ISOLATION, DETECTION, AND PARTIAL CHARACTERIZATION OF *LISTERIA* IN SMOKED SEAFOOD

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BY

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A thesis submitted to the School of Graduate Studies
in partial fulfilment of the requirements
for the degree of Master of Science

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August, 1992

St. John's Newfoundland

ISBN 0-315-82632-0

ABSTRACT

Over a one-year period, 258 samples of smoked fish products were obtained from retail outlets in Newfoundland and processed for *Listeria* using the listeria isolation protocol adopted by Health and Welfare Canada. Direct plating was also carried out. *Listeria* spp. were isolated from 43 of 258 (16.7%) samples, with hot smoked products yielding 25.4% (36/142) of the isolates, and cold smoked products yielding 6% (7/116). Cod had the highest rate of *Listeria* contamination at 46.7%. Of the 43 *Listeria* spp. isolated, 18 (41.9%) were *L. innocua*, 13 (30.2%) were *L. welshimeri*, and 12 (27.7%) were *L. monocytogenes*. Eleven isolates of *L. monocytogenes* were serotype 1/2, and one was 4b. There was an indication that *Listeria* spp., particularly *L. monocytogenes*, occurred more commonly during the cooler months. All *Listeria* isolates were detected after 48h primary enrichment. The secondary enrichment step failed to enhance the isolation rate. Direct plating yielded only 26% of the total isolates. PALCAM, a listeria selective agar, proved more efficient in recovering *Listeria* spp. from smoked fish products.

Multilocus enzyme electrophoresis is an epidemiological tool applied in this study to *Listeria* strains isolated from smoked seafoods to determine if the strains were from a common contamination source. The 57 strains analyzed resulted in 57

distinct electrophoretic types (ETs) indicating many sources of contamination. Major divisions among the four different Listeria spp. (L. innocua, L. monocytogenes, L. welshimeri, L. ivanovii) occurred at genetic distances of 0.72 to 0.85. All 14 enzymes were polymorphic and had a mean genetic diversity of 0.683. The L. monocytogenes strains were not of a single clone nor were they of the same ETs as the two human isolates. This indicates the great need for better sanitation practices in the food processing environment.

Experiments were conducted to study the effects of the cold smoking process and storage on the organism in artificially inoculated cod fillets. In three trials, cod fillets were surface inoculated with L. monocytogenes, cold smoked, vacuum-packaged, stored at 4°C for 3 weeks or at -20°C for 3 months, and quantitatively processed for Listeria using the three-tube most probable number method (MPN). The initial inoculation concentrations were 10^5 MPN/g in trial 1, 10^2 MPN/g in trial 2, and 10^0 MPN/g in trial 3. The organism, relatively stable during the smoking process, increased with storage at 4°C, but decreased in cell number at -20°C. Growth was very slow in trial 3 possibly because of the low inoculum level. Hence, L. monocytogenes contamination in smoked seafoods, not cooked prior to consumption, may pose a health risk to the consumer.

ACKNOWLEDGMENTS

It is my pleasure to express my gratitude to my supervisor, Dr. T. Patel, for his guidance, patience, advice and generosity during the course of this research project. I am very grateful to Mr. Kevin Smart, owner of Seaside Fisheries, for his cooperation, time and generosity. I would also like to thank Mr. Terry St. George, Plant Manager of Seaside Fisheries, for his time and cooperation. Furthermore, my sincere appreciation to: Mr. Melvin Peach at National Sea Products Ltd.; Mr. Lem Ried at Fishery Products International Ltd.; Mr. Ralph Simmons at Golden Shell Fisheries Ltd., and Mr. Fred Hall and Mr. John Isinick at the Nain Fish Plant in Labrador, for their hospitality and time taken to discuss the smoking process at the individual plants. A special thanks is extended to the Marine Institute for lease of equipment and for its help during the period of this project.

I would like to express sincere thanks to Mr. Chris Vriezen and Ms. Annette Morgan for their help with the electrophoresis project and Dr. W.S. Davidson for the use of his laboratory to perform the work. Also, Dr. K. Eggar, Biology Dept., for his contribution with the statistical analysis associated with the electrophoresis project.

Sincere appreciation is forwarded to Dr. S. Ratnam for his patience, guidance, time, and generosity during the course

of this project. A special thanks is rendered to Ms. Kathyrn Bernard and Margaret Bellefeuille at LCDC for their participation in the serotyping of the Listeria strains.

I would like to thank my supervisory committee, Dr. A.M. Martin and Dr. R. Hoover, for their time, guidance and suggestions throughout this project.

Finally, I would like to thank Mr. Jeff Freeman, coordinator of the IRAP-H project, Centre for Fisheries Innovation (CFI), Graduate studies and Department of Fisheries and Oceans for the financial support which made this project a reality.

I would like to devote this thesis to my parents, Mrs. Viola and Ron Dillon, for their support, encouragement, direction and patience over the years of attending Memorial University; I love them dearly.

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ABBREVIATIONS

ADP - adenosine diphosphate

CFU - colony forming units

EDTA - ethylenediaminetetraacetic acid

HPB - Health Protection Branch

LEB - Listeria Enrichment Broth

LPM - Lithium chloride-phenylethanol moxalactam agar

MEE - multilocus enzyme electrophoresis

MTT - dimethylthiazol tetrazolium

NAD - nicotinamide adenine dinucleotide

NADP - nicotinamide adenine dinucleotide phosphate

PALCAM - polymyxin B-acriflavin-lithium chloride - ceftazidime - aesculin - mannitol agar

PMS - phenazine methosulfate

°s - degree saturation of brine

TFTC - too few too count; that is < 30 colonies per plate

TNTC - too numerous too count; that is > 300 colonies per plate

TSA - trypticase soy agar

TSA-YE - trypticase soy agar with yeast extract

TSB-YE - trypticase soy broth with yeast extract

TTC - 2,3,5 - triphenyltetrazolium chloride

USFDA - United States Food and Drug Administration

UVM - University of Vermonth Massauchetts

CHAPTER 1

INTRODUCTION

1.1.0 History

According to the Health Protection Branch of Health and Welfare Canada, Listeria monocytogenes is an environmental contaminant. Food contaminated with Listeria looks, smells, and tastes normal. The World Health Organization has determined that the total elimination of Listeria from all food may be virtually impossible (Moore, 1988).

Listeriosis is the term used to describe the foodborne disease caused by the pathogen L. monocytogenes. Listeria was first seen in tissue sections from patients as early as 1891 by Hayem in France and was first isolated in 1911 in Sweden by Hülphers from rabbit liver; he called it Bacillus hepatis. Listeriosis was first recognized in sheep in 1925. L. monocytogenes was first identified in 1926 by Murray and associates and was originally named Bacterium monocytogenes (Gray and Killinger, 1966; Forsyth, 1991). Harvey Pirie in South Africa discovered the same organism in rodents and called it Listerella hepatolytica in 1927. The first confirmed listeriosis was reported in 1929 by Nyfeldt; he called the organism Bacterium monocytogenes hominis (Gray and Killinger, 1966; Wehr, 1987). However, the organism was

finally given its current name L. monocytogenes in 1940 (Gellin and Broome, 1989; Forsyth, 1991). The first confirmed case of listeriosis in Canada was in a pregnant Russian woman in 1951 who had emigrated from Russia in the previous year (Farber and Losos, 1988). The organism was first regarded as a foodborne pathogen in 1981 (Schlech et al., 1983). Furthermore, L. monocytogenes has been recorded as a reportable disease in Canada since 1989 (Health and Welfare Canada, 1991).

1.1.1 Characteristics

L. monocytogenes is a gram-positive, non-sporeforming rod, exhibiting tumbling motility. It is the only truly recognized pathogen in the genus (Health and Welfare Canada, 1990). However, L. ivanovii and L. seeligeri have been isolated from ill patients as well (Bille and Doyle, 1991; Agriculture Canada, 1990). Both L. monocytogenes and L. ivanovii are pathogenic to mice. The other species include: L. innocua, L. welshimeri, L. grayi, and L. murrayi. Listeria appears to be more heat resistant than the normal vegetative microbes (Moore, 1988; Farber et al., 1988a; 1988b; Health and Welfare Canada, 1990). Controversy exists over whether L. monocytogenes is temperature resistant, particularly during the pasteurization of milk (Bunning et al., 1986; Doyle et al., 1987). The temperature range for growth is -0.4 to 50°C,

with a normal pH range of 5 to 9.6. Under ideal conditions, the organism can withstand a water activity of 0.925. The infectious dose is unknown but it is suspected that it is less than 1000 total organisms. It has been implied that infections occur more often in the summer and fall (Agriculture Canada, 1990; Health and Welfare Canada, 1988; 1989; 1991). The bacterium will grow in salt concentrations up to 10% and will survive in concentrations up to 25% (Health and Welfare Canada, 1990). Freezing has no detrimental effect on the organism (Weagant et al., 1988). Listed in Table 1.0 are biochemical reactions which differentiate the various species (FDA, 1988). Of the 16 recognized serotypes (Table 1.1), 1/2a, 1/2b and 4b account for more than 90% of the cases reported worldwide (Bille and Doyle, 1991; Farber and Peterkin, 1991). Tests for pathogenicity include the ability to cause keratoconjunctivitis in guinea pigs (Anton's test), toxicity in chick embryos, and death in mice (Agriculture Canada, 1990; FDA, 1988). The bacterium L. monocytogenes is ubitiqious in nature. The organism can be present in food in an intracellular state (Forsyth, 1991).

1.1.2 Symptoms of listeriosis

Symptoms of listeriosis involve meningitis, central nervous system infection, septicemia, abortions, still births, and premature labour in the more serious cases. In milder

cases, symptoms are flu-like (Health and Welfare Canada, 1990; Farber and Losos, 1988; van Netten et al., 1988; Cole et al., 1990). The target populations are pregnant women and their fetuses, newborns, elderly, and immunosuppressed. It has been documented that healthy individuals have been also ill due to listeriosis (PHLS Communicable Disease Surveillance Centre, 1990). It has been estimated that L. monocytogenes asymptomatic carriers may vary from 5% in general to 29% of poultry workers, and upto 77% in public health workers (Carnie, 1991). Treatment is in the form of ampicillin with or without an aminoglycoside (Farber and Losos, 1988; Russell, 1991).

1.1.3 Documented outbreaks

The fatality rate has averaged 30% in foodborne outbreaks (Health and Welfare Canada, 1990). Documented outbreaks of listeriosis associated with food in North America began in 1979 in Boston (sources: lettuce, celery, tomatoes) with 5 fatalities from 20 cases (Ho et al., 1986). Cabbage was responsible for the Nova Scotia outbreak in 1981 with 17 deaths from 41 cases (Schlech et al., 1983). A third outbreak occurred in 1983 in Massachusetts from pasteurized milk. From 49 cases, there were 14 fatalities (Fleming et al., 1985). In 1985, an outbreak involving Mexican-style soft cheese in California caused 48 deaths from 142 cases (Linnan et al.,

1988). All four of the outbreaks were caused by L. monocytogenes serotype 4b.

Other documented outbreaks include New Zealand, 1980, of 29 cases there were 9 deaths (Lennon et al., 1984), epidemiologically implicating shellfish and raw fish. In Canton de Vaud, Switzerland, 1983-1987, there were 122 cases with 31 deaths implicating Jalisco cheese (Bille, 1990; Bula et al., 1988). In Philadelphia (1986-1987), there were 36 cases of listeriosis with 16 fatalities epidemiologically linked to ice-cream and salami (Schwartz et al., 1989). In Connecticut, 1989, 9 cases of listeriosis involved 1 death and shrimp was linked to the cause of infection (Riedo et al., 1990). In the United Kingdom (1987-1989), there were more than 300 cases epidemiologically linked to paté (McLauchlin, 1991).

1.1.4 Source of the organism

Listeria is widespread in the environment (van Netten et al., 1988) and therefore, is virtually impossible to eradicate. It is an environmental contaminant found in soil, vegetation, water, sewage, silage, food, humans, and animals. Human carriers exist in up to 5% of the general population as well (Health and Welfare Canada, 1990).

1.2 *Listeria* in Seafoods

1.2.1 *Listeria* in the Fish Environment - Water

As early as 1966, Gray and Killinger (1966) reported *L. monocytogenes* to be present in pond reared rainbow trout. Watkins and Sleath (1981) isolated *L. monocytogenes* from raw and treated sewage, effluent of abattoirs and poultry packing plants, surface waters, including rivers, lakes and canals. One hypothesized cycle of infection for *Listeria* (Brackett, 1988) indicated that contaminated water may infect fish and shellfish which in turn may infect humans through consumption of the contaminated food product (Fig. 1.0).

Listeria species were detected by Colburn et al. (1990) in 81% of freshwater and 33% of marine water samples. *L. monocytogenes* was isolated from 62% of all water samples and was the most predominant of the species collected in Humboldt-Arcata Bay, California. *Listeria* sp. were recovered from 30.4% of sediment samples collected at the same locations with 17.4% of the species being *L. monocytogenes*. *L. innocua* was also detected from 1 of 35 oysters collected during the same sampling, resulting in an incidence of 2.8% (Colburn et al., 1990).

Water samples collected along the U.S. Gulf Coast showed a 5% positive result for *L. innocua* and *L. monocytogenes*. The greatest incidence of *Listeria* sp. from the water occurred at

water temperatures ≤ 20°C. Salinity had no effect on the recovery of Listeria from the estuarine water nor on shrimp sampled from the area. Oysters, however, were not identified as a potential source of Listeria sp. (Motes, 1991). Other investigators (Buchanan et al., 1989; Weagant et al., 1988) also did not recover Listeria from oysters. Approximately 11% of the shrimp sampled were positive for L. monocytogenes (Motes, 1991).

1.2.2 Prevalence of Listeria in seafoods

Several scientists have surveyed the retail markets for seafood products and tested for the prevalence of Listeria. Fuchs and Surendran (1989) sampled thirty-five tropical fish and fishery products. Three of ten samples of fresh products were positive and five of fourteen frozen products also tested positive for Listeria. The fresh products included shrimp and pearl spot, and the frozen contaminated products were seer, peeled prawn meat and black pomfret. No Listeria was recovered from dried, salted fish. More importantly, the Listeria sp. isolated from all samples was L. innocua.

In another study, Listeria cells were isolated from domestic and imported, frozen seafood products (Weagant et al., 1988). Approximately, 61% of the samples tested were positive for Listeria sp. and 26% were positive for L. monocytogenes. The frozen seafood products found to harbour

Listeria were: raw and cooked shrimp; cooked crabmeat; lobster tail; finfish; and surimi based seafoods. The presence of Listeria in frozen products further supports the statement that freezing has no significant effect on the organism (Seeliger and Jones, 1986). Existence of Listeria in frozen seafood previously cooked emphasizes possible post-process contamination. However, the organism is known to be more heat resistant than normal vegetative cells (Health and Welfare Canada, 1990) and may survive the cooking process.

A fish product, ceviche, which is eaten without cooking is a common dish in South America. Raw fish was marinated in lemon juice for at least one hour prior to consumption. The pH of the final product ranged from 4.2 to 5.1 (Fuchs and Sirvas, 1991). The minimum pH to initiate growth of L. monocytogenes in Brain Heart Infusion (BHI) was 4.3-5.2 at 30°C and 5-5.7 at 4°C, depending upon the acidulent used (Farber et al., 1989). Fuchs and Sirvas (1991) surveyed the incidence of Listeria sp. in ceviche products bought from markets in Lima and Callao, Peru. Approximately, 75% of the samples tested contained L. innocua while L. monocytogenes cells were isolated from only 9% of the samples analyzed. Consequently, Listeria can survive the short exposure time to the acidic marinade.

Noah et al. (1991) isolated Listeria from naturally contaminated seafoods consisting of frozen: lobster tails;

shrimp; prawn; breaded shrimp; whole fish; and fish fillets. The processed samples included cooked and refrigerated or frozen crabmeats. Of 211 composites, 60 were positive for Listeria sp. (28%). Approximately, 49% of the raw seafoods were positive for Listeria and only 20% of the processed seafoods contained Listeria.

Wong et al. (1990) conducted a survey in Taiwan of various foods, including seafoods, for the prevalence of Listeria. In that study L. monocytogenes was isolated from 10.5% of the seafoods sampled from local markets. Of the seafoods sampled, frozen and refrigerated fishes, squids, and crabs, L. monocytogenes was isolated only from fish and squid samples. It was noted that the seafood isolates in two other foods, showed higher haemolytic activity than those from other samples when analyzed by both blood agar plates and the microplate method; both yielded similar results. Haemolytic activity has been shown to be mainly related to the virulence factor associated with L. monocytogenes (Gaillard et al., 1986; Kreft et al., 1989). The serotypes from seafood were all types 1 and 4 with the majority being of type 1 (Wong et al., 1990).

Brackett and Beuchat (1990) studied the growth patterns of L. monocytogenes in crabmeat stored at 5 and 10°C to determine the effects, if any, on pathogenicity. Crabmeat was inoculated with 10^4 CFU/g and incubated for 2 weeks. Results

indicated no change in pathogenicity. Also, L. monocytogenes grew well on crabmeat during refrigeration, enhancing the possibility of contaminated seafood acting as a vehicle of infection. Populations reached 4.9×10^6 and 2.0×10^9 for 5 and 10°C, respectively.

Buchanan and associates (1989) found seafood to have the second highest incidence rate of Listeria next to meats at 28%. Listeria was isolated from uncooked, frozen shrimp, cooked and non-pasteurized crabmeat, refrigerated flounder, refrigerated monkfish and refrigerated catfish. The species found were L. innocua, L. welshimeri, and L. monocytogenes. The L. monocytogenes isolates were restricted to two finfish samples with an incidence rate of 11%.

Due to the presence of L. monocytogenes, several fish products have been recalled: frozen canned cooked crabmeat (FDA, 1991); cooked shrimp; frozen cooked shrimp (Anon., 1988b); canned, frozen, fresh and imitation crab meat; smoked salmon; imitation scallops; frozen canned lobster; and surimi products (Anon., 1988a; Farber and Peterkin, 1991).

Jemmi (1990a) detected L. monocytogenes in 12.2% of smoked and fermented fish. Approximately 8.9% of hot smoked fish, 13.6% of cold smoked fish, and 25.8% of fermented fish were contaminated with the pathogenic organism. Serotypes 1/2b , 1/2a, and 4b were implicated in 58.6%, 19.8% and 14.4% of the samples, respectively. The author also noted in

another study of 100 smoked fish that 24% of the smoked samples were contaminated with L. monocytogenes (Jemmi, 1990b). Forsyth (1991) reported this bacterium in raw and cooked, finger shrimps and shellfish in England and Wales. Nitcheva et al. (1990) also found fish to be contaminated with Listeria at an incidence rate of 9.1%. Five producers of smoked fish from areas such as Montreal (Quebec), Vancouver (British Columbia), and Seattle (Washington) have been shut down because of L. monocytogenes contamination. Almost overnight, Listeria has become the number one problem for the entire smoked fish industry (Hadeler, 1990).

1.2.3 Effects of heat on Listeria in seafoods

As previously stated, L. monocytogenes is known to be more heat tolerant than other mesophyllic vegetative bacteria. The heat resistance of Listeria has been controversial particularly in the area of commercial pasteurization of milk. Some authors have suggested that the pathogen survives high-temperature-short-time (HTST) pasteurization which involves heat at 71.7°C for 15s (Doyle et al., 1987). Others have suggested that it can not survive the pasteurization treatment (Bradshaw et al., 1991; Farber et al., 1988a; Farber et al., 1988b; Lovett et al., 1990). The conflicting results have led to a series of thermal resistance studies in other foods, one being seafoods.

Thermal death times for Listeria in crabmeat have been evaluated (Harrison and Huang, 1990). Blue crabs were inoculated with 10^7 cells/g of L. monocytogenes before placing 7.5 g into sausage casings. These were placed at 50, 55 and 60°c in preheated, recirculating waterbaths. D-values based on enumeration of colonies on TSA plates were 40.43, 12.00 and 2.61 min at 50, 55 and 60°C, respectively. When using modified Vogel-Johnson agar, the D-values were 34.48, 9.18 and 1.31 min at 50, 55 and 60°C, respectively. Z-values of 8.40 and 6.99°C were derived from the TSA and modified Vogel-Johnson agar data, respectively. Hence, injured cells were being inhibited on the selective agar. The current pasteurization process for crabmeat in steel and aluminium cans is 30 min at 85°C with a Z-value of 8.89°C for the commercial pasteurization, therefore the commercial process would provide a D-process of several hundred fold. As a result, the process is excessively adequate to inactivate Listeria.

McCarthy et al. (1990) tested the recovery of heat stressed L. monocytogenes from artificially and naturally contaminated shrimp. Listeria was not recovered from the naturally contaminated shrimp containing 10^3-10^5 cells/g boiled at 100°C for 1, 3, or 5 min. However, Listeria was detected in experimentally, internally infected shrimp inoculated with 10^5 cells/g when boiled up to 5 min. The assumption was made

that either the naturally contaminated shrimp were externally contaminated or naturally occurring Listeria is less heat resistant in its natural environment. L. monocytogenes cells did not survive the cook-freeze-thaw process. It has been implied that freezing and heating had a synergistic effect on the lethality of L. monocytogenes; heat injured cells were more susceptible to the additional stress of freezing than uninjured cells. Therefore, when such products undergo heating and freezing, but are still contaminated with Listeria sp., post process contamination may be the most probable mode of infection. However, Listeria has been isolated from frozen, cooked seafood products as previously discussed (Weagant et al., 1988) and, therefore, the additive effect causing lethality is questionable.

In a final study on this topic heated cells of L. monocytogenes (57.8°C, 5 min) were inoculated into surimi , cooked crabmeat, and cooked shrimp. Also, unheated cells were added to cooked and raw shrimp. Listeria was recovered from all the seafood samples and the study revealed the FDA protocol for isolation of Listeria superior to the USFDA protocol when isolating heat-injured cells and vice versa when isolating non-injured cells with high background populations (Lovett et al., 1991).

1.2.4 Growth patterns of *Listeria* in inoculated seafoods

Heat and freeze-injured as well as uninjured *L. monocytogenes* were able to proliferate in inoculated raw oysters (Brackett and Beuchat, 1989; Cassiday et al., 1989); however, in some studies, *Listeria* was rarely isolated from untreated samples (Colburn et al., 1990; Motes, 1991). In another study of fresh crabmeat, frozen crab, frozen langostinoes, and frozen shrimp all supported growth when inoculated with *Listeria* (Hitchins and Tran, 1990). When shrimp, crabmeat, surimi and whitefish were artificially infected with 10^3 cells/g and held at 7°C for 14 days, the concentrations of bacteria reached 10^8 cells/g with no detectable lag ($\leq$ 2 days) (Miller et al., 1990).

Guyer and Jemmi (1991) inoculated salmon with *L. monocytogenes* then cold smoked the experimentally contaminated fish to study the effects of the smoking process on the organism. Three trials were performed with salmon in trials 1, 2 and 3 having inoculation levels of 10^4, 10^1 and 10^2 MPN/g, respectively. It was seen that during the smoking process the numbers stabilized until they were stored at 4 and 10°C for 1 month where growth was initiated in 2 of 3 trials. There was a 2.5 $\log_{10}$ MPN/g increase in trial 1 and a 4.5 $\log_{10}$ MPN/g increase in trial 3. *L. monocytogenes* cells in trial 2 remained stable probably because of the low infections dose used in the salmon. Also, the inoculum used in this trial was

a reference strain as opposed to a wild type originally isolated from salmon, and the reference stain may not have grown as well. This emphasizes the importance of the prevention of pre and post-process contamination.

1.2.5 Recovery methodology of *Listeria* in seafoods

For the recovery of *L. monocytogenes* from foods and environmental samples, Gray and Killinger (1966) proposed the use of cold enrichment. According to their procedure, samples were diluted in nutrient broth and stored at 4°C from which portions were transferred to selective media at 24h and on a weekly basis thereafter and incubated at 35°C. Incubation at 4°C apparently suppresses the growth of most microorganisms, but *Listeria* spp multiply at a very slow rate. One disadvantage of this procedure is that it took up to 3 months to detect the organism.

More recently, the time taken to isolate the organism has been reduced dramatically by use of the incorporation of specific selective agents into enrichment media. The two most currently used protocols in North America are the U.S. Food and Drug Administration (USFDA) method and the U.S. Department of Agriculture (USDA) method (Brackett and Beuchat, 1989; Pusch, 1989). The former, originally developed by Lovett (1987), is used for isolating *L. monocytogenes* from dairy products, seafoods, vegetables, and factory environmental samples. The USDA method, originally developed by McClain and Lee (1987), is recommended for isolation of the foodborne pathogen from meats and poultry products. The main difference

between the two methods is the choice of enrichment broths and isolation agars.

For isolating L. monocytogenes from seafoods the most often selected method is the USFDA protocol (Lovett, 1987). This protocol was later slightly modified by Lovett and Hitchins (1988) and the current USFDA method in practice is that of Hitchins (1990). The Canadian version of the USFDA protocol (Warburton and Farber, 1990) is again a revised version of the procedure published by Hitchins (1990). The first modified USFDA protocol used one enrichment culture, followed by streaking onto the surface of lithium chloride-phenylethanol-moxalactam (LPM) plating media in addition to the usual modified McBride agar medium. The enrichment culture was sampled at 1 and 2 days (Lovett and Hitchins, 1988). The final change was the addition of the listeria selective agar, Oxford and the deletion of McBride agar (Hitchins, 1990). The Canadian version (Warburton and Farber, 1990) involves a two stage enrichment: primary enrichment using enrichment broth (EB) and a secondary using Modified Fraser broth (FB). The selective agars onto which the samples are to be transferred are LPM and Oxford agar. The Fraser broth was developed by modification of the USDA secondary enrichment broth with the addition of lithium chloride and ferric ammonium citrate. Ferric ions react with 6,7-dihydroxycoumarin, the product of esculin hydrolysis, to form a black precipitate. This Fraser broth has also replaced the enrichment broth II as the preferred secondary enrichment of meat and poultry products (Fraser and Sperber, 1988). The

Modified Fraser broth simply has an increase in the amount of acriflavin to make it more selective (Warburton et al., 1991a).

1.2.6 Economic impact of *Listeria* in seafoods

Seafoods were implicated as the leading food in the transmission of foodborne outbreaks for the period 1977-1984 in the United States (Bryan, 1988; Zottola and Smith, 1991). Seafoods contributed approximately 24.8% to foodborne outbreaks while meats ranked second at 23.3% and milk and milk products occurred at 4.2%. Furthermore, shellfish and fish again were the leading foods implicated in foodborne disease outbreaks in the United States in 1983-1987, with an incidence of 22.4%. Meats followed at 13.1%, and dairy products at 5.4%. However, Liston (1990) reported fish and shellfish to be responsible for 10.5% of all foodborne outbreaks and 3.6% of all cases reported for 1978-1987. It is also suggested that this data might have grossly under represented the actual totals for seafood borne illnesses as well as other food borne diseases.

The estimated cost for foodborne illnesses, caused by bacteria in 1987 in the U.S. was 4.8 billion dollars (Zottola and Smith, 1991). This estimate was based on medical costs and productivity losses due to *Salmonella* and *Listeria* outbreaks, and included extrapolated costs for other bacteria based on epidemiological data. *L. monocytogenes* ranked fifth on the descending cost scale with 1,581 recorded cases resulting in a 213 million dollar loss (Zottola and Smith,

1991). Industry costs for contamination of a product with L. monocytogenes can include the cost of: recalling and destroying; reduced consumer demand; investigating the source of the contamination; clean-up; changes in production; liability suits; product spoilage; disrupted work schedules (Miller et al., 1990); and, finally, plant closure.

1.3 Listeria in other foods

Listeria has been recognized as a foodborne pathogen since 1981 (Schlech et al., 1983) and, from that point, there has been tremendous investigations into the organism's growth in foods. Dairy products attracted the widest attention because of its implications in listeriosis outbreaks. L. monocytogenes, L. innocua, and L. welshimeri have been recovered from raw milk (Hayes et al., 1986; Lovett et al., 1987; Beckers et al., 1987; Davidson et al., 1989; Liewen and Plautz, 1988; Slade et al., 1988; Farber et al., 1988a;b), ice-cream (Anon., 1986b;c), and various types of cheese (Anon. 1986a;d; Kvenberg, 1988; Azadian et al., 1989). Vegetables, implicated as vehicles of Listeria contamination in listeriosis outbreaks were also analyzed. Heisich et al.(1989) detected L. innocua in cucumbers, lettuce, mushrooms, potatoes, and radishes; L. welshmeri in cucumbers, potatoes, and radishes; L. monocytogenes in cabbage, cucumbers, potatoes and radishes; finally L. seeligeri was noted in cabbage and radishes. L. monocytogenes and L. innocua were also isolated from prepacked salads (Sizmur and Walker, 1988). Even though meats have not been documented as

sources of contamination resulting in outbreaks, L. monocytogenes and other Listeria species have been isolated from raw, fresh, frozen and processed meat products (Lowry and Tiong, 1988; Skovgaard and Morgen, 1988; Kerr et al., 1990; Barbuti et al., 1989; Johnson et al., 1990; Varabioff, 1990; Kerr et al., 1988a;b; Kerr et al., 1990; Genigeorgis et al., 1990; Bailey et al., 1989; Beer and Yoeman, 1990; Schwartz et al., 1988; Tiwari and Aldenrath, 1990; Gitter, 1976; Nitcheva et al., 1990). It is evident that Listeria is not only ubiquitous in nature but is also ubiquitous in foods.

1.4 Listeriosis in Canada

In 1987, a surveillance program was established within Canada to determine the magnitude of human listeriosis in Canada and the role of food in its transmission by the Bureau of Communicable Disease Epidemiology. In 1987 there was a total of 44 cases of reported listeriosis. The incidence pattern indicated a higher rate of listeriosis in the fall (Health and Welfare Canada, 1988). The highest incidence rate was in the neonate followed by the elderly with an overall case fatality rate of 49%.

In 1988, 60 cases were identified with a total case fatality rate of 40%. Serotype 1/2b contributed to 42% followed by serotype 4b at 29%. Other serotypes causing disease were L. monocytogenes 1/2a, 3a, 3b, and 4c. From Newfoundland, 4 cases were reported; this is a rate of 7 per million of population, the highest across Canada. Again, the highest rate of infection occurred in neonates. Furthermore,

onset of disease showed a higher incidence rate during the fall (Health and Welfare Canada, 1989).

Approximately 63 cases were reported in 1989 with a case fatality rate of 36.5%. The highest number of cases were reported in the fall and none were reported from Newfoundland. The incidence rate was again highest in neonates followed by the elderly. Serotypes 1/2 resulted at 54.3% and serotype 4b at 45.7%. Multilocus enzyme electrophoresis for the most part for isolates in 1988 and 1989 indicated listeriosis was associated with a heterogeneous population of L. monocytogenes (Health and Welfare Canada, 1991).

Surprisingly, healthy individuals are capable of carrying L. monocytogenes in their intestinal tracts (Agriculture Canada, 1990). Listeriosis can also occur as a result of cross infection or cross contamination. The bacterium is not a normal inhabitant of the gut but it can be carried there. Furthermore, L. monocytogenes, L. ivanovii, L. innocua, and L. seeligeri have all been identified from the gut (Forsyth, 1991).

1.5 Smoking process

Smoking has evolved over the centuries; it has changed from a traditional process to a more modern one in the late 20th century. Refrigeration technology aided in the transfer from a well preserved smoked product to a refrigerated product smoking used for mostly flavour. Smoke houses evolved from a traditional smoking kiln constructed of brick in the form of large oversize chimneys in which the fish were hung, fires

ignited on the floor and the success of the venture dependent on the experience of the operator and the weather conditions. By 1939 the mechanical kiln was in use. The Torry Research Station in Aberdeen, Scotland, developed this mechanical kiln and called it the Torry kiln. Eventually the more modern smoker developed with automatic microprocessors or semi-automatic control methods into the system. The latest models are the Afos - English model and Atmos - German model, smokers.

1.5.1 Hot and cold smoke

There are two types of smoking processes: cold and hot. The processes vary only in the temperature at which the products are smoked. Any type of fish can be smoked and is prepared either round, dressed, split, filleted or cut as a steak. The fish can be dry salted or brined. This stage was originally used for its preservation effect but today the salt provides flavour, a change in texture (firmer), and more importantly salt allows for the formation of a pellicle. Brine strength is usually between 60 - 80 °s because greater than this may cause salt to crystallize out on the flesh during drying if not properly rinsed. Several factors affect the penetration of salt: the higher the fat content of the fish, the slower the salt penetration; the thicker the fillet, the slower the penetration of salt as it reaches the centre. Salt penetration is slower at lower temperatures. The stronger the brine, the quicker the penetration; firm resilient fish will not take up salt as quickly; frozen or

damaged fish will take up salt much faster than fresh high quality fish. The salted fish are then racked for 2 1\2 - 3 h at plant temperature or overnight at 1-4°C. The dried fish are then smoked. Smoke production is most often produced by the incomplete combustion of wood usually in the form of sawdust or woodchips. The smoke is made up of vapours and particles; particles contribute nothing to the smoking process. There are 200-400 chemicals in the vapours which cause bactericidal effects (Churchill et al., 1989). During this stage the fish is dried and the chemical constituents of the smoke are deposited on the fish. In a well smoked product, over 90% of the bacteria originally present are killed off by the smoke. The chemicals most commonly found in wood smoke are carbonyls, organic acids, phenols, organic bases, alcohols, hydrocarbons, and such gases as CO_2, CO, O_2, N_2, and N_2O. Beneficial effects include formation of characteristic colour and flavour, as well as bactericidal and antioxidative properties (Daun, 1979). The actual bactericidal action results from the combined effects of heating, drying, and the chemical components of the smoke with the latter playing a more significant role. When found on the surface, smoke constituents have been found to prevent spore formation and growth of many bacteria and fungi and to inhibit viral activities (Asita and Campbell, 1990). Bactericidal properties of smoke components have been found in formaldehyde, acetic acid, creosote, and high-boiling phenols (Messina et al., 1988).

Two types of woods are involved: hardwood (maple, oak,

birch, hickory); and softwood (pine, fir, spruce). Softwoods colour fish more rapidly but impart a more resinous flavour to the fish. The cold smoke is normally carried out at a temperature of 28°C but a hot smoke involves three stages: 1) tempering at 30°C for 1\2 - 1 h; 2) heating at 50°C for 1 - 1 1\2 h; 3) cooking at 80°C for 1\2 - 1 1\2 h. After smoking, products are cooled to room temperature (1-2 h) and then placed in the chill room at 1-4°C either for 12 h or overnight. Messina et al., (1988), have reported liquid smoke (0.25-0.5 %) to be effective in the control of L. monocytogenes in processed meat products. The bacterial population decreased by greater than 99.9% in the vacuum-packaged liquid smoked products stored at 4°C for 72 h.

1.6 Overview

L. monocytogenes is the only one of the seven Listeria species known to be pathogenic to man. L. innocua, L. ivanovii, and L. seeligeri have all been reported to cause infection in animals and man. L. murrayi, L. grayi, and L. welshimeri have not been implicated in listeriosis infections. L. ivanovii is mostly restricted to causing disease in animals and L. innocua has been reported in two such infections (Rocourt and Seeligeri, 1985). However, L. ivanovii has been noted in two human cases of listeriosis as well (Busch, 1971; Rocourt and Seeligeri, 1985). L. seeligeri has been reported to cause listeriosis in one human case (Rocourt et al., 1986). The most important pathogen of the species is L. monocytogenes, a foodborne pathogen affecting primarily

immunosuppressed individuals, elderly, infants, and pregnant women and their fetuses. The disease, listeriosis, results in such major symptoms as septicemia, meningitis, and spontaneous abortions of the fetus in pregnant women and carries an overall fatality rate of 30%.

This foodborne pathogen has been reported in several documented outbreaks (Farber and Peterkin, 1991) caused by consumption of various contaminated foods. Traditional smoked seafoods were heavily smoked solely for preservation. More modern smoked products are held at refrigeration or frozen storage temperatures and, hence, this traditional method of preserving is no longer required. Modern smoked products are lightly salted and lightly smoked imparting specific flavour and texture. L. monocytogenes has been isolated from seafoods as well as smoked seafoods (Jemmi 1990a; 1990b). Some parameters which limit the organism's ability to grow in foods are pH, water activity, and salt content. The latest methodology for Listeria detection in foods is the listeria isolation protocol defined by Health and Welfare Canada (Warburton and Farber, 1990) which takes approximately 10 days to complete. This protocol as well as a similar protocol for isolating Listeria from meats and poultry have come under much investigation for their efficiency of detecting Listeria in various foods (Hayes et al., 1991; in 't Veld and de Boer, 1991; Warburton et al., 1991a;b; Lewis and Corry, 1991). A much more rapid and efficient means of detection is required.

All Listeria spp. have the same structure (Fiedler and Ruhland, 1987); there are serovar overlaps (Fiedler et al.,

1984); lipoteichoic acids are identical (Ruhland and Fiedler, 1987); and fatty acid composition does not vary among species (Raines et al., 1968; Feresu and Jones, 1988). Furthermore, DNA base composition is relatively constant (Seeliger and Jones, 1986) and hence, the pathogenic species, L. monocytogenes, is very often difficult to discriminate from the other Listeria spp. Such methods as Numerical Taxonomy and DNA-DNA hybridization have had success but are not totally adequate. Multilocus enzyme electrophoresis (MEE), an epidemiological tool originating in the 1930's (Tiselius, 1937) is used to investigate genetic variation originally in eukaryotes; recently, it has been employed to study genetic structure and epidemiology of some pathogenic bacteria (Selander et al., 1986). By this method, differentiation of bacterial strains is accomplished by the variation in the electrophoretic mobility of a large number of water soluble metabolites. Variation in mobility is directly associated with allelic variation at specific genes which is the basis for estimating genetic diversity and genetic relatedness and are interpreted as multilocus genotypes of the chromosomal genome (Piffareti et al., 1989).

Serotyping and phagetyping are more commonly applied to Listeria as epidemiological tools for investigation. However, greater than 90% of all cases of listeriosis are caused by serovars 4b, 1/2a, and 1/2b. Hence, serotyping is of limited value (McLauchlin, 1987; Rocourt, 1989) as well, not all strains are typeable (Rocourt et al., 1985). Also, L. seeligeri is non-distinguishable from L. monocytogenes based

on serotyping; they represent the same antigenic structure. Thus, they are only distinguishable based on biochemistry, pathogenicity and genetic make-up (Seeliger and Langer, 1989). The serological analysis can only eliminate possible routes of infection as opposed to indicating the route of infection. Phagetyping is more discriminating than serotypying however, typeability again ranges from less than 50 to greater than 90% according to the sample origin (Audurier and Martin, 1989). Bacteriophages for Listeria spp. were first described by Schultz (1945) and since then many sytems have developed (Audurier et al., 1984; Audurier and Martin, 1989; Loessner and Busse, 1990; Ortel, 1989).

1.6.1 Objectives

The aims of this study were primarily to determine the prevalence of Listeria in commercially produced smoked seafoods, characterize the isolates using biochemical and epidemiological tools, and to determine the fate of L. monocytogenes in artificially inoculated cod fillets during cold smoking and storage temperature.

Table 1.0 Differentiation of *Listeria* species by biochemical reactions.

Species	Hemolysis (beta)	Nitrate reduction	Mannitol	Mannoside	Rhamnose	Xylose	Virulence
L. monocytogenes	+	-	-	+	+	-	+
L. ivanovii	+	-	-	-	-	+	+
L. innocua	-	-	-	+	V	-	-
L. welshimeri	-	-	-	+	V	+	-
L. seeligeri	+	-	-	-	-	+	-
L. grayi	-	-	+		-	-	-
L. murrayi	-	+	+		V	-	-

V = variable reaction

Table 1.1 *Listeria* species and their serovars.

Species	Serovars
L. monocytogenes	1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7
L. ivanovii	5
L. innocua	4ab, 6a, 6b
L. welshimeri	6a, 6b
L. seeligeri	1/2b, 4c, 4d, 6b

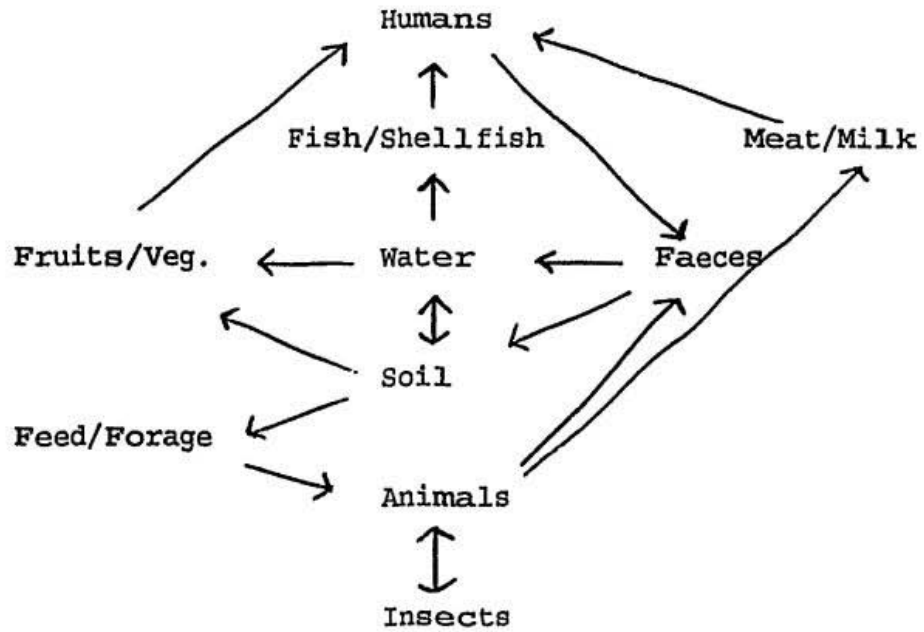

Figure 1.0 One hypothesized cycle of Listeria infection.

CHAPTER 2

MATERIALS AND METHODS

2.1.0 Cultures

Listeria cultures were obtained from Department of Fisheries and Oceans, St. John's, NF. and the other cultures were collected from the Memorial University of NF stock collection. The Listeria cultures were: L. monocytogenes 3a (HPB #59), L. monocytogenes 1/2b (HPB #395), L. innocua (HPB #8), L. ivanovii (HPB #28). Other bacterial cultures consisted of Staphylococcus aureus and Escherchi coli. These cultures were used for controls in various tests. Each was lyophilized for long term storage and subcultured monthly on TSA and stored at 4°C.

2.1.1 Media

Unless specified, all the media used in this work were of reagent or laboratory grade. The following chemicals were obtained from Canlab Division, Baxter Corporation, Mount Pearl, NF: listeria enrichment broth (LEB); UVM listeria enrichment broth (UVM); Oxford listeria selective agar and supplement (Oxford); lithium chloride-phenylethanol moxalactam medium (LPM); coagulase plasma with EDTA; EY-tellurite

enrichment and bacto-TTC.

PALCAM listeria selective agar and supplement (PALCAM), methanol, safranine, crystal violet, iodine, D (+) xylose and mannitol were obtained from BDH Chemicals, Dartmouth, N.S.

The following chemicals sodium chloride, potassium phosphate buffer, α-L-rhamnose, and methyl-α-D-mannopyranoside, ferric ammonium citrate, acriflavin, moxalactam were obtained from Sigma Chemical Company, St. Louis, MO.

The following chemicals were obtained from Fisher Scientific Ltd., Dartmouth, NS: trypticase soy agar (TSA); trypticase soy broth (TSB); purple broth base; motility test medium; enosin methylene blue agar (EMB); baird-parker; acetone; ethanol; filter paper (Whatman No. 3); and yeast extract.

Defibrinated horse and sheep blood were obtained from Woodlyn Laboratories Ltd., Guelph, Ontario.

2.1.2 Smoked fish samples

Hot and cold smoked fish products were obtained from retail outlets in St. John's, Newfoundland: Bidgoods, Goulds; Sobey's Food Village at Ropewalk Lane; Sobey's at the Avalon Mall; Dominion at The Village Mall on Topsail Road; and Dominion on Torbay Road. The samples included: cod (60 samples), mackerel (25), capelin (55), and eels (2) which were

hot smoked; herring (58), salmon (39), charr (11), trout (6), and turbot (2) which were cold smoked. All samples were stored at 4°C and analysed within 3 days of purchase.

Fish were caught in sea waters surrounding Newfoundland and processed locally. The hot smoking process of consisted of either smoking at 38°C for 6-8h, or at a temperature range from 50 to 80°C for several hours up to 48 h. The cold smoking process consisted of smoking at a temperature range of 19°C to 28°C for several hours.

2.1.3 Protocol for detecting Listeria

The Canadian listeria isolation protocol was used to detect Listeria in smoked seafoods as outlined by Warburton and Farber (1990) (Fig. 2.0).

The primary enrichment consisted of homogenizing 25g samples in 225 ml LEB in a stomacher bag for 2 min.(Stomacher Lab Blender 400); the bag was then incubated at 30°C for 48 h. The isolation procedure consisted of subculturing, at 24 and 48h intervals, 0.1 ml of the LEB culture onto PALCAM, Oxford, and LPM agars. PALCAM and Oxford plates were incubated at 35°C for 24-48 h, and LPM plates at 30°C for 24-48h. Secondary enrichment consisted of inoculating, at 24 and 48 h intervals, 0.1 ml of the LEB culture into UVM. The secondary UVM enrichments were incubated for 24-48 h at 35°C and subcultured onto PALCAM, Oxford, and LPM. These media were

incubated as above. Samples were also simultaneously processed by direct plating. Approximately 0.1 ml of the LEB homogenate was spread plated onto PALCAM, Oxford, and LPM media and the plates were incubated as above.

All plates were examined for typical listeria colonies. On PALCAM medium, Listeria typically appear as grey-green colonies with black-brown halo, and with heavy growth, the medium turns black. On Oxford plates, Listeria colonies are dark grey-greenish colour with a black halo and a sunken centre. As with PALCAM medium, with heavy growth, the originally green medium turns black. On LPM plates, Listeria colonies typically appear as whitish-blue piles of crushed glass having a blue-grey iridescence when using the Henry technique (Fig. 2.1) (Henry, 1933).

Typical colonies from each plate were subcultured onto TSA with 0.6% yeast extract and incubated at 30°C for 24 h. These cutlures were used to carry out further confirmatory tests including Gram staining, motility, catalase, and carbohydrate fermentation tests. The carbohydrate fermentation tests were carried out in purple broth base with 5% sugar solutions consisting of mannitol, mannoside, rhamnose, and xylose. Both horse blood and sheep blood agar media were used to determine haemolysis with appropriate controls, L. innocua (no haemolysis around stab), L. monocytogenes (slight zone), and L. ivanovii (well defined

zone).

2.1.4 Serotyping

All L. monocytogenes strains were serotyped according to the procedure of Seeliger and Höhne (1979).

2.1.5 Salt content

Eleven gram samples were removed from the smoked seafood products and homogenized in the stomacher with 99 ml distilled water for 1 min. in stomacher bags. Salt analysis was conducted on the blended samples using the prestandardized Salt Meter 10 (Presto-Tek corporation); the probe of the meter was simply placed in the stomacher bags containing the samples.

2.1.6 pH

Eleven gram samples were removed from the smoked seafood products and homogenized in the stomacher with 99 ml distilled water for 1 min. in stomacher bags. The pH was read with a prestandardized pH meter (Accumet pH Meter, Model No. 915, Fisher Scientific); the probe of the meter was placed in the stomacher bags containing the samples.

2.1.7 Water activity

Shavings of the smoked seafood products were placed in

the containers used for water activity readings in the CX-1 Decagon Water Activity Unit (Decagon Devices Inc., Pullman Washington, Model No. 0290344). Efficiency of the water activity unit was verified with a saturated salt solution.

2.1.8 Total aerobic counts

Approximately 0.1 ml of the LEB homogenate or dilution was spread plated onto TSA in duplicates. The TSA plates were incubated at 30°C for 24-48 h. Colonies on TSA were enumerated using a colony counter (Model 225, Technilab Instruments, Pequannock, New Jersey). Total aerobic counts were determined according to the methods of Busta et al. (1984). Dilutions of 10^1, 10^3 and 10^5 were used.

2.1.9 Faecal coliforms

Eosin methylene blue agar (EMB) plates were spread plated with 0.1 ml of the LEB blended sample and incubated at 35°C for 24-48h. Colonies on EMB were examined for a metallic green sheen. This test was conducted according to the confirmation tests for E. coli described by Mehlman (1984).

2.1.10 Staphylococci

Baird-Parker containing egg yolk with tellurite plates were spread plated with 0.1 ml of the LEB blended sample and incubated at 35°C for 24-48h. Black colonies with or without

haemolysis were verified as Staphylococcus spp. by the coagulase plasma test. The methods were carried out according to the methods of Tatini et al. (1984).

2.2.0 Chemicals

The following chemicals were obtained from Sigma Chemical Company, St. Louis: acetic acid (glacial); cis-aconitic acid; D L-alanine; ADP; amaranth dye; arsenic acid heptahydrate (sodium salt); l-aspartic acid; boric acid; citric acid monohydrate; cobalt chloride; l-cysteine hydrochloride; EDTA (disodium); fast blue B salt (o-Dianisidine); fast black K salt; fast blue BB salt; fast blue RR salt; fructose 1,6-diphosphate; d-fructose 6-phosphate (disodium); fumaric acid (potassium salt); glucose; glucose 1- phosphate; glucose 1,6-diphosphate; glucose 6-phosphate (disodium salt); glucose 6-phosphate dehydrogenase; l-glutamic acid; glyceraldehyde 3'phosphate dehydrogenase (Rabbit); glycine; hexokinase; hydrogen peroxide; inosine; dl-isocitric acid monohydrate (trisodium); isocitrate dehydrogenase; α-ketoglutaric acid (free acid);lithium hydroxide; lithium lactate; magnesium chloride hexahydrate; maleic acid; malate dehydrogenase (pigeon); manganese chloride; mannose 6-phosphate (barium salt); dimethylthiazol tetrazolium (MTT); NAD; NADP; alfa-naphthyl acetate; beta-naphthyl acetate; alfa-naphthyl acid phosphate (monosodium); beta-naphthyl acid phosphate

(monosodium); l-peptides (l-Phenylalanine-l-Leucine); peroxidase; phenazine methosulfate (PMS); 6-phosphogluconic acid (Barium salt); phosphoglucose isomerase; potassium acetate; potassium hydroxide; potassium iodide; potassium phosphate (monobasic); pyridoxal 5'phosphate; snake venom (Crotalus Atrox); sodium acetate trihydrate; sodium azide; sodium hydroxide; sodium phosphate (monobasic); sodium phosphate (dibasic); sodium sulfide; triosphosphate isomerase; TRIS; and xanthine oxidase.

Hydrolysed starch was obtained from Connaught Laboratories, Willowdale, Ontario.

2.2.1 Bacterial strains

A total of 57 isolates were analyzed (Table 3.3.0) including 43 strains isolated from smoked seafoods; 1 L. monocytogenes strain isolated from raw cod fish; 4 Listeria sp. obtained from Department of Fisheries and Oceans; 2 ATCC L. monocytogenes strains; 7 L. monocytogenes strains obtained from LCDC, Ottawa. In total there were 24 L. monocytogenes strains, 19 L. innocua, 13 L. welshimeri and 1 L. ivanovii. The strains originated from humans, animals, food, and the environment.

2.2.2 Starch gel electrophoresis

Preparation of lysate, electrophoresis, and selective enzyme staining were performed according to the procedures described by Selander et al. (1986) and the experimental protocol was done as described by Kephart (1990). However, for enzyme preparation, Listeria strains were grown up in 10ml TSB for 24h at 30°C and the culture was then inoculated into 200ml of TSB and incubated as above before centrifuging prior to sonicating as opposed to growing the cells overnight in 200 ml broth prior to sonicating. Enzymes analyzed were: 6PG, 6-phosphogluconate dehydrogenase; GD2, glutamate dehydrogenase; ALD, alanine dehydrogenase; PGM, phosphoglucomutase; ADK, adenylate kinase; ACP, acid phosphatase; IPO, indophenol oxidase; α-EST, α-naphthyl propionate esterase; GP2, NADP-dependent-glyceraldehyde-phosphate dehydrogenase; IDH, isocitrate dehydrogenase; G6P, glucose-6-phosphate dehydrogenase; LDH, lactate dehydrogenase; CAT, catalase; PGI, phosphoglucose isomerase. Staining procedure for catalase was done according to the procedure of Harris and Hopkinsons (1976). Buffer A was used for enzymes 6PG, GD2, ALD, PGM, GP2, IDH, PGI, Buffer B for G6P, IPO, LDH and Buffer F for ADK, ACP, α-EST, and CAT (App. A 1.2).

2.2.3 Preparation of enzyme extracts

Listeria were grown overnight at 37°C in 200ml of TSB and

cells were harvested by centrifugation at 15,000 x g for 10 min. Supernatant was discarded and cells were suspended in 2 ml of a buffer solution containing 1mM EDTA, 10mM Tris and 0.5mM NADP at pH 6.0. Cells were lysed by sonication (Braun Sonic 2000) for 5 min. After lysis and centrifugation (Model No. Sorval SC5 (superspeed refrigerated centrifuge) at 30,000 x g for 20 min., aliquots of the supernatant were transferred to three to four 1.5 ml Eppendorf tubes and stored at -70°C until required for electrophoresis.

2.2.4 Horizontal starch gel

Starch (48 g) and gel buffer (420 ml) was mixed in a 2 L erlenmyer flask and heated over a bunsen burner just beyond boiling point with vigorous stirring and aspirated for 1 min. (or until very large bubbles appeared). The degassed liquid was immediately poured into a lucite gel mold, wrapped in plastic film to prevent desiccation, and cooled at room temperature for 2 h or overnight at 4°C. The gels were used within 24 h of preparation.

2.2.5 Loading of gel

Individual Whatman No. 3 filter paper (5x5 mm) wicks were dipped into samples of lysate and excess was blotted onto filter paper. The filter paper dips were inserted at 3 mm intervals in a continuous slit cut in the gel and the

migration front of the buffer line was tracked with amaranth dye prepared according to Selander et al. (1986) and inserted at one or both ends. A constant voltage was maintained and the gel was kept cool by circulating cold water.

2.2.6 Staining

Following electrophoresis, approximately 3-4 horizontal slices (1-2 mm thick) were cut from the gel with a thin wire and incubated individually at 37°C in various enzyme staining solutions. Each stain was of a sufficient volume to stain a single gel slice. Stained gels were incubated at 37°C in the dark until bands appeared (mins - hours) after which the staining solution was removed and the gel slice was rinsed with water (except when stain had been applied in an agar overlay). The gel was then fixed in a 1:5:5 mixture of acetic acid:methanol:water. After fixing and destaining, gels were sealed in plastic bags with the gel buffer and 0.3% sodium azide and stored at 4°C.

2.2.7 Statistical analysis

Each electromorph was assigned a number according to increasing distance travelled from the cathode which corresponded to the relative anodal migration (Fig. 3.3.0). The absence of such electromorph was given a 0. Each unique combination of electromorphs was designated as an ET.

Numerical Taxonomy program (Rohlf, 1992) was used to compute genetic distance between electrophoretic types (ETs). The genetic distance was computed as the proportion of loci at which dissimilar alleles occurred. Clustering of ETs was accomplished using the average linkage method and a matrix of coefficients of pairwise genetic distances. The genetic diversity for each enzyme locus among ETs was calculated as $h = (1-\Sigma x_i^2)\,[n/n-1)]$, where x_i is the frequency of the i^{th} allele and n is the number of ETs (Nei, 1978).

2.3.0 Materials used for smoking

Fresh cod fillets were obtained from National Sea Products Ltd., St. John's. Hickory sawdust was purchased from the Marine Institute, St. John's and fine salt and brinometers from Charles R. Bell Ltd., St. John's.

2.3.1 Preparation of inoculum

For all trials, a wildtype strain isolated from smoked cod, L. monocytogenes 1/2 was used. Inocula were prepared from cultures grown in TSB overnight at 30°C and were diluted to provide concentrations of : 10^6 cells/ml in trial 1, 10^2 cells/ml in trial 2, and 10^1 cells/ml in trial 3.

2.3.2 Inoculation and cold smoking of cod fillets

Fresh local cod fillets, were vacuum-packaged, frozen,

stored at -20°C, and thawed at 4°C overnight when required. Twelve fillets of 300g each were uniformly surface inoculated with 3 ml of the specific inoculum broths and left to dry for 10 min. at room temperature. The inoculated cod fillets were then placed in a 70°s brine at a ratio of 1:1 fish fillet to brine for 5 mins. The fillets were removed and allowed to air dry at 5°C for 2-3h. Finally, the cod were cold smoked (Afos Mini-Kiln Smoker, Manor Estate, Anlaby . Hull, England, Patent No. 1184363) at 25-30°C for 3-4 h. The smoked products were vacuum-packaged (Multivac, Model No. A 300/32, Sepp Haggenmüller KG, West Germany) and one portion was stored at 4°C for 3 weeks and the other at -20°C for 3 months.

2.3.3 Sampling for analysis

Approximately 10g samples were removed from the fillets using a sterile knife. Samples were taken at initial, inoculation, brining, and smoking stages. Samples were removed weekly for 3 weeks from smoked cod stored at 4°C and monthly for 3 months for product stored at -20°C.

2.3.4 Enumeration of L. monocytogenes cells on smoked cod fillets

L. monocytogenes was enumerated by a three-tube most probable number (MPN) technique. The samples were homogenized for 2 min. in a stomacher with 90 ml of 0.85% physiological

saline. A total of 10 ml, 1 ml, and 0.1 ml of the homogenate were dispensed in University Vermonth broth (UVM) supplemented with 0.1 ml ferric ammonium citrate and 0.1 ml acriflavin, and incubated for 48h at 37°C. Upon production of a black precipitate within the UVM broth, 0.1 ml of the lowest dilution was spread plated onto PALCAM agar. Further confirmation of L. monocytogenes included catalase test, gram staining, and carbohydrate fermentation tests using xylose, rhamnose, mannitol, and mannoside.

3.1 Existing Newfoundland smokers

The commercially licensed seafood smoking operations in Newfoundland were visited and the current smoking procedures were documented. There are 7 fish processing companies presently involved in smoking fish: Labrador Inuit Development Corporation, Golden Shell Fisheries Ltd., National Sea Products Ltd., Seaside Fisheries Ltd., Fishery Products International Ltd., Cape Charles Smoked Products, and Bidgoods (See Table A 2.0). However, the last two plants were not visited.

3.1.1 Nain Fisheries in Nain, Labrador

The existing smoker in Nain is an Afos model and has been in use for approximately 15 years. The smoked products are sold in Newfoundland and Labrador by Provincial Fisheries and

sold locally in Labrador and to Quebec by the Labrador Inuit Development Corporation. Provincial Fisheries operate the plant in the spring and summer, and the Labrador Inuit Development Corporation operate the plant during the fall and winter. The LIDC purchase frozen charr from the provincial government in fall and winter to smoke. In 1990, they smoked 15,000 lb (7200 kg) charr. The products smoked were salmon and charr. The fish were frozen in the dressed state and kept at -25°C until ready for use. They were then sliced using a bandsaw while in the frozen state, which rendered a better quality fillet. They were thawed, placed in an 80°s brine for 10-15 min., placed on racks and left overnight in the cool processing area. The fillets were smoked for 9-12h at 80°-90°F (cold smoked). The smoker has 2 doors equipped with two trolleys which can handle 500 lb (240 kg). fillets each. They were left in the processing area to cool before being vacuum-packaged in cryovac bags, frozen and stored at -25°C. The bags were packed in 30 lb (14.4 kg) cardboard boxes. The hardwood sawdust used was of a hickory flavour (Swift Edible Oil Company, Chicago, Illn.). The Nain Plant does not have a large market for its product. Smoked charr sold at $8.00/lb and smoked salmon at $10.00/lb.

3.1.2 Golden Shell Fisheries in Hickman's Harbour

Golden Shell Fishery has been established for the last

three years; however, the owner, Mr. Ralph Simmons has been smoking fish for nearly 50 years. The smoker is typical of the traditional style: fish were hung vertically on racks and the smoke generated from the sawdust on the floor of the smoke house, planks of wood were laid on the floor and local sawdust, mainly fir, was placed on top in load fulls; these planks of wood were set on fire and the sawdust on top produced a smouldering hot smoke. Mr. Simmons' smoker has been in use for the last 18-19 years. The products presently smoked are herring and capelin.

One 45 kg bag of rock salt is used in salting capelin placed in a 1500 lb (720 kg) polyethylene fish container. The container bottom is finely layered with this salt and a net full of capelin is placed on top. Salt is sprinkled about and mixed through before another net load of capelin are dumped into the grey container. The process continues until the fish container is full. The container is then topped off with a small amount of seawater. The capelin are left for up to a day before undergoing two seawater washes. The capelin are hung on nails through the eyes on wooden racks and left to dry in the sun for a day. The dried capelin are hot smoked for 18-24h. The smoke house can handle upto 400 lb (192 kg) capelin at any one time. The smoked capelin are placed in plastic bags immediately after smoking. Kippers however, require a cooling period before packaging. Kippers and smoked

capelin are sold locally and to the mainland. The herring are brined at 90°s for 1h or at 30°s overnight. They are washed, placed vertically on racks, and smoked immediately for 1-2 days. There are no controls over the consistency of the product such as relative humidity or temperature. The process is controlled by the experience of the operator. The kippers (smoked herring) are placed on foam trays and saran wrapped. Smoked capelin is sold at approximately 90 cents/ 1/2 lb and kippers at $1.00/pr.

3.1.3 National Sea Products Ltd. in Arnold's Cove

The existing smoker at National Sea Products is an Afos (Model No. 200) with four doors and four trolleys and has been operating since the plant opened in 1979. It can handle 3000-4000 lb (1920 kg) fish fillets per load. The products smoked are cod, herring and mackerel with capelin and salmon smoked only upon request. The sawdust used is local (North Harbour), mainly softwood fir. There are no relative humidity controls nor temperature controls connected to the smoker; the only control is that of the experience of the operator. There is a temperature gauge. Fish is smoked either fresh or is frozen and later smoked. The products are mostly hot smoked at a constant temperature of 38°C. The cod requires the use of 5% Annatto dye in its brine to impart a more favourable colour. Cod, herring, and mackerel are brined at 70° s for 7-

8 mins. or 14-15 mins. for larger cod, removed and left to dry for 2-3 h in the processing area and then 2-3 h in the chill room. Before smoking they are left at plant temperature again for 1 h. The fish are smoked for 6-8 h before chilling for 1-2 days prior to packaging. The smoked cod are placed in 15 lb (72 kg) cardboard cartons layered with polysheets and blast frozen for 18h before storing.

3.1.4 Fishery Products International in Dildo

FPI in Dildo also has an Afos smoker (Model No. 120) and it has been periodically used for approximately 12 years by FPI since they purchased the plant from H. B. Nickerson in the late 1970's. The smoker was bought new by Mr. Nickerson. It has 4 doors and 4 trolleys each consisting of 48 racks. The smoker has a capacity of 2500 lb (1200 kg) fish. The heater controls allow for either a cold smoke or hot smoke. A temperature gauge is attached. FPI smoke only for 2-3 weeks each year around November to produce product for Christmas. The smoked product is sold to FPI stores in St. John's, NF. In 1990, they smoked 10,000 lb (4800 kg) in 2 weeks. Salmon was the only product smoked and it was purchased from Labrador. The frozen salmon were thawed, filleted and placed in small pans and layered with a mixture of sodium chloride, potassium chloride (Reheis Chemical Company, Midlothian, Texas 76065, USA), and sugar. Fillets were rolled in the mixture

and then placed skin down in the pans before layering. They were left for 2.5h in the chill room prior to washing. The fillets were racked and left to dry in the chill room for 3 h. Cold smoking occurred for 2.5 h before reversing racks and then smoked for another 2.5 h. The smoked fish were chilled for 2-3h before vacuum packaging, blast frozen (-40°C) for 12-15 h, placed in 20 kg boxes and stored at -25°C. Hardwood sawdust was used (PWI, Richelieu, Quebec).

3.1.5 Seaside Fisheries at Coley's Point in Bay Roberts

The Afos smoker (Model No. 25) was purchased in 1989 from St. Pierre and has been used in the plant at Seaside Fisheries since. The smoker has a capacity of 700 lb (336 kg) fish. Salmon, mackerel, herring, and charr are the usual materials smoked. Fresh, farmed salmon is imported from B.C., Chili, and other places, filleted and dry salted with fine salt for 2-3 h depending on size and then racked and dried overnight. The fillets are smoked for 6-7 h at 19°C, vacuum-packaged and either blast frozen or frozen in cold storage at -25°C. The sawdust used is local and mostly fir. Labrador charr is smoked in the same manner as salmon and is purchased from Labrador. Mackerel is hot smoked and herring is cold smoked.

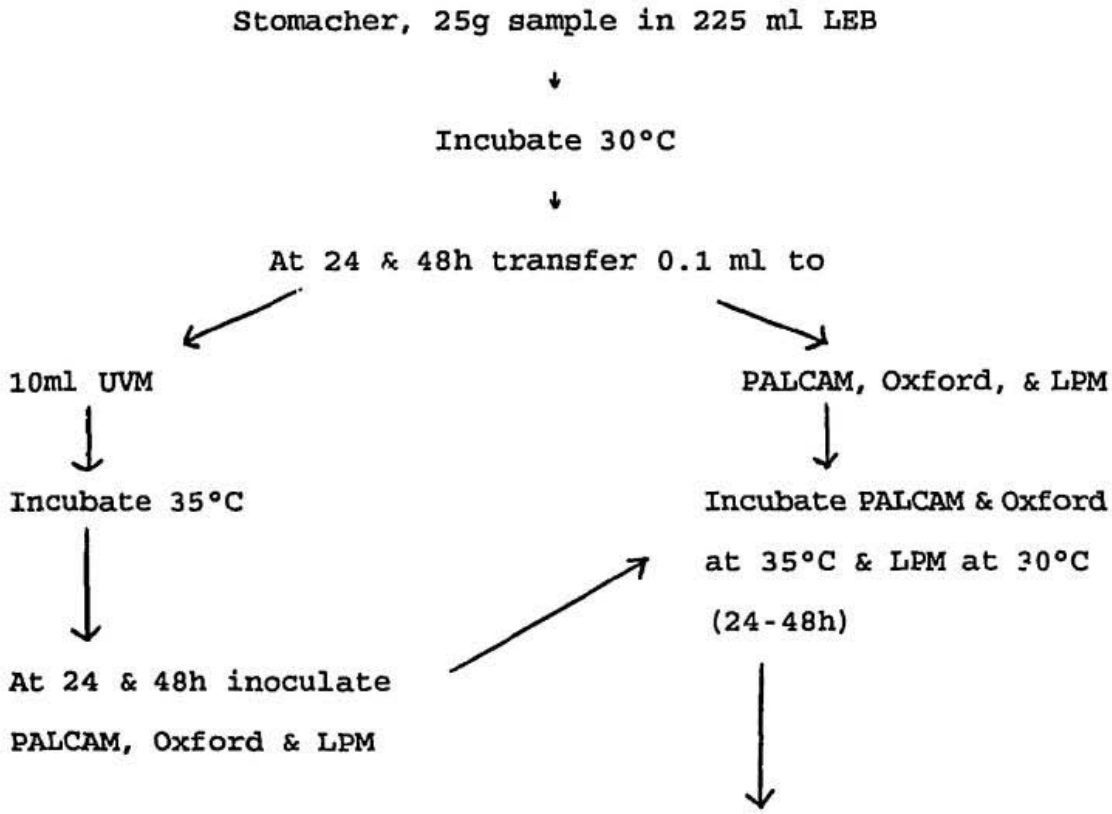

Figure 2.0 A flow diagram illustrating the isolation procedure for Listeria.

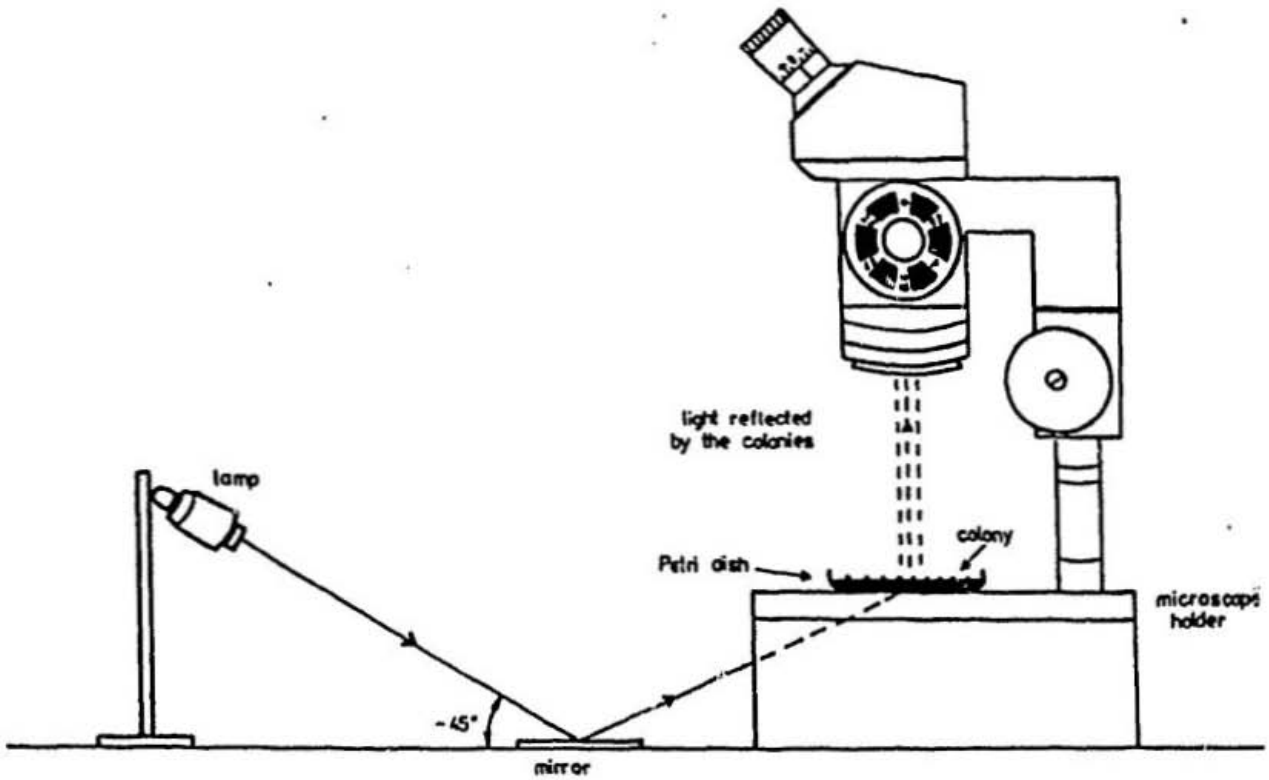

Figure 2.1 Henry technique used to examine LPM plates for Listeria.

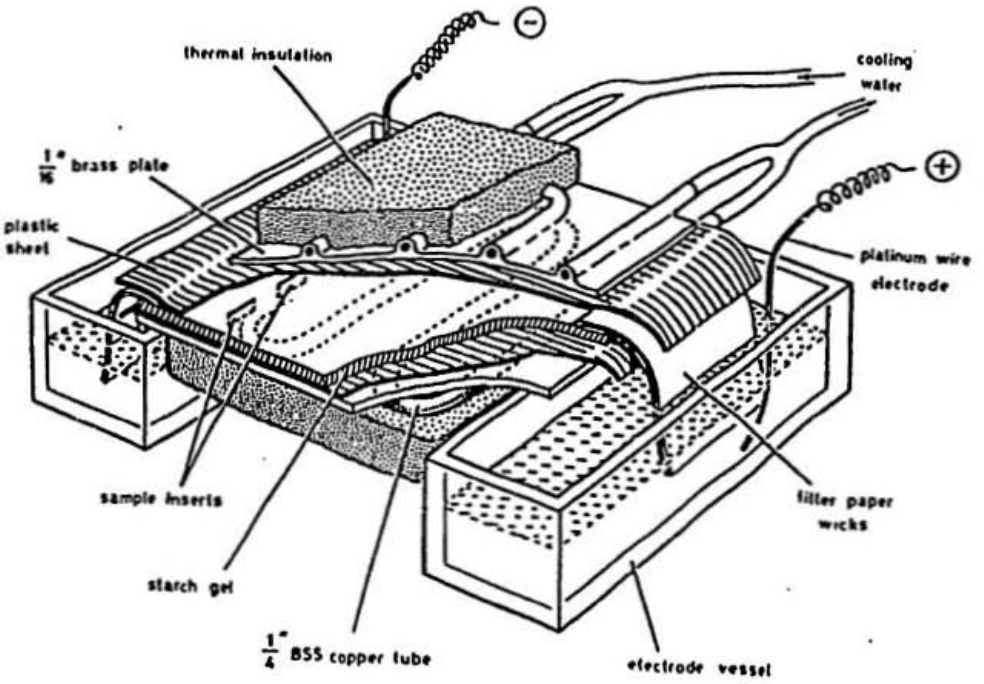

Figure 2.2 Electrophoresis apparatus.

CHAPTER 3

RESULTS

3.1 Prevalence of *Listeria* in surveyed smoked seafood products

Over a period of one year, a total of 258 samples of smoked seafood products were obtained, with a monthly average of 22 samples. Of these, 142 were hot smoked and 116 were cold smoked (Tables A 3.1-3.12).

A total of 142 of the 258 (55%) samples were tested to determine water activity, salt content, and pH. The water activity ranged from 0.704 to 0.979 with capelin samples uniformly recording lower levels. The salt content readings had a narrow range of 2.39 to 4.77% for the most part, with the exception of those of turbot and capelin samples at 11.42% and 14.39%, respectively. The pH readings ranged from 5.18 to 6.75 (Table 3.1.0).

Listeria spp. were isolated from 43 of 258 (16.7%) samples processed; hot smoked samples yielded 36 isolates (36/142 or 25.4%), and cold smoked samples yielded 7 isolates (7/116 or 6%). Of the nine species of seafood tested, *Listeria* spp. were isolated in cod, mackerel, kippers, capelin, salmon, and turbot with cod samples yielding the highest number of isolations (28/60 or 47%) (Fig. 3.1.0). No

Listeria spp. were isolated from either charr, trout, or eel. Of the 43 Listeria spp. isolated, 18 (41.9%) were L. innocua, 13 (30.2%) were L. welshimeri, and 12 (27.9%) were L. monocytogenes. L. monocytogenes was isolated only from cod (11/60 or 18.3%) and herring (1/58 or 1.7%) samples (Fig. 3.1.0). Serotyping of the 12 L. monocytogenes isolates indicated 11 of them belonging to serotype 1/2; the remaining single isolate was found to be 4b. Of the 11 serotype 1/2 strains, one was non-ß-hemolytic. Listeria spp. were more commonly found during the cooler months with a dramatic increase during the month of October with 60% of the samples tested yielding Listeria spp; L. monocytogenes was isolated from 15% of samples during this month (Fig. 3.1.1). Although Listeria spp. occurred during the warmer months, L. monocytogenes was not detected during this period.

The major contaminated smoked seafood was cod and the major producer of the cod was producer D, who had the highest rate of L. monocytogenes contamination for smoked products processed at the individual plants (Fig. 3.1.3). Listeria spp. were however, found in products processed from all plants. Again, Listeria spp. were found in products obtained from all of the supermarkets, although no L. monocytogenes was found in supermarkets A nor B (Fig. 3.1.2). Quality indicators of smoked seafoods such as total aerobic counts, coliforms, and S. aureus were measured. Total aerobic counts

ranged from 0 to > 10^8 cells/g, with 45% of the samples having counts $\geq 10^5$ cells/g (Tables A 3.1 to A 3.12). Coliforms were observed in 17 (6.6%) samples and S. aureus was noted in 44 (17%) samples (Tables A 3.1 to A 3.12). Listeria spp. were noted to be associated with very high aerobic counts in 35 (81%) of the Listeria positive samples. The other 8 (19%) samples positive for Listeria had very low aerobic counts most often too few too count (Tables A 3.1 to A 3.12).

All 43 isolates of Listeria spp. were detected after 48 h primary enrichment in LEB (Table A 3.13). The secondary enrichment in UVM failed to enhance the isolation rate; in fact, this additional step led to false positive rate of 49%. Direct plating of homogenates onto the three selective media detected only 26% of the total isolates. In positive Listeria cultures obtained by direct plating, the plate count ranged from 100 to 30,000 bacteria per gram samples (Table A 3.14). When comparing the three selective media for their ability to recover Listeria spp. following primary enrichment, PALCAM was found to be superior to both Oxford and LPM; all 43 isolates were recovered on PALCAM while Oxford yielded 38 isolates and LPM 37 isolates (Tables 3.2.2 and A 3.13). By the direct plating method, however, none of the selective media individually recovered all isolates of Listeria spp.

Table 3.1.0 Water activity, salt content, and pH of smoked seafood products.

Product	A_w	Salt(%)	pH
Caplin(25)[a]	0.704 ± 0.058[b]	14.39 ± 3.20[b]	5.84 ± 0.34[b]
Charr(9)	0.976 ± 0.011	3.70 ± 2.22	5.56 ± 0.31
Cod(28)	0.979 ± 0.009	3.82 ± 1.48	6.75 ± 0.50
Eel(2)	0.965 ± 0.001	2.39 ± 0.55	5.18 ± 0.37
Kippers(25)	0.958 ± 0.017	4.77 ± 1.34	5.69 ± 0.51
Mackerel(19)	0.955 ± 0.011	4.28 ± 1.10	5.68 ± 0.26
Salmon(26)	0.972 ± 0.010	3.66 ± 1.25	6.09 ± 0.33
Trout(6)	0.958 ± 0.008	4.37 ± 0.35	6.22 ± 0.24
Turbot(2)	0.834 ± 0.030	11.42 ± 0.82	6.49 ± 0.17

[a] Figure in parenthesis indicates number of samples analyzed

[b] Mean ± standard deviation

A_w Water activity

Table 3.1.1 Differentiation of *Listeria* species from smoked seafoods based on biochemical and confirmation tests.

Strain*	Product	CHO utilization				Motility	Haem. (ß)	Identification
		Rhm[e]	Xyl[f]	Mat[g]	Mas[h]			
BGM 1	2 Macker.[a]	+	-	-	+	+	-	L. innocua
DTC 1	14 Cod[a]	+	-	-	+	+	-	L. innocua
SRM 1	18 Macker.[a]	+	-	-	+	+	-	L. innocua
SRM 2	2 Macker.[b]	+	-	-	+	+	-	L. innocua
DTC 2	14 Cod[b]	+	-	-	+	+	-	L. innocua
SRCP 2	11 Capelin[b]	+	-	-	+	+	-	L. innocua
SRC 3	14 Cod[d]	+	-	-	+	+	-	L. innocua
FPC 3	16 Cod[d]	+	-	-	+	+	+	L. monocytogenes 4b
BGK 4	6 Kippers[i]	-	+	-	+	+	-	L. welshimeri
SAC 4	11 Cod[i]	+	-	-	+	+	+	L. monocytogenes 1/2
SRC 4	19 Cod[i]	+	-	-	+	+	-	L. innocua
SACP 5	1 Capelin[j]	+	+	-	+	+	-	L. welshimeri
SAT 5	2 Turbot[j]	+	+	-	+	+	-	L. welshimeri
SAK 5	3 Kippers[j]	+	+	-	+	+	-	L. welshimeri
SAC 5	4 Cod[j]	+	-	-	+	+	-	L. innocua
DRC 5	12 Cod[j]	+	-	-	+	+	+	L. monocytogenes 1/2
DTC 5	13 Cod[j]	+	+	-	+	+	-	L. welshimeri
DTS 5	14 Salmon[j]	+	+	-	+	+	-	L. welshimeri
DTK 5	15 Kippers[j]	+	+	-	+	+	-	L. welshimeri
SRM 5	16 Mackerel[j]	+	+	-	+	+	-	L. welshimeri
SRC 5	17 Cod[j]	+	-	-	+	+	+	L. monocytogenes 1/2
SRK 5	19 Kippers[j]	+	-	-	+	+	+	L. monocytogenes 1/2
SRCP 5	20 Capelin[j]	+	+	-	+	+	-	L. welshimeri
DTK 6	1 Kippers[k]	+	+	-	+	+	-	L. welshimeri
DTC 6	4 Cod[k]	+	+	-	+	+	-	L. welshimeri
SRC 6	14 Cod[k]	+	-	-	+	+	+	L. monocytogenes 1/2

Table 3.1.1 continued

Strain*	Product	CHO utilization				Motility	Haem. (£)	Identification
		Rhm[e]	Xyl[f]	Mat[g]	Mas[h]			
SRM 6	15 Mackerel[k]	+	+	-	+	+	-	L. welshimeri
SAC 7	8 Cod[l]	+	-	-	+	+	+	L. monocytogenes 1/2
DTC 7	16 Cod[l]	+	-	-	+	+	-	L. innocua
SRC 8	3 Cod[m]	+	-	-	+	+	-	L. innocua
SAC 8	5 Cod[m]	+	-	-	+	+	-	L. monocytogenes 1/2
DTC 8	11 Cod[m]	+	+	-	+	+	-	L. welshimeri
SRC 9	4 Cod[n]	+	-	-	+	+	-	L. innocua
SAC 9	5 Cod[n]	+	-	-	+	+	-	L. innocua
DVC 9	17 Cod[n]	+	-	-	+	+	+	L. monocytogenes 1/2
DTC 9	22 Cod[n]	+	-	-	+	+	-	L. innocua
DTC 10	6 Cod[o]	+	-	-	+	+	-	L. innocua
SRC 10	15 Cod[o]	+	-	-	+	+	-	L. innocua
DVC 10	17 Cod[o]	+	-	-	+	+	+	L. monocytogenes 1/2
DVC 11	13 Cod[p]	+	-	-	+	+	+	L. monocytogenes 1/2
DTC 11	15 Cod[p]	+	-	-	+	+	+	L. monocytogenes 1/2
DVC 12	1 Cod[q]	+	-	-	+	+	-	L. innocua
DTC 12	19 Cod[q]	+	-	-	+	+	-	L. innocua

* The letter code for the strain refers to the bacterial strain and the number refers to a month with 1 beginning with June month;

[a] June, 1991, [b] July, 1991, [c] variable, [d] August, 1991, [e] Rhamnose, [f] Xylose, [g] Mannitol, [h] Mannoside, [i] September, 1991, [j] October, 1991, [k] November, 1991, [l] December, 1991, [m] January, 1992, [n] February, 1992], [o] March, 1992, [p] April, 1992, [q] May, 1992

Table 3.1.2 Comparison of *Listeria* selective media for the isolation of the strains during enrichment and direct plating.

Media	Primary enrichment (% recovery)	Direct Plating (% recovery)
PALCAM	100	80
Oxford	88.4	80
LPM	86	60

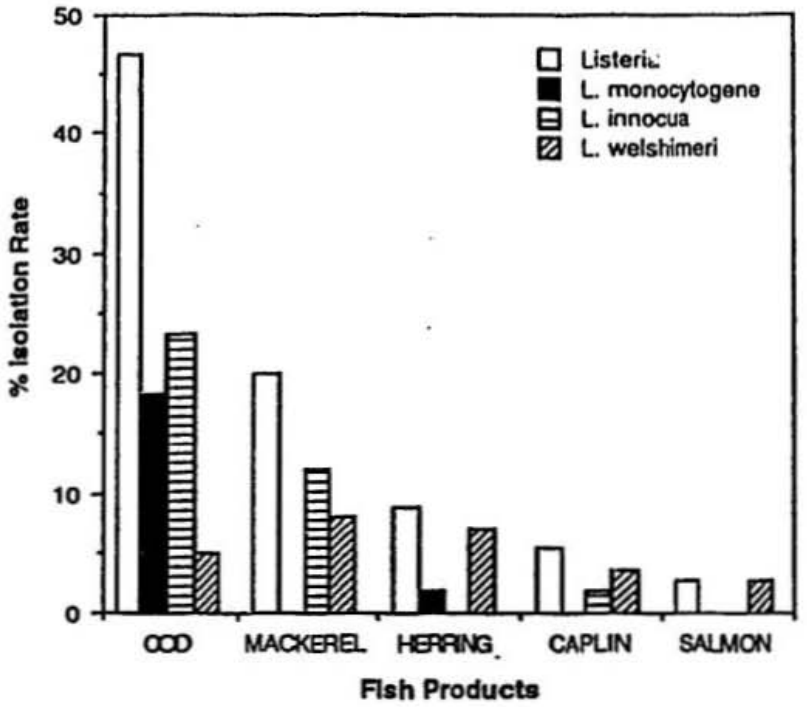

Figure 3.1.0 Isolation rate of Listeria spp. in smoked seafood products.

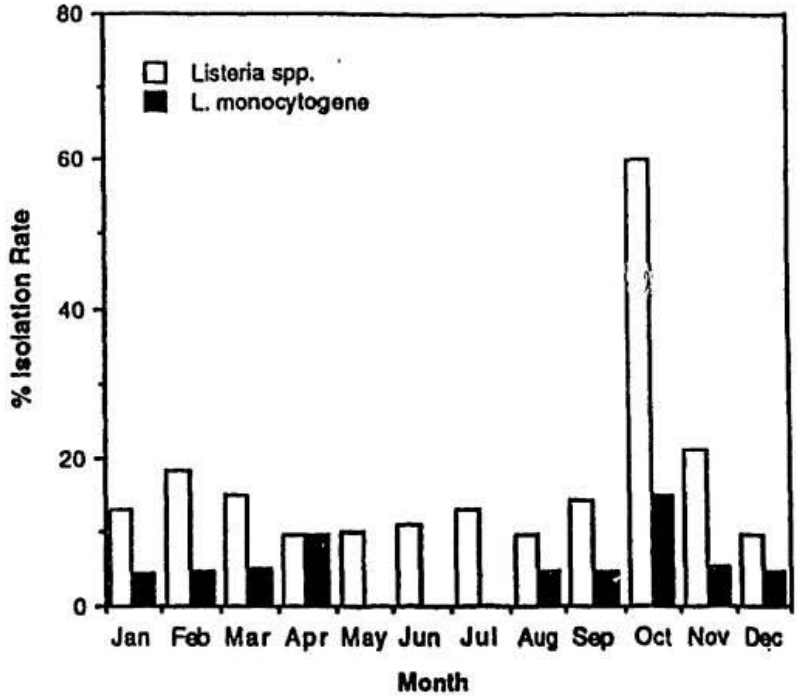

Figure 3.1.1 Seasonal variation on the prevalence of *Listeria* spp. in smoked seafood.

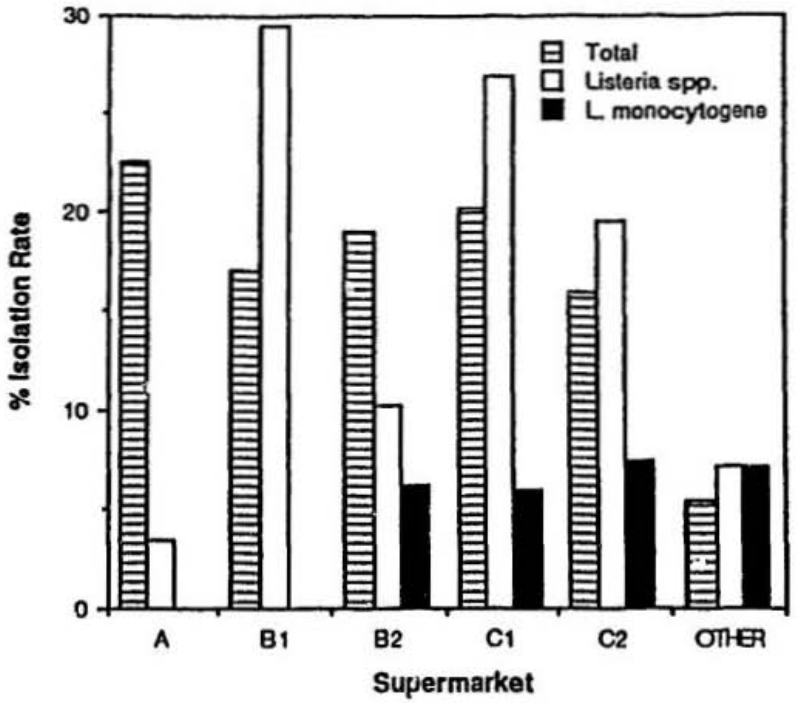

Figure 3.1.2 Variation of Listeria contamination in smoked seafoods according to the place of purchase. Total - represents total product of 258 samples purchased from each supermarket.

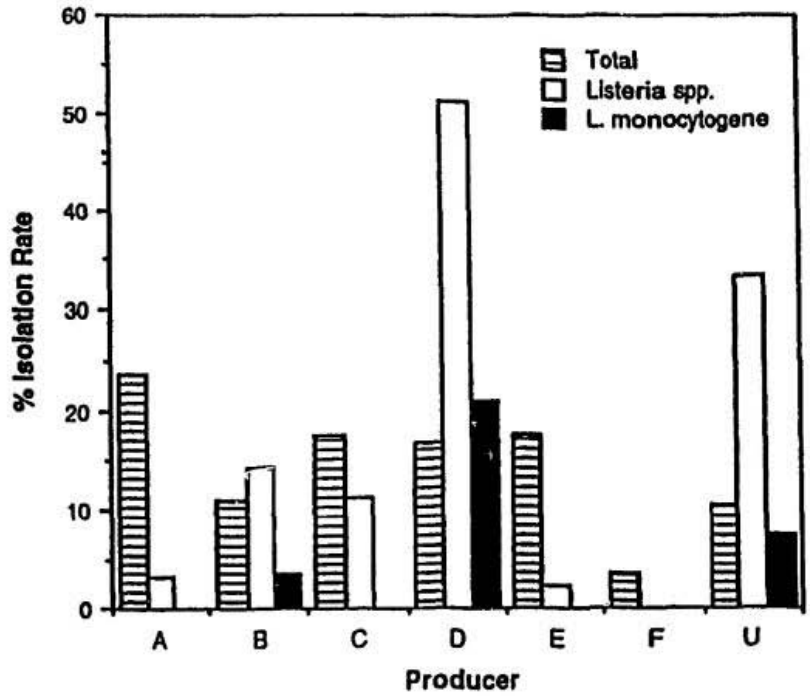

Figure 3.1.3 Variation of Listeria contamination in smoked seafoods according to the commercial producer. Total - represents total product of 258 samples processed at each smoking plant.

3.2 Characterization of *Listeria* spp. isolated from smoked seafoods by using multilocus enzyme electrophoresis

Fifty-seven strains were analyzed resulting in 57 ETs, hence each strain was distinct. The genetic relatedness is presented in Fig. 3.2.1 with corresponding data in Table 3.2.1 and the sources of the strains are provided in Table 3.2.0. Each of the four *Listeria* spp. fell into separate clusters with major divisions separated at genetic distances of 0.72 to 0.85. The genetic distance between two ETs belonging to the same specific cluster did not exceed 0.70 (cluster 1). Furthermore, ETs represented by atypical strains such as SAC8 (non-ß-haemolytic), ATCC 15313 and 35152 also non-ß-haemolytic, did not fall into separate categories. There was no correlation noted among serovars (1/2, 1/2b, 4b, and 3a). There were three clusters of *L. monocytogenes* strains (cluster 2) of which no significant trend was observed. For enzymes that had no activity (null alleles), the respective strains were grown a second time to prepare a new batch of enzymes, but the result remained the same. All of the 14 enzyme loci tested were polymorphic. The mean number of alleles per locus was 5.5 with a mean genetic diversity of 0.683. The number of alleles ranged from 3 (PGI and 6PG) to 9 (IDH) (Table 3.2.2).

Table 3.2.0 Electrophoretic types and source of *Listeria* strains.

Strain	Source	*Listeria* species	Serovar	Electrophoretic type
BGM 1	Mackerel	*L. innocua*		1
DTC 1	Cod	*L. innocua*		2
SRM 1	Mackerel	*L. innocua*		3
SRM 2	Mackerel	*L. innocua*		4
DTC 2	Cod	*L. innocua*		5
SRCP 2	Capelin	*L. innocua*		6
SRC 3	Cod	*L. innocua*		7
FPC 3	Cod	*L. monocytogenes*	4b	8
BGK 4	Kippers	*L. welshimeri*		9
SAC 4	Cod	*L. monocytogenes*	1/2	10
SRC 4	Cod	*L. innocua*		11
SACP 5	Capelin	*L. welshimeri*		12
SAT 5	Turbot	*L. welshimeri*		13
SAK 5	Kippers	*L. welshimeri*		14
SAC 5	Cod	*L. innocua*		15
DVC 5	Cod	*L. monocytogenes*	1/2	16
DTC 5	Cod	*L. welshimeri*		17
DTS 5	Salmon	*L. welshimeri*		18
DTK 5	Kippers	*L. welshimeri*		19
SRM 5	Mackerel	*L. welshimeri*		20
SRC 5	Cod	*L. monocytogenes*	1/2	21
SRK 5	Kippers	*L. monocytogenes*	1/2	22
SRCP 5	Capelin	*L. welshimeri*		23
DTK 6	Kippers	*L. welshimeri*		24
DTC 6	Cod	*L. welshimeri*		25
SRC 6	Cod	*L. monocytogenes*	1/2	26
SRM 6	Mackerel	*L. welshimeri*		27
SAC 7	Cod	*L. monocytogenes*	1/2	28
DTC 7	Cod	*L. innocua*		29
SRC 8	Cod	*L. innocua*		30
SAC 8	Cod	*L. monocytogenes*	1/2 (non-ßhem)	31
DTC 8	Cod	*L. welshimeri*		32

Table 3.2.0 continued

Strain	Source	Listeria species	Serovar	Electrophoretic type
SRC 9	Cod	L. innocua		33
SAC 9	Cod	L. innocua		34
DVC 9	Cod	L. monocytogenes	1/2	35
DTC 9	Cod	L. innocua		36
DTC 10	Cod	L. innocua		37
SRC 10	Cod	L. innocua		38
DVC 10	Cod	L. monocytogenes	1/2	39
DVC 11	Cod	L. monocytogenes	1/2	40
DTC 11	Cod	L. monocytogenes	1/2	41
DVC 12	Cod	L. innocua		42
DTC 12	Cod	L. innocua		43
Raw Fish	Cod	L. monocytogenes	1/2	44
LM3a	DFO	L. monocytogenes	3a	45
LM 1/2b	DFO	L. monocytogenes	1/2b	46
Livan	DFO	L. ivanovii		47
Linnoc	DFO	L. innocua		48
LM 15313	ATCC	L. monocytogenes		49
LM 35152	ATCC	L. monocytogenes	1/2	50
Swab1/2	Swab	L. monocytogenes	1/2	51
HUM1/2b	Human	L. monocytogenes	1/2b	52
HUM4b	Human	L. monocytogenes	4b	53
SS1/2b	Smoked salmon	L. monocytogenes	1/2b	54
SS4b	Smoked salmon	L. monocytogenes	4b	55
LOB1/2b	Lobster	L. monocytogenes	1/2b	56
LOB4b	Lobster	L. monocytogenes	4b	57

Notes: 1) ETs 1 to 43 are Listeria strains isolated from smoked seafoods

2) ETs 45 to 48 are Listeria strains collected from Department of Fisheries and Oceans, St. John's, NF

3) ETs 51 to 57 are Listeria strains collected from LCDC, Ottawa, Canada

Table 3.2.1 Allele profiles of 57 ETs of *Listeria* species.

		No. of alleles at the following enzyme loci:												
Strain	ET	6PG	GD2	ALD	PGM	ADK	ACP	IPO	EST	GP2	IDH	G6P	LDH	CAT	PGI
SRM2	4	2	3	3	2	2	1	1	5	2	8	2	2	1	1
SRC8	30	3	3	2	2	2	1	1	5	2	7	2	2	1	1
INNO	48	3	3	2	1	2	4	1	5	2	5	3	2	2	2
SAC5	15	2	3	2	1	2	1	1	5	2	4	2	4	0	2
DTC2	5	2	3	2	1	2	4	1	5	2	7	2	4	1	2
SRC3	7	2	3	2	1	2	4	1	5	2	7	2	4	1	1
DTC7	29	2	4	2	1	3	4	1	5	2	7	2	4	0	2
SRC4	11	3	3	2	3	2	4	1	5	2	0	2	4	0	1
BGM1	1	3	4	2	3	2	0	1	4	2	7	2	4	2	2
DTC1	2	2	4	2	2	2	3	1	5	1	7	3	4	3	2
DTC9	36	2	4	2	1	2	3	1	5	3	5	3	4	3	2
SRC9	33	2	4	2	1	2	1	1	5	3	7	3	4	2	2
SAC9	34	1	4	0	1	5	1	1	5	3	7	2	4	3	2
SRCP2	6	3	4	3	0	2	3	1	5	2	0	1	3	3	2
SRM1	3	2	4	3	0	2	4	1	5	2	0	2	4	4	2
DVC12	42	2	2	2	2	2	2	1	3	3	2	2	3	1	2
DTC12	43	2	2	2	2	2	1	1	3	3	2	1	2	1	1
DTC10	37	3	3	3	1	1	4	3	4	2	2	2	2	4	1

Table 3.2.1 continued

Strain	ET	No. of alleles at the following enzyme loci: 6PG	GD2	ALD	PGM	ADK	ACP	IPO	EST	GP2	IDH	G6P	LDH	CAT	PGI
SRC10	38	3	2	2	1	1	4	2	4	2	2	2	1	3	1
SAC4	10	2	3	3	1	4	1	2	2	3	6	1	2	2	1
DVC11	40	1	3	2	1	4	3	2	2	3	5	1	4	2	1
LM3A	45	2	3	4	2	4	0	2	3	3	5	1	1	3	1
SWAB1/2	51	1	3	3	2	4	1	2	3	3	5	1	5	4	1
LOB1/2B	56	1	2	3	2	4	1	3	2	3	5	1	4	3	1
SS1/2B	54	1	4	3	2	4	1	2	1	4	0	1	4	3	1
SRC5	21	2	3	2	3	4	2	2	1	3	4	1	4	0	1
DVC5	16	2	3	3	0	4	2	2	1	3	0	1	4	3	2
FPC3	8	2	3	3	0	4	0	2	5	1	3	1	4	3	1
LM1/2B	46	3	3	4	1	4	1	2	3	2	2	1	4	0	1
35152	50	2	3	0	2	4	3	2	3	0	2	1	0	0	1
15313	49	2	3	0	1	4	4	2	3	2	0	0	0	2	1
SRK5	22	2	4	3	3	5	1	2	3	2	5	1	4	3	1
SS4B	55	2	3	4	5	5	1	2	2	2	5	1	4	2	1
LOB4B	57	2	3	3	5	5	1	2	3	2	5	2	5	2	1
HUM4B	53	2	3	3	2	5	1	2	3	2	7	3	4	3	2
SAC8	31	2	4	3	0	5	1	2	2	2	0	1	4	1	2
SAC7	28	2	4	3	2	5	2	2	2	2	7	1	4	1	2
SRC6	26	2	5	3	0	4	2	4	2	2	5	1	4	1	2

Table 3.2.1 continued

Strain	No. of alleles at the following enzyme loci:														
	ET	6PG	GD2	ALD	PGM	ADK	ACP	IPO	EST	GP2	IDH	G6P	LDH	CAT	PGI
HUM1/2B	52	2	1	4	2	4	1	5	2	1	5	1	4	3	2
DTC11	41	3	2	4	2	4	2	2	3	2	5	2	4	2	2
DVC9	35	2	3	2	2	3	2	3	3	2	1	2	4	3	1
RF	44	2	3	2	2	3	3	3	0	3	1	1	4	3	1
DVC10	39	2	3	3	2	3	3	3	2	2	2	2	4	3	1
DTS5	18	1	4	6	3	1	1	4	2	3	1	2	4	0	2
SAK5	14	1	5	6	3	1	2	4	1	3	1	2	4	4	2
SAT5	13	3	3	6	3	1	1	1	2	2	5	2	4	3	1
DTC5	17	1	3	6	3	1	1	3	2	2	5	1	4	3	1
DTC6	25	1	3	6	3	2	1	4	2	2	5	2	4	2	1
DTK6	24	1	3	6	4	1	1	4	2	2	5	2	4	2	3
DTC8	32	1	0	6	4	1	1	4	2	3	5	2	4	0	1
SRM6	27	1	0	6	4	1	1	4	2	3	5	2	4	1	2
BGK4	9	1	4	6	2	1	1	3	2	3	5	2	4	2	1
DTK5	19	1	1	6	4	1	1	3	4	1	5	1	4	2	1
SRCP5	23	2	3	6	3	1	1	4	2	2	4	1	3	0	1
SRM5	20	2	3	6	3	1	0	4	2	2	7	1	4	0	1
SACP5	12	2	3	6	3	1	3	1	2	2	7	2	5	1	1
IVAN	47	1	4	1	3	3	0	5	5	3	7	4	5	3	3

Table 3.2.2 Number of alleles and genetic diversity at 14 enzyme loci among 57 ETs of *Listeria* spp.

Enzyme locus[a]	No. of alleles	Genetic diversity
6PG	3	0.595
GD2	6	0.635
ALD	7	0.762
PGM	6	0.785
ADK	6	0.839
ACP	5	0.718
IPO	5	0.745
α-EST	6	0.757
GP2	5	0.578
IDH	9	0.817
G6P	5	0.528
LDH	6	0.495
CAT	5	0.778
PGI	3	0.524
Mean	5.5	0.683

[a] 6PG, 6-phosphogluconate dehydrogenase; GD2, glutamate dehydrogenase; ALD, alanine dehydrogenase; PGM, phosphoglucomutase; ADK, adenylate kinase; ACP, acid phosphatase; IPO, indophenol oxidase; α-EST, α-naphthyl propionate esterase; GP2, NADP-dependent-glyceraldehyde-phosphate dehydrogenase; IDH, isocitrate dehydrogenase; G6P, glucose-6-phosphate dehydrogenase; LDH, lactate dehydrogenase; CAT, catalase; PGI, phosphoglucose isomerase.

Figure 3.2.0 Determination of ETs based on the electrophoretic mobility of a large number of enzymes. Illustration is of enzyme 6PG.

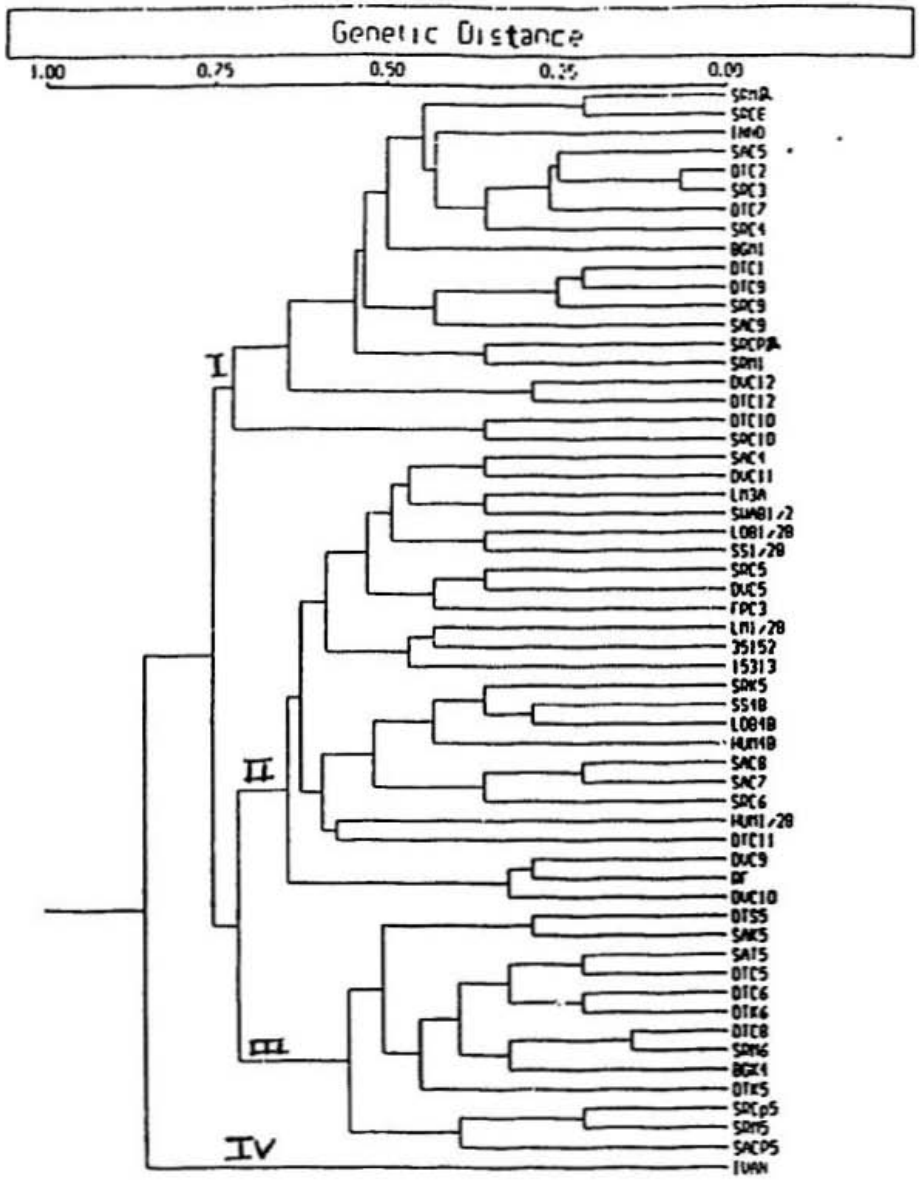

Figure 3.2.1 Genetic relationships among 57 ETs of four Listeria species. The dendrogram was generated by the average-linkage method of clustering from a matrix of pairwise coefficients of genetic distances, based on electrophoretically demonstrable allelic variation at 14 enzyme loci. The roman numerals indicate the main clusters: I , L. innocua, II, L. monocytogenes, III, L. welshimeri, IV, L. ivanovii.

3.3 Fate of *Listeria* in artificially inoculated cod fillets during smoking and storage temperatures

Trial 1

In trial 1, the inoculum was 2.8 x 10^6 MPN/g which remained relatively stable during the smoking process but decreased slightly to 10^5 MPN/g after 1 week of storage at 4°C. Under these conditions, the cell counts increased from 3.9 x 10^5 to 7.8 x10^7 MPN/g over a three week period (Fig. 3.3.0). The smoked cod stored at -20°C showed a decrease in cell numbers from a 10^4 MPN/g after the first month to 2.9 x 10^3 MPN/g after 3 months (Fig. 3.3.1). It should be noted that confidence intervals for 3 tube MPNs are very broad.

Trial 2

The inoculum for trial 2 was approximately 10^2 (110) MPN/g. It remained stable during brining and decreased slightly after smoking. Weekly analysis of the samples stored at 4°C revealed a growth increase of the organism from 7.8 x 10^2 (week 1) to 5.6 x 10^4 MPN/g after 3 weeks of storage (Fig. 3.3.0). Frozen storage at -20°C caused a decrease in cell number to 10^1 MPN/g. Gradual decreases occurred during month 2 but stabilized or increased slightly during month 3 (Fig. 3.3.1). Similar patterns of cell counts were observed in each trial.

Trial 3

Trial 3 had a very small inoculum, 2 MPN/g. The organism was still detectable at these low numbers. Upon storage at 4°C, the organism grew slowly, never growing above 10^1 MPN/g (Fig. 3.4.0). Frozen storage at -20°C caused a decrease in cell numbers almost to nil, making detection difficult. The cell counts remained at less than 10^0 MPN/g through the 3 months (3.3.1).

Storage effects

At 4°C, the organism increased in numbers by 1-2 $\log_{10}$ cycles per week for inoculum levels above 100 MPN/g (Fig. 3.3.0). In contrast the frozen storage of the finished product at -20°C showed a decrease in cell numbers in each trial but then remained stable at low numbers (Fig. 3.3.1).

Physicochemical analysis

The pH, water activity, and salt content ranged from 6.46 - 6.69, 0.942 - 0.967, and 3.99 - 5.97%, respectively (Table 3.3.0). These parameters expressed no significant influence on the organisms viability.

Table 3.3.0 Water activity (A_w), pH, and salt content of the smoked cod samples stored at -20°C and at 4°C.

Trial	Stored at 4°C			Stored at -20°C		
	A_w[a]	pH[a]	Salt (%)[a]	A_w[a]	pH[a]	Salt (%)[a]
1	0.948±0.009	6.46±0.048	4.84±0.398	0.947±0.028	6.54±0.058	5.41±0.673
2	0.942±0.010	6.46±0.046	3.99±0.631	0.942±0.030	6.62±0.076	5.97±1.580
3	0.952±0.008	6.49±0.052	5.01±0.801	0.967±0.002	6.69±0.101	4.47±0.607

[a] Mean ± standard deviation of five replicates

A_w water activity

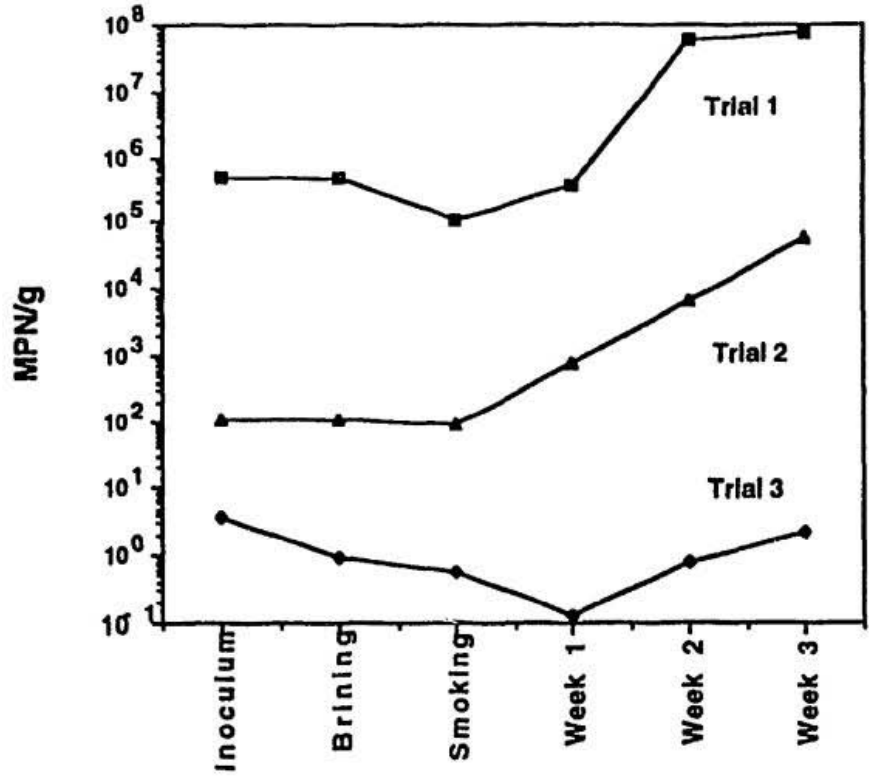

Figure 3.3.0 The survival rate of L. monocytogenes artificially inoculated onto cod fillets, smoked and stored at 4°C for three weeks determined using the MPN technique.

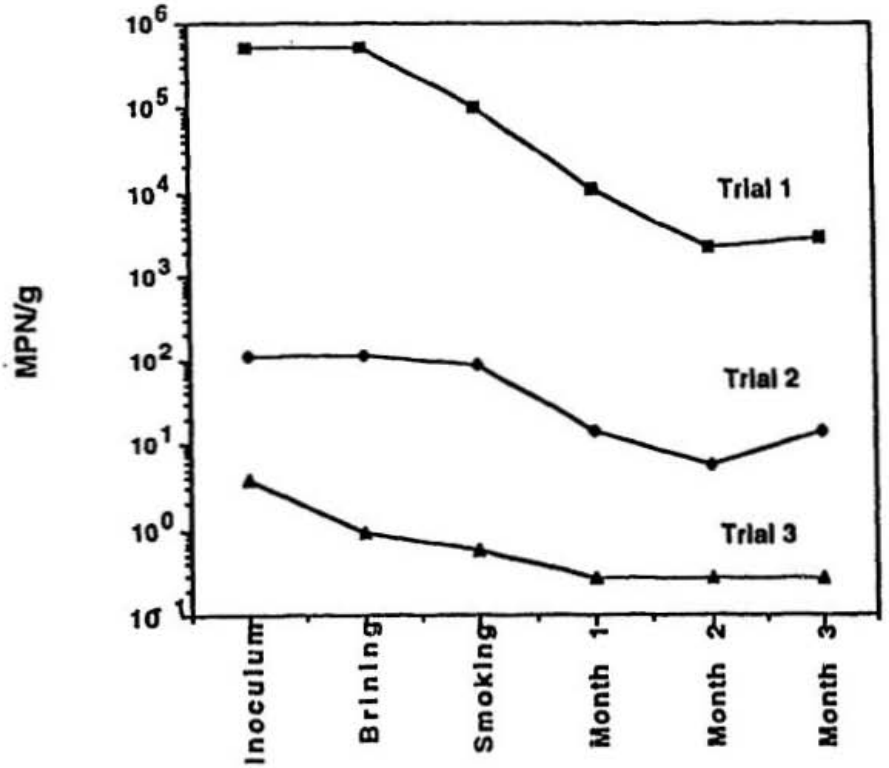

Figure 3.3.1 The survival rate of L. monocytogenes artificially inoculated onto cod fillets, smoked and stored at -20°C for three months determined using the MPN technique.

CHAPTER 4

DISCUSSION

4.1 Prevalence of Listeria in smoked seafoods

L. monocytogenes has been detected in smoked, fermented, and marinated seafood products (Jemmi, 1990a;b). Listeria species have been found to harbour in fresh and marine waters as well as raw and processed seafood products. Hence, it is not surprising that the foodborne pathogen L. monocytogenes and its sister non-pathogenic strains have also been detected in smoked seafoods surveyed from the Newfoundland market. It is evident that almost all foods investigated such as dairy foods (Azadian et al., 1989; Hayes et al., 1986; James et al., 1985: Lovett et al., 1987), vegetables (Heisick et al., 1989; Sizmur and Walker, 1988), and meats (Barbuti et al., 1989; Kerr et al., 1990; Lowry and Tiong, 1988; Pini and Gilbert, 1988; Varabioff, 1990) for the foodborne pathogen, have had positive results.

Overnight L. monocytogenes has become a critical problem for the smoked seafood industry (Hadeler, 1990). Smoked seafood are often prepared as ready-to-eat food commodities and hence, may pose a consumer health risk if the foods were contaminated with L. monocytogenes. The smoke treatment applied to seafood is one of two processes: cold smoke which

consists of a temperature of ≤ 28°C; and a hot smoke which has three heating stages, tempering at 30°C, heating at 50°C and cooking at 80°C (Churchill et al., 1989). The cold smoking process does not provide enough heat to kill bacteria. However, the smoke itself has been reported to have a bactericidal effect, killing up to 90% of the surface microflora in a well smoked product. The hot smoke process should be sufficient to destroy L. monocytogenes among numerous other bacteria. The reported hot smoking process in Newfoundland seldom follows the outlined hot smoking process. Therefore, it is possible to assume that the pathogen would not be destroyed during the hot smoke process. Naturally, there is always the problem of post-process contamination in any processed food product. Guyer and Jemmi (1991) studied the effect of cold smoking on L. monocytogenes in salmon and found that there was no significant effect on the organism. Listeria was found in both hot and cold smoked seafoods in this survey.

The present data on water activity, salt content, and pH of the hot and cold smoked seafood products suggest that these should be good bacterial substrates, and permit the proliferation of Listeria spp. Nevertheless, Listeria spp. have been isolated from dry products (Doyle et al., 1985; Farber et al., 1990), and although the ideal pH range for Listeria spp. is 5 to 9, they have been found to survive and

grow in much lower pH levels (Farber et al., 1989; Parish and Higgins, 1989; Fuchs and Sirvas, 1991), as well as being resistant to alkaline pH (Doyle, 1988). These, in fact, attest to the wide spread distribution of Listeria spp. in nature.

There appears to be some sort of predilection of Listeria spp. for salt water fish. Our test samples yielded L. innocua, L.welshimeri, and L.monocytogenes, and these same Listeria spp. have been reported to occur in fish by other workers as well (Buchanan et al., 1989; Hartemink and Georgsson, 1991). The high rate of contamination in cod was linked to the one commercial producer. It should be noted that the smoked cod is considered a hot smoked product even though the product is smoked at a constant temperature of 38°C.

Information concerning Listeria contamination among different species of seafood is limited. Our observation shed some light on the species-specific distribution of Listeria spp. However, this requires additional studies. A higher isolation rate of Listeria spp. during cooler months seems to coincide with a trend of listeriosis occuring in the fall (Health and Welfare Canada, 1988; 1989; 1991). A similar trend in the seasonal occurrence of Listeria spp. has been found in a study dealing with raw milk (Lovett et al., 1987). Nevertheless, there is contrary evidence that the pattern of

seasonal distribution may not be as distinct (Ralovich, 1984). It is difficult to pinpoint the major source of infection since smoked seafoods flow through the hands of processors as well as retailers before reaching the consumer. Hence, cross contamination is possible at any time. Therefore, an education system on sanitary practices to help eliminate Listeria is needed at both levels of suppliers and producers.

The serotypes of L. monocytogenes commonly causing listeriosis cases and outbreaks are 4b, 1/2a, and 1/2b (Farber and Peterkin, 1991). These serotypes account for more than 90% of all cases recorded (Bille and Doyle, 1991). The serotypes of L. monocytogenes for this survey were 4b and 1/2. These strains could serve as potential outbreak sources.

The presence of high total aerobic counts, coliforms, and S. aureus indicates lack of sanitary practices and the greater need of exercising good manufacturing practices (GMPs) among industry as well as at the retail level since a majority of the smoked seafoods are repackaged at the supermarket.

The USFDA Listeria isolation protocol (Hitchins, 1990) calls for a 48h primary enrichment in enrichment broth followed by plating onto listeria selective agars Oxford and LPM. This protocol was modified by Warburton and Farber (1990) to include a two stage enrichment, 48h primary enrichment and 48h secondary enrichment in modified Fraser broth. A group of workers at Health and Welfare Canada

examined the Canadian listeria isolation protocol in laboratories across Canada and found that 92% of all Listeria strains were detected within 24h of primary enrichment (Warburton et al., 1991b). A second study by Warburton et al. (1991a) revealed similar results. They also concluded that although useful, the secondary enrichment was not very selective (Warburton et al., 1991b). These results coincide with those found in this study. The secondary enrichment step only added to unnecessary work. This secondary enrichment broth turns from a yellow straw colour to a black precipitate in the presence of Listeria; ferric ions react with the product of aesculin hydrolysis (6,7-dihydroxycoumarin) to form a black precipitate (Fraser and Sperber, 1988). However, some other bacteria in foods such as enterococci cause the same reaction. This is eliminated by the addition of lithium chloride to the secondary enrichment broth, originally UVM, producing Fraser broth.

Conflicting data exists regarding the superiority of the newest listeria selective agars, LPM (Lee and McClain, 1987), Oxford (Curtis et al., 1989), and PALCAM (Van Netten et al., 1989). Oxford and LPM are most widely used in North America, whereas PALCAM is most widely applicable in Europe (Farber and Peterkin, 1991). Oxford medium was reported to be better than LPM (Tiwari and Aldenrath, 1990) and McBride agar (Curtis et al., 1989). When LPM was first formulated, it appeared to be

the superior agar for Listeria selectivity (Lee and McClain, 1987). However, with the two latest media, Oxford and PALCAM, LPM seems less dominating. It has been reported that PALCAM is more selective than Oxford and LPM (Van Netten et al., 1989). LPM medium requires the use of the Henry technique (Henry, 1933) and when compared with the other two media, it is much more difficult to distinguish Listeria colonies from other bacteria. Listeria colonies give a whitish-bluish crushed glass appearance using the Henry technique with LPM. PALCAM and Oxford utilize aesculin hydrolysis which result in black-green colonies with sunken centres and black halos after 24 and 48h incubation at 35 to 37°C typical of Listeria (Bille and Doyle, 1991). These agars can immediately give suspected Listeria colonies by simple observation of the plate. The existing controversy may be due in part to the type of foods being analyzed, the numbers and types of strains typical of that particular food sample, and of course the methods of the scientists. This study revealed PALCAM to detect all strains isolated after enrichment whereas some strains did not grow on Oxford or LPM. It is obvious that for Listeria detection in foods, enrichment as opposed to direct plating is necessary as seen in this study and that of others (WHO, 1988). The numbers of Listeria in foods are often reported to be very low and hence, can exist undetected on the selective agars during direct plating.

Some strains of L. monocytogenes are non-ß-haemolytic as seen in this study. The virulence factors of such strains are not completely understood nor known. The ATCC 15313 L. monocytogenes strain serotype 1/2 is haemolytic on rabbit blood, but non-haemolytic on sheep, horse, and bovine blood, and is avirulent (Van der Kelen and Lindsay, 1990). Njoku-Obi et al. (1963) reported low haemolytic, high virulent variants of L. monocytogenes. Listeriolysin, a haemolysin, has been noted to be greatly associated with the virulence factor of L. monocytogenes (Kuhn et al., 1988, Rocourt and Seeliger, 1987; Schlech, 1988; Pine et al., 1987; Cossart et al., 1989), however, the evidence for this being the only virulence factor is inconclusive and doubtful (Gellin and Broome, 1989; Ralovich, 1989; Van der Kelen and Lindsay, 1990). Parrisuis et al. (1986) have noted that human pathogenic Listeria strains produce more than one haemolysin different from listeriolysin O. L. seeligeri, is weakly haemolytic but avirulent and L. ivanovii is strongly haemolytic but regarded as a major pathogen of animals and rare in humans (Bille and Doyle, 1991). However, L. seeligeri has been isolated from a human listeriosis case and L. ivanovii also has been reported to cause disease in man (Schönberg, 1989). Some difficulty was encountered with ß-haemolysis as a confirmation test. L. monocytogenes strains isolated from smoked seafoods gave a very narrow zone of haemolysis most often undetectable. It is

often necessary to remove the colony to determine the haemolysis (Seeliger and Jones, 1986). Consequently, it becomes difficult to distinguish between the non-pathogenic strain L. innocua and L. monocytogenes which give the same carbohydrate fermentation reactions. Some blood, notably that of sheep, contain antibodies against L. monocytogenes (Seeliger and Jones, 1986). Although, an easy method for detecting the ß-haemolysis from blood agar plates is to place the plates upside down on a light source. The light will shine through the narrow zone of haemolysis which can be compared to the inoculated controls.

4.2 Characterization of Listeria spp. isolated from smoked seafoods by multilocus enzyme electrophoresis

Previous studies have reported that L. monocytogenes contains a great number of ETs with MEE but only one to a few clones have actually been reported to cause listeriosis outbreaks (Piffaretti et al., 1989). Greater than 90% of the outbreaks have been caused by serovars 4b, 1/2a, and 1/2b (Bibb et al., 1990). A strain of the same ET has been noted to be responsible for outbreaks in Switzerland, 1983-1987 (Bille, 1990; Piffaretti et al., 1989); California, 1985 (Linnan, 1988; Piffaretti et al., 1989) and Nova Scotia, 1981 (Schlech et al., 1983; Piffaretti et al., 1989). A separate ET caused listeriosis outbreaks in Massachusetts in 1979 (Ho

et al., 1986; Bibb et al., 1989) and again in 1983 (Flemming et al., 1985; Bibb et al., 1989). In Philadelphia, 1987, 11 ETs were found ruling out a common food source as a cause of outbreak (Bibb et al., 1989). In this study the strains isolated from humans do not correspond to any identical ETs from contaminated foods. Non-matching ETs in epidemiological experiments with foods and patients indicate that the particular food under study is not the source of contamination. It has been suggested that small, unrecognized clusters of disease from a single source are likely to exist (Bibb et al., 1989).

The fact, that there is not a common branching of ETs, suggests that there is multiple contamination of the smoked fish products from various sources. Boerlin and Piffaretti (1991) found that an ET1 strain causing listeriosis in 49% of animals was not the same ET isolated from the meat products; they concluded that the contamination of the meat products was actually from the processing environment and did not originate from the animals themselves. Hence, it seems more likely that the contamination of the food products in this study occurred most likely at processing and postprocessing stages. This proves the need for greater care and sanitation during the handling and processing of the various stages of smoked products.

Separate clusters of Listeria spp. have been observed in

a similar study with MEE (Boerlin et al., 1991) as was found in this study. Most studies to date of MEE on Listeria are mostly with L. monocytogenes only. These authors have found two clusters to occur with L. monocytogenes based on two groups of serovars: 1/2a, 1/2c, 3a ; and 1,2b, 4b, 3b, 4a (Bibb et al., 1990; Piffarette et al., 1989; Boerlin and Piffaretti, 1991). They also found no ET' to contain isolates from more than one serogroup indicating the possible stability of the enzymes composing the flagellar antigens. In this study, the strains of L. monocytogenes were only typed to 1/2 and were not designated a letter, hence making any conclusions on the serovars impossible. However, it was noted that the two major clusters had both 1/2b and 4b serovars providing no distinction among them. However, others have included in their analysis 16 to 21 enzymes. The present study investigated only 14 enzymes and this fact may account for some difference. Furthermore, the one study of MEE which analyzed all Listeria spp., observed L. welshimeri linking to L. innocua prior to clustering with L. monocytogenes (Boerlin et al., 1991). However, in this study, L. welshimeri clustered with L. monocytogenes before clustering with L. innocua. The pattern of these three then clustering with L. ivanovii agreed with the results of the previous study (Boerlin et al., 1991). Again, the difference may lie with the number of enzymes analyzed (14 versus 18) as well as the

fact that the genetic distance data in this study was not weighed with the reciprocal of the mean genetic diversity for each enzyme locus.

In this study 20 enzymes were assayed but only 14 were adequate enough for scoring. It appeared that the enzyme fumarase (FUM) produced almost the same patterns as indophenol oxidase (IPO); however, fumarase should give violet bands (Harris and Hopkinson, 1976) and IPO white bands. In this study FUM gave white bands and therefore, was assumed inaccurate. On occasion a wave in the migration front would result in inconsistencies and the gels would have to be redone. Using the protocols outlined by Selander et al. (1986), the enzymes: GOT (glutamic-oxalacetic transaminase), PEP (peptidases), ACO (aconitase), ALO (aldolase) and ß-ESt (esterases) did not work. Possible reasons could be that the buffer systems were inappropriate; Boerlin et al. (1991)stained GOT according to the procedures described by Harris and Hopkinson (1976); for PEP Buffer A was used but Harris and Hopkinson (1976) reported use of sodium phosphate buffer (pH 7.5) and in another study (Bibb et al., 1990) used a Tris/citrate buffer, pH 7.2. Perhaps using ß-EST staining with ß-naphthyl propionate instead of ß-naphthyl acetate (Boerlin et al., 1991) may solve the problem. In addition Tris/maleic anhydride buffer, pH 7.2 (Harris and Hopkinson, 1976) may be used. Bibb et al. (1990) tested 16 enzymes and

IDH, NP1, NP2, and ACP were assayed in pH 6.7 Tris citrate gels (Buffer B) and all others were in pH 8.2 tris citrate gels (approximately buffer A, pH 8.0). In this study, IDH worked well in Buffer A and ACP was done in Buffer F. Also, many workers culture bacteria in 100ml nutrient broth and dilute the harvested bacteria in 2ml of buffer before sonicating. In this study, cells were grown in 10ml TSB and incubated at 30°C for 24h before inoculating into 200ml TSB and growing for 24h under the same conditions to concentrate the cells. Sonication took place at full power for 5 min as opposed to 2-3 mins. Only very faint bands occurred with ACO and ALO making scoring difficult.

It has been noted by other researchers (Boerlin and Piffaretti, 1991; Kephart, 1990) that results from MEE are often irreproducable partly because they are subjected to quantitative inaccuracy. This variation may occur because of the differences in strain history, buffer systems (Wendel and Weeden, 1989), insufficient experience of the technicians with MEE, inefficient analytical equipment such as pH meters and electrophoresis apparatus, as well as insufficient cooling of the gel during electrophoresis (Kephart, 1990). The researchers have not always used the same enzymes nor numbers in there studies of MEE with Listeria spp. (Bibb et al., 1989; Boerlin et al., 1991; Boerlin and Piffaretti, 1991; Piffaretti et al., 1989; Bibb et al., 1990). Furthermore, the methods

were those of Selander et al. (1986) followed by all; but for the most part the authors did not inform the reader of the buffer systems used for each enzyme. In the procedures of Selander et al. (1986) several buffer systems are reported for most enzymes. Hence, in this study an educated guess had to be taken for the particular buffer systems to be used. Before any method is to be used, it should be consistent in all aspects to avoid irreproduceable data which is already a reported mishap for electrophoresis (Carr and Johnson, 1980; Gordon et al., 1988). It is necessary that the application of MEE with Listeria be well standardized.

MEE has been used successfully for many years as a tool for studying genetic variation in eukaryotes. The epidemiological tool has recently been applied to prokaryotes (Selender et al., 1986). This investigative tool has more recently been applied to Listeria spp. in the event of determining the food responsible for listeriosis outbreaks. The tool to date has been found valuable in defining the possible routes of infection in foods as well as assessing environmental clones (Borelin and Piffaretti, 1991). All strains are typeable with MEE which allows distinct separation of Listeria spp. which are phenotypically similar and indicates the genetic relatedness within the species. Furthermore, there is minimal subjectivity to evolutionary convergence (Selander et al., 1986).

4.3 Fate of *Listeria* in artificially inoculated cod fillets during smoking and storage temperature

Messina et al. (1988) reported *L. monocytogenes* to be reduced by 99.9% during the addition of liquid smoke to frankfurters. Beltran et al. (1989) also reported similar antibacterial effects of smoking on sardine fillets. Traditional smoking of fish products has been noted to destroy more than 90% of the surface microflora in a well smoked product. More modern products, however, are lightly salted and smoked for flavour and not for preservation reasons. In this study, the smoking process had no detrimental effect on the foodborne pathogen, *L. monocytogenes*. Even with the lowest possible number of cells per gram of flesh, the organism was still detectable.

Listeria's ability to survive in various foods depends on several combined parameters such as temperature, pH, salt content and water activity. *L. monocytogenes* has been reported to survive in up to 25.5% salt (Health and Welfare Canada, 1990) and is therefore very salt resistant. The average salt concentration of 5% had no effect on the organism's ability to proliferate. *Listeria* is capable of growing in a wide pH range of 5-9, but has been known to survive in much lower pH systems (Conner et al., 1986; Farber et al., 1989; Parish and Higgins, 1989). Hence, the average pH of 6.5 of the samples analyzed in this study, had no

influence on the organism. It has been documented that the closer the pH to 7.0 in meats, the better the organism grew (Glass and Doyle, 1989; Grau and Vanderlinde, 1990). The minimum water activity for Listeria growth was recorded at 0.92 (Health and Welfare Canada, 1990). Although, the organism has been detected in alfalfa tablets (Farber et al., 1990) as well as nonfat dry skim milk powder (Doyle et al., 1985). Thus, the pathogen can withstand very dry environments. The average water activity for the smoked fillets in this study was 0.950, and as a result there was no effect on the organism. Listeria has a growth temperature range of -.4 to 50°C (Health and Welfare Canada, 1990). It has been published that low temperatures slow down metabolism and growth and thereby allow Listeria to survive low pH values in conjunction with high salt concentrations longer than if at higher temperatures (Cole et al., 1990; Conner et al., 1990).

Cold smoking is conducted at temperatures $\leq$ 28°C; and therefore, provides an adequate environment for bacterial growth. No growth was observed during the smoking process in any of the trials conducted during this investigation. Similar observations have been reported by Guyer and Jemmi (1991). It is obvious that smoking has played some role in this stabilization of growth during the process. Listeria has been detected in hot smoked products as well (Jemmi, 1990a;1990b). A hot smoking process eventually involves

cooking the product at 80°C for 0.5 to 1 h (Churchill et al., 1989). This organism has been noted to be more heat resistant than the normal vegetative cells of other bacteria (Doyle et al., 1987); although much controversy exists over the organisms heat resistance, particularly its ability to survive pasteurization of milk (Bradshaw et al., 1991; Doyle et al., 1987; Lovett et al., 1990). It has been reported that cooking foods to an internal temperature of 70°C for 2 min. is adequate to ensure destruction of L. monocytogenes (Mackey and Bratchell, 1989). However, conflicting data exists as previously described (McCarthy et al., 1990). Listeria have also been reported to be less sensitive to irradiation than gram negative bacteria; some strains have survived as high as 4KGy radiation (Tarjan, 1990; Huhtanen et al., 1989). However, L. monocytogenes was found to be sensitive to short wave UV-energy but not long wave UV-energy (Yousef and Marth, 1988). UV-energy is ideally used to decontaminate the atmosphere in food processing areas and hence may be an alternative which requires more research to decontaminate Listeria from food products.

Freezing at -20°C of the smoked cod fillets did indicate an initial decrease in cell number and eventually a dormant stage. Listeria has been isolated from frozen seafoods (Weagant et al., 1988) so it is not surprising to discover the organism's viability at very low numbers observed in the

present study. Bille and Glausser (1988) also noted a decrease of 10 to 100 cells of L. monocytogenes in soft cheese. However, other studies have reported no effect of freezing on the organisms in foods (Guyer and Jemmi, 1991; Kaya and Schmidt, 1989).

Vacuum packaging appeared to have no effect on the organism in this study as well as in other studies (Bentley et al., 1989; Glass and Doyle, 1989; Grau and Vanderlinde, 1992; Harrison et al., 1991). Also, other methods of packaging such as controlled atmosphere storage, plastic wrap or modified atmosphere packaging did not have any significant effect on the organism's ability to grow (Berrang et al., 1989; Ingham et al., 1990; Wimpfheimer et al., 1990; Kallander et al., 1991).

The presence of Listeria in smoked cod, as well as other smoked products, indicates the need for a exceptional sanitation procedures and careful handling during and after processing. Farber (1991) reported the presence of L. monocytogenes in smoked salmon. The organism was found to grow very well in artificially inoculated smoked salmon (Guyer and Jemmi, 1991; Farber, 1991). Some workers (Farber, 1991) insist that no health risk is imposed because of the low numbers of organisms and short shelf life of the product unless temperature abuse occurs. However, some smoked products have a shelf life up to 1 month and very often the

temperatures of refrigerated cabinets at retail level can be anywhere from 2 to 10°C. Furthermore, not knowing the infectious dose makes it difficult to conclude which products are not a health risk. Most smoked fish products are cooked prior to consumption and depending on method of cooking, the risk may be reduced. Guyer and Jemmi (1991) concluded that there may be a potential risk if such products are stored for a long period of time. Based on this study, if the seafoods have low bacterial numbers < 50 cells/g then the organism may not grow as well as when the numbers of cells are > 100 cells/ g of flesh over a three week period indicating that there could be a potential health risk.

CHAPTER 5

CONCLUSIONS

Approximately 5 smokers in Montreal, Vancouver and Seattle have been shut down already because of Listeria contamination. Unless the problem can be eradicated, the entire industry may have to be closed. Almost overnight Listeria has become the number one problem for the entire smoking industry that depends on a share of the US market (Hadeler, 1990). USFDA require a zero tolerance for Listeria in any ready-to-eat food entering the US. This report verifies the prevalence of Listeria in hot and cold smoked seafood products and concludes that cold smoking has no bactericidal or bacteriostatic effect on Listeria. However, proper hot smoking could destroy Listeria spp.; but the problem of post-process contamination poses a problem.

More research is needed to determine which products are potential health risks for listeriosis and the numbers of organisms present and required to cause illness. Good manufacturing practices (GMPs) for smoked fish products are essential and would help to reduce if not eliminate contamination of the product. Palumbo and Williams (1990) concluded from their study that Listeria could persist in plant environments for long periods if the temperature is low

and the organism is protected by various foods. Also, efficient temperature controls would alleviate the possibility of placing products under temperature abuse. It would be ideal to determine the major source of contamination so as to reduce and possibly eliminate Listeria contamination from foods. Slade (1992) has reported the ubiquity of Listeria spp. in food production environments particularly in cool, damp environments on conveyors, floors and drains.

Finally, well defined methods of Listeria detection and isolation as well as epidemiological testing is still required. The fundamentals of the protocols should be consistent and well established. The FDA protocol requires refinement since L. monocytogenes strains are arising with atypical characteristics on which the FDA protocol basis much of its emphasizes (Swartz et al, 1991). As well, a more rapid method of detection is essential so as to prevent unnecessary detention of products. Furthermore, with regards to epidemiological testing, MEE appears to be the most promising tool to date for determining the causative agent of listeriosis outbreaks. However, this method still needs to be perfected and the protocol made consistent to produce more accurate and reliable data.

Wilkinson (1989) stated: "The aetiology of listeriosis will not be properly understood until we have better information on the incidence, levels of contamination, and

conditions of survival and destruction of listeria in a wide variety of foods" in the British Medical Journal.

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APPENDIX

Appendix A 1.0 Formulations of media used in the detection of Listeria.

1) Listeria Enrichment broth - Bacto tryptone 17.0 g, bacto soytone 3.0 g, dextrose 2.5 g, sodium chloride 5.0 g, potassium phosphate, dibasic 2.5 g, bacto yeast extract 6.0 g, cycloheximide 0.05 g, acriflavin HCl 0.015 g, nalidixic acid 0.04 g, distilled water 1.0 L.

2) UVM Listeria Enrichment Broth - Bacto tryptose 10.0 g, bacto beef extract 5.0 g, bacto yeast extract 5.0 g, sodium chloride 20.0 g, potassium phosphate, monobasic 1.35 g, sodium phosphate, dibasic 9.6 g, esculin 1.0 g, nalidixic acid 0.02 g, acriflavin HCl 0.012 g, distilled water 1 L. Stock Solution - Acriflavin (1.2 mg/ml in distilled water) and Ferric ammonium citrate (5% in distilled water).

3) PALCAM Listeria Selective Agar - Peptone 23.0 g, starch 1.0 g, sodium chloride 5.0 g, agar 13.0 g, mannitol 10.0 g, ammonium iron (III) citrate 0.5 g, aesculin 0.8 g, dextrose 0.5 g, lithium chloride 15.0 g, phenol red 0.08 g, distilled water 1.0 L. PALCAM Listeria Selective Supplement - polymyxin B-sulphate 0.01 g, ceftazidime 0.02 g, acriflavin 0.005 g per litre of PALCAM agar.

4) Oxford Listeria Selective Agar - Columbia agar base 39.0 g, aesculin 1.0 g, iron (III) ammonium citrate 0.5 g, lithium chloride 15.0 g, and bacto agar 2.0 g, distilled water 1.0 L. Oxford Listeria Selective Supplement - cycloheximide 0.4 g, colistin sulphate 0.02 g, acriflavin 0.005 g, cefotetan 0.002 g, fosfomycin 0.01 g.

5) Lithium Chloride-Phenylethanol-Moxalactam Medium (LPM) - Bacto tryptose 10.0 g, bacto beef extract 3.0 g, sodium chloride 5.0 g, lithium chloride 5.0 g, phenylethanol 2.5 g, glycine anhydride 10.0 g, bacto agar 15.0 g, distilled water 1 L. The LPM agar used was in dehydrated form.

Appendix A 1.1 Media preparation for Listeria detection protocol

Blood agar: horse blood or sheep blood plates consisted of 5% blood in TSA.

Carbohydrate fermentation broth: 9 mls of purple broth base was dispensed into 16 x 125 mm tubes containing a Durham tube, then autoclaved at 121°C for 15 min. Carbohydrates were filter sterilized as 5% solutions and 1 ml was added to 9 ml broth base to yield 0.5% carbohydrate in broth.

LPM: The moxalactam stock solution consisted of 1 g of moxalactam sodium salt in 100 ml of 0.1 M potassium phosphate buffer pH 6.0. The filter sterilized stock solution was stored at -20°C in 1 ml aliquots in Eppendorf tubes. Approximately 2 ml was added to LPM per litre before pouring plates.

UVM: Added 0.1 ml of acriflavin (0.06%) and ferric ammonium citrate (5%) stock solutions to 10 mls (16 x 150 mm) of UVM immediately before use.

Oxford agar: Added 2 vials of supplement to 1 L of Oxford agar before pouring plates. The vials required a 1:1 mixture of 5 ml of ethanol and sterile distilled water to be dissolved.

PALCAM agar: Added 2 vials of supplement to 1 L of PALCAM agar immediately before pouring plates. The vials required 1 ml of sterile distilled water to be dissolved.

Motility Test Medium: Rehydrated and sterilized according to the manufacturer's instructions. Added 6 ml medium in 16 x 125 mm screw-cap test tubes.

Trypticase soy agar with 0.6% yeast extract (TSA-YE): Trypticase soy agar 40.0 g, yeast extract 6.0 g, and distilled water 1.0 L.

Trypticase soy broth with 0.6% yeast extract (TSB-YE): Trypticase soy broth 30.0 g, yeast extract 6.0 g, and distilled water 1.0 L.

Appendix A 1.2 Buffer systems for electrophoresis

Buffer A

ELECTRODE BUFFER	
TRIS-CITRATE (PH 8)	
Tris	83.20g
Citric acid monohydrate	33.09g
Water	1 L
GEL BUFFER	
TRIS-CITRATE (PH 8)	
Electrode buffer diluted	1:29
VOLTAGE	
130 v	

Buffer B

ELECTRODE BUFFER	
TRIS-CITRATE (PH 6.3)	
Tris	27.00g
Citric acid monohydrate	18.07g
Water	1 L
(adjust pH with NaOH)	
GEL BUFFER	
TRIS-CITRATE (PH 6.7)	
Tris	00.97g
Citric acid monohydrate	00.63g
Water	1 L
(adjust pH with NaOH)	
VOLTAGE	
150 v	

Buffer C

ELECTRODE BUFFER	
BORATE (PH 8.2)	
Boric acid	18.50g
NaOH	02.40g
Water	1 L
GEL BUFFER	
TRIS-CITRATE (PH 8.7)	
Tris	09.21g
Citric acid monohydrate	01.05g
Water	1 L
VOLTAGE	
250 v	

Buffer F

ELECTRODE BUFFER

TRIS-MALEATE (PH 8.2)

Tris	12.10g
Maleic acid	11.60g
Disodium EDTA	03.72g
$MgCl.H_2O$	02.03g
Water	1 L

(adjust pH with 5.15g of NaOH)

GEL BUFFER

TRIS MALEATE (PH 8.2)

Electrode buffer diluted 1:9

VOLTAGE

100 v

Appendix A 1.3 Preparation of chemicals for staining

Solution A	Isocitric acid 1.0M 2.94g of DL-isocitric acid. water (trisodium salt) in 100ml water
Solution B	Tris hydrochloride 0.2M pH 8 24.2g Tris in 1 L water; adjust pH with HCl
Solution C	$MgCl_2$ 0.1M 2.03g of $MgCl_2.6H_2O$ in 100 ml water
Solution D	NAD 1g NAD-free acid in 100 ml water
Solution E	NADP 1g of disodium NADP in 100 ml water
Solution F	Dimethylthiazol tetrazolium (MTT) solution 1.25g in 100 ml water (Keep in refrigerator and in dark bottles)
Solution G	Phenazine methosulfate (PMS) solution 1g in 100 ml water (Keep in refrigerator and in dark bottles)
Solution H	Glycyl-glycine 0.1M pH 7.5 11.3g glycine plus 1 L water; adjust pH with 1M KOH
Solution I	Sodium phosphate pH 7.0 Mix equal parts of 27.6g of $NaH_2PO_4.H_2O$ (monobasic) in 1 L water and 53.6 g $Na_2HPO_4.7H_2O$ in 1 L water. Then dilute mixture 1:25 with water.
Solution J	Sodium acetate pH 5.0 0.05M 6.8g of sodium acetate.3 H_2O in 1 L water; adjust pH to 5.0 with about 2 ml HCl
Solution K	Agar overlay: 500 mg of agar in 25 ml of 0.2 M Tris hydrochloride buffer pH 8.0
Solution L	$MnCl_2$ (0.25 M). 4.9 g $MnCl_2.4H_2O$ in 100 ml water
Solution M	TRIS acetate (0.1 M, pH 7.5) 12.11 g TRIS in 1000 ml water

add acetic acid (till pH 7.5)
add 19.63 g KAc.
add 333 mg $CoCl_2$
add 35.2 mg l-cysteine HCl

Appendix A 1.4 Enzyme preparations

1) OXIDOREDUCTASES

A) ISOCITRATE DEHYDROGENASE (IDH)
Buffer A

Staining

Isocitric acid 1.0M[A*]	2ml
Tris hydrochloride 0.2M pH 8[B]	50 ml
$MgCl_2$ 0.1M[C]	2 ml
NADP[E]	

* The letter refers to the type of staining solutions described in Appendix A 1.3.

B) 6-PHOSPHOGLUCONATE DEHYDROGENASE (6GP)
Buffer A

Staining

6-phosphogluconic acid (barium salt)	10mg
Tris hydrochloride 0.2M pH 8[B]	20 ml
$MgCl_2$ 0.1M[C]	10 ml
NADP[E]	

C) GLUCOSE 6-PHOSPHATE DEHYDROGENASE (G6P)
Buffer B

Staining

Glucose 6 phosphate (disodium salt hydrate)	100mg
Tris hydrochloride 0.2M pH 8[B]	50 ml
$MgCl_2$ 0.1M[C]	1 ml
NADP[E]	

D) GLYCERALDEHYDE-PHOSPHATE DEHYDROGENASE (GP2)
Buffer A

Staining

Fructose 1,6 - diphosphate	100mg

Tris hydrochloride 0.2M pH 8[B]	40 ml
$Na_2HAsO_4.7H_2O$	50 mg
NADP[E] or NAD[D]	

E) DL-LACTATE DEHYDROGENASE (LDH)
Buffer B

Staining

DL-Lithium lactate	330 mg
Glycyl-glycine 0.1M pH 7.5[H]	50 ml
NAD[D]	

F) ALANINE DEHYDROGENASE (ALD)
Buffer A

Staining

DL-Alanine	50 mg
Sodium phosphate pH 7.0[I]	50 ml
NAD[D]	

G) GLUTAMATE DEHYDROGENASE (NADP) (GD2)
Buffer A

Staining

L-Glutamic acid	200 mg
Tris hydrochloride 0.2M pH 8[B]	50 ml
NADP[E]	

H) INDOPHENOL (IPO) (superoxide dismutase)
Buffer B

Staining

Stain in light at room temperature

Tris hydrochloride 0.2M pH 8[B]	40 ml
$MgCl_2$ 0.1M[C]	2 ml
NAD[D]	

I) CATALASE (CAT) (Harris and Hopkinson, 1976)
Buffer F

Staining

Incubate gel slice for 15 min. in 50 ml of a solution containing 1.5 ml of 50% solution of hydrogen peroxide and 750 mg of sodium sulphite. Pour off solution, rinse gel slice

with water, and immerse it in a freshly made 1.5% solution of potassium iodide (750 mg in 50 ml water). Mix gently and remove stain solution when white zones appear on dark-blue background.

2) TRANSFERASES

B) GLUTAMIC-OXALACETIC TRANSAMINASE (GOT)
Buffer A

Staining

L-Aspartic acid	50 mg
Pyridoxal 5'-phosphate	1 mg
α-Ketoglutaric acid	100 mg
Tris hydrochloride 0.2 M pH 8[B]	50 ml
Fast Blue BB salt	100 mg

C) ADENYLATE KINASE (ADK)
Buffer F

Staining

Glucose	100 mg
ADP	25 mg
Hexokinase	1 mg
Glucose 6-phosphate dehydrogenase	15 U
NADP	1 mg
Tris hydrochloride 0.2M pH 8[B]	25 ml
$MgCl_2$ 0.1M[C]	1 ml
MTT[F]	0.6 ml
PMS[G]	0.6 ml

D) PHOSPHOGLUCOMUTASE (PGM)
Buffer A

Staining

Glucose 1-phosphate & Glucose 1,6-diphosphate	5 mg

(The glucose 1-phosphate preparation contains sufficient glucose 1,6-diphosphate for the staining reaction)

NADP	0.5 mg
Tris hydrochloride 0.2M pH 8[B]	5 ml
$MgCl_2$ 0.1M[C]	5 ml
Water	25 ml
MTT[F]	1.0 ml

PMS[G]	0.5 ml

3) HYDROLASES

A) ESTERASES (EST) α & ß
Buffer F

Staining

α-/ß-naphthyl acetate or α-/ß-naphthyl propionate (1% sol. in acetone)	1.5 ml
Sodium phosphate pH 7.0[I]	40 ml
Fast Blue RR salt	25 mg

B) ACID PHOSPHATE (ACP)
Buffer F

Staining

α-Naphthyl acid phosphate	50 mg
Sodium acetate pH 5.0 0.05M[J]	50 ml
Black K salt	20 mg

C) PEPTIDASES (PEP)
Buffer A

Staining

L-Phenylalanyl-L-leucine	20 mg
Peroxidase	10 mg
Snake venom	10 mg
Tris hydrochloride 0.2M pH 8[B]	50 ml
$MnCl_8$ 0.25 M	0.5 ml
o-Dianisidine dihydrochloride	10 mg
Agar overlay[K]	

4) LYASES

A) FUMARASE (FUM)
Buffer C

Staining

Fumaric acid (potassium salt)	50 mg
Malate dehydrogenase	50 U
NAD	20 mg
Tris hydrochloride 0.2M pH 8[B]	50 ml
MTT[F]	1.0 ml
PMS[G]	0.5 ml

B) ACONITASE (ACO)
Buffer A

Staining

Cis-Aconitic acid	20 mg
Isocitrate dehydrogenase	5 U
NADP	10 mg
Tris hydrochloride 0.2M pH 8[B]	15 ml
$MgCl_2$ 0.1M[C]	10 ml
MTT[F]	1.0 ml
PMS[G]	0.5 ml
Agar overlay[K]	

5) ISOMERASES

B) PHOSPHOGLUCOSE ISOMERASE (PGI)
Buffer A

Staining

Fructose 6-phosphate	10 mg
Glucose 6-phosphate dehydrogenase	3 U
NADP	6 mg
Tris hydrochloride 0.2M pH 8[B]	25 ml
$MgCl_2$ 0.1M[C]	0.3 ml
MTT[F]	1.0 ml
PMS[G]	0.5 ml
Agar overlay[K]	

Appendix A 2.0 Fish smoking company addresses.

Company Name and Address

Fishery Products International Ltd.
P.O. Box 550
St. John's, NF
A1C 5L1
Contact: William Wells, Executive Vice-President
Telephone: 709-570-0000
Fax: 709-570-0209

Golden Shell Fisheries Ltd.
Hickman's Harbour, Trinity Bay
A0C 1P0
Contact: Ralph Simmons, Owner
Telephone: 709-547-2518
Telephone: 709-368-5805

National Sea Products Ltd.
P.O. Box 89
Arnold's Cove, NF
A0B 1A0
Contact: Bruce Wareham, VP Production
Telephone: 709-463-2445
Fax: 709-463-2300

Paul Pye, Trading in the Style & Manner of
Cape St. Charles
Cape Charles Smoked Product
Cape Charles, NF
A0K 1T0
Contact: Paul Pye
Telephone: 709-921-6327

Seaside Fisheries Ltd.
Coley's Pt., Conception Bay, NF
A0A 1Y0
Contact: Kevin Smart, Manager
Telephone: 709-786-7393
Telephone: 709-334-2868

Nain Fisheries
Nain, Labrador
A0P 1L0
Contact: Ed Smith, Assistant Director
Telephone: 709-576-3748

Table A 3.1 Microbiological analysis water activity, salt content and pH of the smoked seafood products sampled from various supermarkets and industry for the month of June '91.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Kippers	-	-	-	-	0.956	4.72	5.09
2 Mackerel	-	-	TNTC	+	0.962	4.39	5.77
3 Capelin	3600b/g	-	-	-	0.659	12.32	6.63
4 Cod	-	TNTC	TNTC	-	0.987	3.61	7.03
5 Salmon	1 CFU	-	-	-	0.939	6.86	5.85
6 Salmon	2 CFU	-	TNTC	-	0.963	3.05	5.97
7 Charr	-	-	-	-	0.985	2.92	5.47
8 Kippers	7 CFU	-	11 CFU	-	0.943	5.22	6.17
9 Cod	-	-	TNTC	-	0.981	3.43	7.14
10 Mackerel	-	-	TNTC	-	0.945	3.27	5.59
11 Capelin	-	-	-	+	0.715	10.60	6.62
12 Charr	-	-	-	-	0.988	2.12	5.55
13 Capelin	-	-	-	-	0.735	15.81	5.65
14 Cod	-	8 CFU	TNTC	+	0.983	3.02	7.00
15 Kippers	-	-	-	-	0.944	4.93	5.21
16 Charr	-	-	-	-	0.994	2.85	5.89
17 Cod	6 CFU	-	TNTC	-	0.983	3.02	7.00
18 Kippers	-	-	-	-	0.942	5.20	5.17
19 Kippers	23600b/g	-	TNTC	-	0.982	4.06	6.09
20 Capelin	-	-	-	-	0.687	11.90	6.28
21 Capelin	-	-	TNTC	-	0.744	10.73	5.99
22 Capelin	6500b/g	-	6800b/g	-	0.693	13.13	6.09
23 Capelin	-	-	-	-	0.702	10.25	6.21

[1] Staphylococcus aureus
[2] Total aerobic counts at 1:10 dilution on Trypticase Soy Agar (TSA)
A_w Water activity

Table A 3.2 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets and industry for the month of July '91.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Kippers	-	-	-	-	0.956	4.72	5.09
2 Mackerel	-	-	TNTC	+	0.962	4.39	5.77
3 Capelin	3600b/g	-	-	-	0.659	12.32	6.63
4 Cod	-	TNTC	TNTC	-	0.987	3.61	7.03
5 Salmon	1 CFU	-	-	-	0.939	6.86	5.85
6 Salmon	2 CFU	-	TNTC	-	0.963	3.05	5.97
7 Charr	-	-	-	-	0.985	2.92	5.47
8 Kippers	7 CFU	-	11 CFU	-	0.943	5.22	6.17
9 Cod	-	-	TNTC	-	0.981	3.43	7.14
10 Mackerel	-	-	TNTC	-	0.945	3.27	5.59
11 Capelin	-	-	-	+	0.715	10.60	6.62
12 Charr	-	-	-	-	0.988	2.12	5.55
13 Capelin	-	-	-	-	0.735	15.81	5.65
14 Cod	-	8 CFU	TNTC	+	0.983	3.02	7.00
15 Kippers	-	-	-	-	0.944	4.93	5.21
16 Charr	-	-	-	-	0.994	2.85	5.89
17 Cod	6 CFU	-	TNTC	-	0.983	3.02	7.00
18 Kippers	-	-	-	-	0.942	5.20	5.17
19 Kippers	23600b/g	-	TNTC	-	0.982	4.06	6.09
20 Capelin	-	-	-	-	0.687	11.90	6.28
21 Capelin	-	-	TNTC	-	0.744	10.73	5.99
22 Capelin	6500b/g	-	6800b/g	-	0.693	13.13	6.09
23 Capelin	-	-	-	-	0.702	10.25	6.21

[1] Staphylococcus aureus
[2] Total aerobic growth at dilution 10
A_w Water activity

Table A 3.3 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets and industry for the month of August '91.

Product	*S.aureus*[1]	*E. coli*	TSA[2]	*Listeria*	A_w	Salt(%)	pH
1 Kippers	-	-	-	-	0.970	3.02	5.60
2 Mackerel	-	-	-	-	0.961	2.67	5.71
3 Capelin	-	-	-	-	0.661	11.32	5.68
4 Cod	-	-	$3.5x10^5$	-	0.969	5.80	6.54
5 Salmon	-	-	TNTC	-	0.975	3.15	5.85
6 Salmon	-	-	-	-	0.965	3.92	5.75
7 Salmon	-	-	TNTC	-	0.981	2.19	6.07
8 Kippers	15 CFU	-	TNTC	-	0.976	2.99	6.52
9 Cod	-	$6.7x10^3$	TNTC	-	0.984	3.55	6.99
10 Kippers	-	-	2 CFU	-	0.929	5.70	5.18
11 Capelin	-	-	-	-	0.655	15.95	5.77
12 Kippers	-	-	-	-	0.941	4.71	5.51
13 Capelin	-	-	-	-	0.657	13.68	5.99
14 Cod	-	-	TNTC	+	0.984	3.06	7.49
15 Kippers	2 CFU	-	3.5 CFU	-	0.936	6.23	5.11
16 Cod	-	-	2.5 CFU	+	0.975	4.12	6.61
17 Cod	6 CFU	-	TNTC	-	0.987	3.62	7.40
18 Kippers	-	-	-	-	0.976	4.02	5.51
19 Cod	-	2 CFU	TNTC	-	0.989	2.51	7.19
20 Capelin	-	-	-	-	0.692	23.20	5.83
21 Capelin	-	-	1 CFU	-	0.703	16.19	5.77

[1] A dilution factor of 1000 was used as opposed to 10 dilution for *Staphylococcus*
[2] Total aerobic growth at dilution 1000
A_w Water activity

Table A 3.4 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets and industry for the month of September '91.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Capelin	1 CFU	-	TNTC	-	0.703	14.74	5.55
2 Capelin	1 CFU	-	4 CFU	-	0.745	14.42	5.55
3 Salmon	-	-	3 CFU	-	0.965	6.10	5.81
4 Kippers	-	-	$5.5x10^5$	-	0.966	7.79	5.89
5 Kippers	13 CFU	-	2 CFU	-	0.976	3.37	6.09
6 Kippers	18 CFU	-	$6.6x10^5$	+	0.965	3.63	6.03
7 Capelin	-	-	-	-	0.718	18.21	5.61
8 Turbot	-	-	TNTC	-	0.863	10.60	6.32
9 Cod	9 CFU	2 CFU	$1.5x10^6$	-	0.988	4.25	7.08
10 Cod	-	-	TNTC	-	0.980	4.00	7.00
11 Cod	-	-	TNTC	+	0.973	6.08	7.16
12 Capelin	-	-	1 CFU	-	0.708	16.20	5.67
13 Salmon	-	-	1 CFU	-	0.972	3.65	5.91
14 Mackerel	-	-	TNTC	-	0.967	3.39	5.37
15 Kippers	-	-	-	-	0.935	7.14	5.21
16 Salmon	-	-	-	-	0.952	6.71	7.45
17 Kippers	-	-	9 CFU	-	0.969	6.47	6.30
18 Salmon	2 CFU	-	$1.2x10^6$	-	0.975	3.43	5.71
19 Cod	TNTC	13 CFU	TNTC	+	0.970	7.25	7.02
20 Cod	-	-	21 CFU	-	0.949	6.90	5.61
21 Capelin	6 CFU	-	2 CFU	-	0.944	7.98	5.71

[1] A dilution factor of 1000 was used as opposed to 10 dilution for Staphylococcus
[2] Total aerobic growth at dilution 1000
A_w Water activity

Table A 3.5 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets and industry for the month of October '91.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Capelin	-	-	5 CFU	+	nd[3]	nd	nd
2 Turbot	-	-	$1.3x10^7$	+	0.804	12.23	6.66
3 Kippers	-	-	$1.7x10^8$	+	nd	nd	nd
4 Cod	-	6 CFU	$1.3x10^8$	+	nd	nd	nd
5 Cod	-	-	1 CFU	-	nd	nd	nd
6 Charr	-	-	$1.6x10^6$	-	0.970	2.58	5.85
7 Salmon	-	-	$1.1x10^8$	-	0.975	2.98	6.01
8 Capelin	-	-	-	-	nd	nd	nd
9 Kippers	-	-	$4.5x10^5$	-	0.978	2.68	6.32
10 Capelin	-	-	-	-	0.676	17.37	5.57
11 Salmon	-	-	$1.3x10^8$	-	nd	nd	nd
12 Cod	-	1 CFU	$4.9x10^7$	+	nd	nd	nd
13 Cod	-	-	$1.0x10^7$	+	0.982	2.97	7.12
14 Salmon	-	-	$2.3x10^8$	+	nd	nd	nd
15 Kippers	-	-	-	+	nd	nd	nd
16 Mackerel	-	-	3 CFU	+	0.950	4.76	5.87
17 Cod	-	1 CFU	$6.1x10^7$	+	nd	nd	nd
18 Salmon	-	-	$1.3x10^6$	-	nd	nd	nd
19 Kippers	$1.4x10^4$	-	$2.0x10^6$	+	nd	nd	nd
20 Capelin	-	-	4 CFU	+	nd	nd	nd

[1] Staphylococcus aureus
[2] A dilution factor of 10^3 and 10^5 were used as opposed to 10 dilution for total aerobic counts
[3] not done
A_w water activity

Table A 3.6 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets and industry for the month of November '91.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Kippers	-	-	$2.1x10^6$	+	nd[3]	nd	nd
2 Capelin	-	-	-	-	nd	nd	nd
3 Salmon	$2.1x10^3$	-	$1.6x10^8$	-	nd	nd	nd
4 Cod	TNTC	-	TNTC	+	nd	nd	nd
5 Trout	-	-	-	-	0.959	4.55	6.02
6 Cod	-	-	$3.4x10^8$	-	nd	nd	nd
7 Capelin	-	-	-	-	0.723	12.62	6.02
8 Capelin	-	-	$8.0x10^4$	-	nd	nd	nd
9 Mackerel	$1.3x10^3$	-	$2.0x10^4$	-	nd	nd	nd
10 Kippers	$1.5x10^3$	-	$7.0x10^4$	-	nd	nd	nd
11 Capelin	-	-	-	-	nd	nd	nd
12 Kippers	-	-	$8.1x10^7$	-	0.989	3.05	6.77
13 Salmon	-	-	$1.0x10^4$	-	0.971	3.63	6.15
14 Cod	-	-	$4.5x10^5$	+	0.981	1.85	6.53
15 Mackerel	-	-	$5.4x10^7$	+	0.953	3.65	5.50
16 Salmon	-	-	$3.2x10^8$	-	nd	nd	nd
17 Cod	-	-	$1.8x10^5$	-	nd	nd	nd
18 Kippers	$2.9x10^3$	-	$4.0x10^4$	-	nd	nd	nd
19 Capelin	-	-	$8.0x10^4$	-	nd	nd	nd

[1] Staphylococcus aureus
[2] A dilution factor of 10^3 and 10^5 were used as opposed to 10 dilution for total aerobic counts
[3] not done
A_w water activity

Table A 3.7 Microbiological analysis, water activity, salt content, and pH of the smoked seafoods products sampled from various supermarkets and industry for the month of December '91.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Mackerel	-	-	-	-	nd[3]	nd	nd
2 Cod	-	-	$3.0x10^6$	-	0.976	3.25	6.47
3 Salmon	-	-	TNTC	-	nd	nd	nd
4 Kippers	$2.0x10^2$	-	$3.4x10^5$	-	nd	nd	nd
5 Capelin	-	-	-	-	nd	nd	nd
6 Capelin	$1.0x10^2$	-	$8.0x10^4$	-	nd	nd	nd
7 Kippers	-	-	$7.1x10^5$	-	nd	nd	nd
8 Cod	-	-	$2.5x10^6$	+	nd	nd	nd
9 Mackerel	-	-	$2.6x10^5$	-	nd	nd	nd
10 Kippers	-	$2.5x10^4$	$1.3x10^6$	-	0.956	4.78	6.08
11 Cod	-	-	$1.0x10^4$	-	nd	nd	nd
12 Salmon	$1.0x10^2$	-	-	-	0.969	3.16	6.36
13 Charr	$3.0x10^2$	-	-	-	nd	nd	nd
14 Mackerel	$1.2x10^3$	-	$8.0x10^5$	-	0.958	3.60	5.98
15 Charr	-	-	$3.0x10^4$	-	nd	nd	nd
16 Cod	$4.8x10^3$	-	TNTC	+	nd	nd	nd
17 Capelin	-	-	-	-	0.660	12.98	6.13
18 Charr	-	-	$5.0x10^4$	-	0.978	2.50	6.08
19 Cod	-	-	$1.0x10^4$	-	nd	nd	nd
20 Capelin	-	-	-	-	nd	nd	nd
21 Kippers	$3.0x10^4$	-	$1.4x10^5$	-	nd	nd	nd

[1] Staphylococcus aureus
[2] A dilution factor of 10^3 and 10^5 were used as opposed to 10 dilution for total aerobic counts
[3] not done
A_w water activity

Table A 3.8 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets and industry for the month of January '92.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Capelin	-	-	-	-	nd[3]	nd	nd
2 Kippers	-	-	-	-	nd	nd	nd
3 Cod	-	-	$1.3x10^6$	+	nd	nd	nd
4 Trout	-	-	$1.7x10^8$	-	0.956	5.01	6.15
5 Cod	-	$8.0x10^2$	TNTC	+	nd	nd	nd
6 Mackerel	-	$3.0x10^4$	TNTC	-	nd	nd	nd
7 Salmon	-	-	-	-	nd	nd	nd
8 Kippers	-	-	TNTC	-	0.961	3.56	5.15
9 Capelin	-	-	-	-	nd	nd	nd
10 Eels	-	-	$1.7x10^8$	-	0.964	1.84	4.81
11 Cod	-	-	$9.0x10^6$	+	0.977	3.51	5.94
12 Capelin	-	-	-	-	nd	nd	nd
13 Kippers	-	-	-	-	nd	nd	nd
14 Capelin	-	-	-	-	nd	nd	nd
15 Mackerel	-	-	-	-	nd	nd	nd
16 Cod	-	-	-	-	nd	nd	nd
17 Salmon	-	-	$9.5x10^7$	-	0.977	2.68	6.10
18 Kippers	-	-	$2.2x10^5$	-	nd	nd	nd
19 Mackerel	-	-	-	-	0.946	6.44	5.55
20 Salmon	-	-	-	-	nd	nd	nd
21 Cod	-	-	$1.2x10^8$	-	nd	nd	nd
22 Kippers	-	-	-	-	nd	nd	nd
23 Capelin	-	-	-	-	0.629	14.44	5.38

[1] Staphylococcus aureus
[2] A dilution factor of 10^3 and 10^5 were used as opposed to 10 dilution for total aerobic counts; [3] not done; A_w water activity

Table A 3.9 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets for the month of February '92.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Capelin	-	-	$6.5x10^5$	-	0.650	14.32	5.45
2 Kippers	-	-	$8.4x10^5$	-	nd[3]	nd	nd
3 Salmon	-	-	TNTC	-	0.979	3.15	6.25
4 Cod	-	-	TNTC	+	nd	nd	nd
5 Cod	-	-	TNTC	+	0.982	3.25	6.01
6 Salmon	-	-	1 CFU	-	nd	nd	nd
7 Mackerel	-	-	-	-	nd	nd	nd
8 Kippers	-	-	3 CFU	-	0.972	3.75	5.05
9 Capelin	-	-	-	-	nd	nd	nd
10 Cod	-	-	TNTC	-	nd	nd	nd
11 Kippers	-	-	-	-	nd	nd	nd
12 Capelin	-	-	-	-	nd	nd	nd
13 Salmon	-	-	-	-	nd	nd	nd
14 Trout	-	-	$6.4x10^5$	-	0.969	4.02	6.05
15 Herring	-	-	1 CFU	-	0.980	3.65	5.15
16 Salmon	-	-	$9.4x10^5$	-	nd	nd	nd
17 Cod	-	-	$5.6x10^7$	+	nd	nd	nd
18 Mackerel	-	-	1 CFU	-	0.958	4.53	5.65
19 Kippers	-	-	10 CFU	-	nd	nd	nd
20 Capelin	-	-	-	-	nd	nd	nd
21 Salmon	-	-	1 CFU	-	nd	nd	nd
22 Cod	-	-	3 CFU	+	nd	nd	nd

[1] Staphylococcus aureus

[2] A dilution factor of 10^3 and 10^5 were used as opposed to 10 dilution for total aerobic counts

[3] not done

A_w water activity

Table A 3.10 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets for the month of March '92.

Product	*S.aureus*[1]	*E. coli*	TSA[2]	*Listeria*	A_w	Salt(%)	pH
1 Charr	-	-	-	-	0.966	3.55	5.40
2 Salmon	-	-	TNTC	-	0.970	3.21	6.40
3 Cod	-	-	-	-	nd[3]	nd	nd
4 Herring	-	-	$4.9x10^5$	-	nd	nd	nd
5 Kippers	-	-	$3.6x10^5$	-	nd	nd	nd
6 Cod	-	-	6 CFU	+	nd	nd	nd
7 Kippers	-	-	-	-	nd	nd	nd
8 Salmon	-	-	TNTC	-	0.975	3.15	6.10
9 Mackerel	-	-	$9.0x10^7$	-	0.960	3.75	6.12
10 Kippers	-	-	-	-	nd	nd	nd
11 Cod	-	$5.0x10^3$	$7.0x10^7$	-	nd	nd	nd
12 Capelin	-	-	$7.5x10^5$	-	nd	nd	nd
13 Kippers	-	-	TNTC	-	nd	nd	nd
14 Mackerel	-	-	-	-	0.940	4.85	5.89
15 Cod	-	-	4 CFU	+	nd	nd	nd
16 Capelin	-	-	-	-	nd	nd	nd
17 Cod	-	-	$9.1x10^7$	+	nd	nd	nd
18 Salmon	-	-	$9.3x10^5$	-	0.980	3.25	6.20
19 Kippers	-	-	-	-	nd	nd	nd
20 Capelin	-	-	-	-	nd	nd	nd

[1] *Staphylococcus aureus*
[2] A dilution factor of 10^3 and 10^5 were used as opposed to 10 dilution for total aerobic counts
[3] not done
A_w water activity

Table A 3.11 Microbiological analysis, water activity, salt content, pH of the smoked seafood products sampled from various supermarkets for the month of April '92.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Cod	-	-	8 CFU	-	nd[3]	nd	nd
2 Kippers	-	-	1 CFU	-	nd	nd	nd
3 Salmon	-	15 CFU	$9.1x10^7$	-	nd	nd	nd
4 Capelin	-	-	-	-	nd	nd	nd
5 Charr	-	-	$2.6x10^8$	-	0.963	3.95	5.24
6 Cod	-	-	6 CFU	-	nd	nd	nd
7 Capelin	-	-	-	-	nd	nd	nd
8 Kippers	-	-	18 CFU	-	nd	nd	nd
9 Mackerel	-	-	-	-	0.947	5.19	5.78
10 Trout	-	-	$6.6x10^5$	-	0.961	4.35	6.63
11 Kippers	-	-	-	-	nd	nd	nd
12 Capelin	-	-	2 CFU	-	nd	nd	nd
13 Cod	-	-	$1.28x10^8$	+	nd	nd	nd
14 Kippers	-	-	-	-	nd	nd	nd
15 Cod	-	-	$2.18x10^8$	+	nd	nd	nd
16 Mackerel	-	-	1CFU	-	0.930	5.21	5.94
17 Capelin	-	-	-	-	nd	nd	nd
18 Mackerel	-	-	-	-	0.956	3.52	6.07
19 Trout	-	-	-	-	0.941	3.98	6.43
20 Capelin	-	-	-	-	nd	nd	nd
21 Kippers	-	-	TNTC	-	nd	nd	nd

[1] Staphylococcus aureus

[2] A dilution factor of 10^3 and 10^5 were used as opposed to 10 dilution for total aerobic counts

[3] not done

A_w water activity

Table A 3.12 Microbiological analysis, water activity, salt content, and pH of the smoked seafood products sampled from various supermarkets for the month of May '92.

Product	S.aureus[1]	E. coli	TSA[2]	Listeria	A_w	Salt(%)	pH
1 Cod	-	-	$8.3x10^7$	+	nd[3]	nd	nd
2 Kippers	-	-	-	-	nd	nd	nd
3 Capelin	-	-	$3.0x10^5$	-	nd	nd	nd
4 Mackerel	-	-	-	-	0.957	4.60	5.25
5 Kippers	1 CFU	-	$9.1x10^5$	-	nd	nd	nd
6 Cod	-	-	$3.0x10^7$	-	nd	nd	nd
7 Trout	-	-	$6.6x10^5$	-	0.961	4.30	6.01
8 Capelin	-	-	-	-	nd	nd	nd
9 Salmon	-	-	-	-	0.982	3.21	6.10
10 Kippers	-	-	-	-	nd	nd	nd
11 Capelin	-	-	-	-	nd	nd	nd
12 Kippers	-	-	$3.0x10^5$	-	nd	nd	nd
13 Salmon	-	-	$8.3x10^7$	-	0.979	3.40	6.20
14 Charr	-	-	TNTC	-	0.982	3.05	5.49
15 Cod	3 CFU	-	6 CFU	-	nd	nd	nd
16 Capelin	-	-	-	-	nd	nd	nd
17 Capelin	-	-	-	-	nd	nd	nd
18 Mackerel	-	-	$7.8x10^5$	-	0.975	3.15	5.50
19 Cod	-	-	TNTC	+	nd	nd	nd
20 Kippers	-	-	1 CFU	-	nd	nd	nd

[1] Staphylococcus aureus

[2] A dilution factor of 10^3 and 10^5 were used as opposed to 10 dilution for total aerobic counts

[3] not done

A_w water activity

Table A 3.13 Analysis of the FDA lisetria isolation protocol.

Product	LEB (1) 24 h[1] Palcam	Oxford	LPM	LEB (2) 48 h[2] Palcam	Oxford	LPM	UVM1[3]	UVM2[4]	Listeria species
2 Mackerel[6]	3800	5700	3700	TNTC	TNTC	TNTC	+	+	+
5 Salmon[6]	-	-	-	-	-	-	+	+	-
9 Cod[6]	-	-	-	-	-	-	+	+	-
14 Cod[6]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
16 Salmon[6]	-	-	-	-	-	-	-	+	-
17 Cod[6]	-	-	-	-	-	-	+	+	-
18 Mackerel[6]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
20 Cod[6]	-	-	-	-	-	-	-	+	-
21 Cod[6]	-	-	-	-	-	-	+	+	-
23 Cod[6]	-	-	-	-	-	-	+	+	-
2 Mackerel[7]	31	-	TNTC	TNTC	-	TNTC	+	+	+
4 Cod[7]	-	-	-	-	-	-	+	+	-
8 Kippers[7]	-	-	-	-	-	-	-	+	-
9 Cod[7]	-	-	-	-	-	-	-	+	-
11 Capelin[7]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
14 Cod[7]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
19 Kippers[7]	-	-	-	-	-	-	+	+	-
7 Salmon[8]	-	-	-	-	-	-	-	+	-
8 Kippers[8]	-	-	-	-	-	-	+	+	-
9 Cod[8]	-	-	-	-	-	-	+	+	-
14 Cod[8]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
15 Kippers[8]	-	-	-	-	-	-	-	+	-
16 Cod[8]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTc	+	+	+
17 Cod[8]	-	-	-	-	-	-	-	+	-

Table A 3.13 continued

Product	LEB (1) 24 h[1] Palcam	Oxford	LPM	LEB (2) 48 h[2] Palcam	Oxford	LPM	UVM1[3]	UVM2[4]	Listeria species
19 Cod[8]	-	-	-	-	-	-	+	+	-
3 Salmon[9]	-	-	-	-	-	-	+	+	-
4 Kippers[9]	-	-	-	-	-	-	+	+	-
5 Kippers[9]	-	-	-	-	-	-	+	+	-
6 Kippers[9]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
8 Turbot[9]	-	-	-	-	-	-	+	+	-
9 Cod[9]	-	-	-	-	-	-	+	+	-
10 Cod[9]	-	-	-	-	-	-	+	+	-
11 Cod[9]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
13 Salmon[9]	-	-	-	-	-	-	+	+	-
18 Salmon[9]	-	-	-	-	-	-	+	+	-
19 Cod[9]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
21 Capelin[9]	-	-	-	-	-	-	+	+	-
1 Capelin[10]	TNTC	TNTC	-	TNTC	TNTC	-	+	+	+
2 Turbot[10]	TNTC	TNTC	-	TNTC	TNTC	-	+	+	+
3 Kippers[10]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
4 Cod[10]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
9 Kippers[10]	-	-	-	-	-	-	-	+	-
12 Cod[10]	$6.8x10^3$	$4.0x10^3$	$4.7xx10^3$	TNTC	TNTC	TNTC	+	+	+
13 Cod[10]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
14 Salmon[10]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
15 Kipper[10]	TNTC	-	-	TNTC	-	-	+	+	+
16 Mackrl[10]	$2.2X10^4$	$2.3X10^4$	-	TNTC	TNTC	-	+	+	+
17 Cod[10]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
19 Kippers[10]	$7.3X10^3$	$7.1X10^3$	-	$9.5X10^3$	$1.3X10^4$	-	+	+	+
20 Capelin[10]	TNTC	TNTC	-	TNTC	TNTC	-	+	+	+
1 Kippers[11]	TNTC	TNTC	TNTC	-	-	-	+	-	+
4 Cod[11]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
6 Cod[11]	-	-	-	-	-	-	-	+	-

Table A 3.13 continued

Product	LEB (1) 24 h[1] Palcam	Oxford	LPM	LEB (2) 48 h[2] Palcam	Oxford	LPM	UVM1[3]	UVM2[4]	Listeria species
1 Kippers[11]	TNTC	TNTC	TNTC	-	-	-	+	-	+
4 Cod[11]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
6 Cod[11]	-	-	-	-	-	-	-	+	-
12 Kippers[11]	-	-	-	-	-	-	+	+	-
13 Salmon[11]	-	-	-	TNTC	-	-	-	-	-
14 Cod[11]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
15 Mackrl[11]	-	-	-	TNTC	TNTC	TNTC	-	+	+
19 Capelin[11]	-	-	-	-	-	-	-	+	-
2 Cod[12]	-	-	-	-	-	-	+	+	-
4 Kippers[12]	-	-	-	-	-	-	+	+	-
8 Cod[12]	$2.6x10^4$	$2.6x10^4$	$2.4x10^4$	TNTC	TNTC	TNTC	+	+	+
11 Cod[12]	-	-	-	-	-	-	-	+	-
12 Salmon[12]	-	-	-	-	-	-	-	+	-
14 Mackarel[12]	-	-	-	-	-	-	+	+	-
16 Cod[12]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
21 Kippers[12]	-	-	-	-	-	-	-	+	-
3 Cod[13]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
5 Cod[13]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
11 Cod[13]	$1.3x10^4$	$1.5x10^4$	$9.7x10^3$	TNTC	TNTC	TNTC	+	+	+
16 Cod[13]	-	-	-	-	-	-	+	+	-
4 Cod[14]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
5 Cod[14]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
10 Cod[14]	-	-	-	-	-	-	-	+	-
12 Capelin[14]	-	-	-	-	TNTC	-	-	-	-
17 Cod[14]	TNTC	-	TNTC	TNTC	-	TNTC	+	+	+
19 Kippers[14]	-	-	-	-	-	-	-	+	-
22 Cod[14]	$7.6x10^3$	$9.6x10^3$	$1.1x10^4$	TNTC	TNTC	TNTC	+	+	+

Table A 3.13 continued

Product	LEB (1) 24 h[1] Palcam	Oxford	LPM	LEB (2) 48 h[2] Palcam	Oxford	LPM	UVM1[3]	UVM2[4]	Listeria species
4 Herring[15]	-	-	-	-	-	-	-	+	-
6 Cod[15]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
11 Cod[15]	-	-	-	-	-	-	-	+	-
15 Cod[15]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
17 Cod[15]	TNTC	-	TNTC	TNTC	-	TNTC	+	+	+
18 Salmon[15]	-	-	-	-	-	-	-	+	-
1 Cod[16]	-	-	-	$3.4x10^3$	-	-	-	-	-
3 Salmon[16]	-	-	-	-	-	-	-	+	-
13 Cod[16]	1CFU	-	3CFU	TNTC	-	TNTC	+	+	+
15 Cod[16]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+
1 Cod[17]	$1.5x10^4$	10CFU	$1.1x10^4$	TNTC	TNTC	TNTC	+	+	+
6 Cod[17]	-	-	-	-	-	-	+	+	-
19 Cod[17]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	+	+	+

[1] Transfer of 0.1 ml from 24 h incubation in LEB to Listeria selective agars;
[2] Transfer of 0.1 ml from 48 h incubation in LEB to Listeria selective agars;
[3] UVM 1 is the broth to which 0.1 ml of culture is transferred from LEB 1;
[4] UVM 2 is the broth to which 0.1 ml of culture is transferred from LEB 2;
[6] Samples from the month of June, 1991;
[7] Samples from the month of July, 1991;
[8] Samples from the month of August, 1991;
[9] Samples from the month of September, 1991;
[10] Samples from the month of October, 1991;
[11] Samples from the month of November, 1991;
[12] Samples from the month of December, 1991;
[13] Samples from the month of January, 1992;
[14] Samples from the month of February, 1992;
[15] Samples from the month of March, 1992;
[16] Samples from the month of April, 1992; [17] Samples from the month of May, 1992.

Table A 3.14 Number of Listeria positives by direct plating of smoked seafood homogenates.

Product	Direct Plating		
	PALCAM	Oxford	LPM
14 Cod[a]			
16 Cod[a]			
6 Kippers[b]			
11 Cod[b]	-	$5.7X10^3$	-
19 Cod[b]			
1 Capelin[c]			
2 Turbot[c]			
3 Kippers[c]	TNTC	TNTC	TNTC
4 Cod[c]			
12 Cod[c]			
13 Cod[c]			
14 Salmon[c]	TNTC	TNTC	TNTC
15 Kipper[c]			
16 Mackrl[c]			
17 Cod[c]	2 CFU	-	-
19 Kippers[c]			
20 Capelin[c]			
1 Kippers[d]	TNTC	TNTC	TNTC
4 Cod[d]	1 CFU	3 CFU	0 CFU
13 Salmon[d]			
14 Cod[d]			
15 Mackrl[d]			
8 Cod[e]			
16 Cod[e]			
3 Cod[f]			
5 Cod[f]	$7.2x10^3$	-	$5.3x10^3$
11 Cod[f]			
4 Cod[g]	-	1 CFU	-
5 Cod[g]			
17 Cod[g]			
22 Cod[g]			

Table A 3.14 continued

Product	Direct Plating		
	PALCAM	Oxford	LPM
6 Cod[h]			
15 Cod[h]			
17 Cod[h]			
13 Cod[i]			
15 Cod[i]	2 CFU	2 CFU	3 CFU
1 Cod[j]			
19 Cod[j]	2 CFU	1 CFU	2 CFU

[a] August samples, 1991, positive by enrichment;
[b] September samples, 1991, positive by enrichment;
[c] October samples, 1991, positive by enrichment;
[d] November samples, 1991, positive by enrichment;
[e] Decembersample, 1991, positive by enrichment;
[f] January samples, 1992, positive by enrichment;
[g] February samples, 1992, positive by enrichment;
[h] March samples, 1992, positive by enrichment;
[i] April samples, 1992, positive by enrichment;
[j] May samples, 1992, positive by enrichment;