 4°C for 28 days or 10° C for 9 days *(47)*. The results indicate that nisin and ALTA™ 2341 retarded growth of *L. monocytogenes* in the vacuum-packaged product. However, under 100% CO_2, *L. monocytogenes* growth was prevented for all nisin and ALTA™ 2341 treated samples *(47)*.

In another study, Nilsson et al., *(38)* added nisin (500 or 1000 IU/g) to cold smoked salmon inoculated with six strains of *L. monocytogenes* (~10^3 CFU/g), vacuum packaged the salmon and stored it at 5°C. Under vacuum-packaging conditions, growth of *L. monocytogenes* was delayed but not prevented (i.e., *L. monocytogenes* increased to 10^8 CFU/g in 8 days). However, packaging under 100% CO_2 with added nisin (500 or 1000 IU/g) resulted in a 1 to 2 log reduction in *L. monocytogenes* numbers and an 8 and 20 day lag phase, respectively *(38)*.

Chemical growth inhibitors

Pelroy et al. *(40)* used comminuted raw salmon, inoculated with 10 *L. monocytogenes*/g, to determine the effects of combinations of sodium lactate, sodium chloride, and sodium nitrite on *L. monocytogenes* growth. The samples were vacuum packaged and stored at 5° C or 10°C. The results indicate that a combination of 2% sodium lactate and 3% water phase salt (WPS) inhibited the growth of *L. monocytogenes* stored at 5°C for 50 days. At 10°C, total growth inhibition of *L. monocytogenes* for 35 days required 3% sodium lactate and 3% WPS, or 2% sodium lactate and 125 ppm sodium nitrite $NaNO_2$ *(40)*. However, industry experience suggest that it is difficult to achieve sufficient levels of sodium lactate (i.e., 2–3%) in the finished product to control *L. monocytogenes* growth. In the United States, $NaNO_2$ is approved for use only in smoked cured tunafish (10 ppm) and smoked cured salmon, chubs, sablefish, and shad, with concentrations not to exceed 200 ppm *(2)*.

Competitive lactic acid bacteria flora

The use of competitive lactic acid bacteria to control growth of *L. monocytogenes* on smoked fish products has not been given GRAS status by the FDA; however, some data indicates that it has potential for controlling growth of *L. monocytogenes* on finished product. For example, in one study, *Lactobacillus sake* strain LKES5 and four strains of *Carnobacterium piscicola* were inoculated on cold smoked salmon *(37)*. The authors reported that inoculum levels of ~2 × 10^6 CFU/g of a bacteriocin-producing strain of *Carnobacterium piscicola* (A9b) and a non bacteriocin-producing strain (A10a) caused no undesirable sensory changes, and controlled the growth in cold smoked salmon of *L. monocytogenes* strain O157 inoculated at ~2 × 10^2 CFU/g. However, *L. sake* LKES5 caused strong sulfurous flavors in the cold smoked salmon product. In a separate study, Duffes et al. *(17)* reported that *Carnobacterium pisicola* V1 was bactericidal and *C. divergens* V41 was bacteriostatic to *L. monocytogenes* in vacuum packaged cold smoked salmon stored at 4°C and 8°C, respectively.

Irradiation

Research conducted on seafood products since the mid-1950s has demonstrated that ionizing radiation can help maintain the safety, quality and freshness of seafood products *(1)*. The World Health Organization (WHO) reported that irradiation is an effective process that can improve the safety and quality of our food supply *(51)*. The Centers for Disease Control and Prevention (CDC) stated that irradiation can prevent foodborne illnesses, and that overwhelming evidence indicates that when irradiated, foods are not made dangerous and the nutritional values remain unchanged *(9)*. In the United States, the FDA has approved irradiation for wheat flour, white potatoes, pork, fruits and vegetables, poultry, and fresh and frozen uncooked red meat, but not seafood *(9)*. Petitions have been submitted to the FDA to approve irradiation of molluscan shellfish (1999) and crustaceans (2000), but approval has not been granted *(15)*.

SUMMARY

Listeria monocytogenes can be a contaminant on raw fish and can be present on finished products if there is no kill step in its processing. The presence of *L. monocytogenes* on finished product can also occur through post-processing contamination from the plant environment, and/or from poor personnel hygiene or poor food handling practices. Raw product testing or pathogen reduction treatments can help to reduce or eliminate *L. monocytogenes* on raw material and possibly reduce the levels of *L. monocytogenes* in the plant environment. Similarly, pathogen reduction treatments can also reduce or eliminate *L. monocytogenes* on finished product.

A number of treatments are approved by the FDA for use on raw materials or on finished product. They are also likely to be available to most smoked seafood processors and have been demonstrated to control or reduce numbers of *L. monocytogenes*. However, they require validation under actual commercial processing conditions. Treatments for raw material include: (1) washing raw fish with water containing chlorine; and (2) treating raw fish with calcium hydroxide solution (pH 12). Treatments for finished product include: (1) freezing to stop growth; and (2) addition of approved chemical growth inhibitors.

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