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THE EFFECT OF ANTIMICROBIALS ON THE GROWTH OF *LISTERIA MONOCYTOGENES* IN BRINE AND SELECTED MEAT PRODUCTS.

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Dje Yobouet

May 1990

AG-VET-MED.
Thesis
90
.Y628

DEDICATION

The author dedicates this thesis to his uncles Tonton Bendhedou Koffi Paul, Tantie Bendhedou A. Rosalie, Tonton Assan Kouame Arsene and his wife, to his father, mother brothers and sisters, to Azian Asso Micheline and to St Etienne de Koumassi..

ACKNOWLEDGMENTS

The author extends his thanks to the Ivorian government for the financial support throughout his studies. His is also grateful to his major professor, Dr. P. M. Davidson who gave him all the help he needed to accomplish his research work. His gives thanks to his committee, Dr. F. A. Draughon and M. J. Riemann for their support. The author would like to give thanks to Dr. S. L. Melton, Dr. J. Mount, Dr. Merched Chaar and Jim Wempe for their help. He thanks also his lab mates Emayat, Cyrus, Ruth, Ying, Vijay, Kim, Mohamed and Rong. His gratitude goes to Mr. Jake Taylor as well.

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CHAPTER I

INTRODUCTION

Listeria has been recognized as a human and animal pathogen for over 50 years. Recent foodborne listeriosis outbreaks (Fleming et al., 1985; James et al., 1985) have given an increasing importance to this bacterium. To date, a majority of listeriosis outbreaks have been associated with dairy products. However, there is increasing evidence that meat and poultry products are major sources of this organism. Recovery of the pathogen in meat processing plants and in meat products (Johnson, 1988; Cox et al., 1989; Genigeorgis et al., 1989; Farber et al., 1989) are tangible reasons to believe that both raw and ready-to-eat meat products are susceptible to contamination. A recent study by the Centers for Disease Control (CDC) (Schwartz et al., 1988) emphasized this point. Based upon epidemiological data, the CDC reported that consumption of chicken and raw hot dogs was associated with sporadic listeriosis.

The primary method used for the prevention of growth of pathogens in foods is low temperature storage (refrigeration). However, due to its psychrotrophic

nature, L. monocytogenes is able to grow relatively rapidly at 4-7°C. It is therefore desirable to have post-process safeguards which would prevent the proliferation of Listeria and other foodborne pathogens during refrigerated storage or temperature abuse. The most effective method of controlling microorganisms in these circumstances is the use of antimicrobial agents. Additives such as sodium chloride, organic acids and their salts, sulfites and sodium nitrite have been used as food preservatives for centuries. However, very little research has been done on the control of Listeria in meat products.

The antimicrobial action against bacteria of esters of p-hydroxybenzoic acid is well established. However, their effect on Listeria was not recognized until a recent study by Payne et al. (1989). They determined the minimum inhibitory concentration (MIC) of methyl and propyl parabens for Listeria and found that propyl paraben inhibited L. monocytogenes in a model milk system at 4°C (Payne et al., 1989).

This study was undertaken in order to determine the effect of methyl paraben, propyl paraben and sodium lactate on Listeria monocytogenes Scott A and ERM, when inoculated into salt brine and model meat systems at 4°C, 24°C and 35°C.

CHAPTER II

LITERATURE REVIEW

THE GENUS *LISTERIA*

Taxonomy

In 1926, Murray, first described the infection caused by *L. monocytogenes* in laboratory animals (rabbits and guinea pigs) (Murray et al., 1926). Because the infection was characterized by monocytosis, he named the causative agent *Bacterium monocytogenes*. Three years later, Nyfeldt (1929) described the same type of disease in humans. Pirie gave the name *Listeria hepatolytica* to a similar organism which was isolated from the liver of infected gerbils. *Listeria* got its final generic name in honor of Lord Lister (Pirie, 1940).

Since these first discoveries, scores of investigators have worked to characterize and classify this organism. The taxonomic position of the genus *Listeria* is far from clear. It was originally classified in the family *Corynebacteriaceae* as a single species *Listeria monocytogenes* (Breed et al., 1957), then as "genera of uncertain affiliation" (Buchanan et al., 1974). Currently,

Listeria is classified in Bergey's Manual of Systematic Bacteriology (Seeliger et al., 1986) as a regular, non-sporeforming gram-positive rod, with eight species: L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri, L. murrayi, L. grayi and L. denitrificans (Skerman et al., 1980; Moore et al., 1985). Typical biochemical characteristics of these species are shown in Table 1. Among them, L. monocytogenes, L. ivanovii, L. innocua and L. seeligeri have been found to be human and animal pathogens (McLauchlin, 1987). Listeria monocytogenes has been reported to cause 98 and 87% of human and animal listeriosis, respectively (Seeliger, 1985).

Listeria monocytogenes

Listeria monocytogenes is a small gram positive, microaerophilic, non-sporeforming rod with rounded ends measuring 0.4-0.5 um by 0.5-0.2 um. It is slightly curved when grown in some media. It occurs singly in "V" shaped or parallel pairs and in short chains. It is motile with 4 peritrichous flagella at ambient temperature (25°C), however, no motility is observed at 37°C (Seeliger, 1986). What makes this organism important to the food industry is its ability to grow at refrigerator temperatures (4-6°C).

Table 1. Biochemical Characteristics of Species of *Listeria*

Species	Hemolysis[a]	Nitrate Red	Acid Production From D-mannitol	L-rhamnose	D-xylose
L. monocytogenes	+	-	-	+	-
L. ivanovii	+	-	-	-	+
L. innocua	-	-	-	v[b]	+
L. welshimeri	-	-	-	v	+
L. seeligeri	v	-	-	-	+
L. grayi	-	-	+	-	-
L. murrayi	-	+	+	v	-
L. denitrificans	-	+	-	-	+

[a] Hemolysis on horse blood
[b] v = variable

This species contains 13 serovars (Table 2). The type 4b has been implicated the most in human infections (McLauchlin, 1987).

Reservoirs of Listeria monocytogenes

1. Environment

Listeria monocytogenes is a ubiquitous organism, isolated from soil, plants, dust, sewage and silage. Weis et al. (1975) showed the presence of the bacterium in both cultivated and uncultivated areas. Their study, done in West Germany, demonstrated that 20.9% of 779 soil and plant samples analyzed contained Listeria. Welshimer and Donker (1971) suggested that L. monocytogenes was a saprophytic organism living in decayed vegetations such as corn, soybeans (Welshimer, 1968), wild grasses and leaves of shrubs (Weis et al., 1975).

Silage is one of the major environmental sources of L. monocytogenes (Gray, 1960; Fenlon, 1985). Animal listeriosis due to silage-borne *Listeria* has been documented (Dijkstra, 1975; Fenlon, 1986). The incidence level of the organism in silage has been estimated to be 12,000 cells/g (Gronstol, 1979; Fenlon, 1986). It was

Table 2. Serovars of Listeria spp.

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	6a, 6b, 4ab, undesignated
L. welshimeri	6a, 6b
L. seeligeri	1/2b, 4c, 4d, 6b, undesignated

reported that L. monocytogenes could survive in silage for 10-12 years (Dijkstra, 1975).

Sewage (Watkins et al., 1981; Al-Ghazali et al., 1986) and effluents from meat and poultry processing plants (Watkins et al., 1981) have been reported to contain L. monocytogenes. Other water sources such as lakes and rivers may be infected by the organism (Dijkstra, 1982).

2. Animals

Listeria monocytogenes is common in animals and the infection is similar to that encountered in humans. Scientists have suggested that both wild and domestic animals may be contaminated with the pathogen through feed (Gray, 1960; Gronstol, 1979; Fenlon, 1985; Gitter, 1985) or the consumption of other infected animals. A great number of animals including mammals, birds, crustaceans and fish have been reported to harbor L. monocytogenes (Table 3). Gregorio (1974) reported that 43 species of domestic and wild animals, 22 species of birds, fish, crustaceans and insects were carriers of the pathogen. There is also evidence that L. monocytogenes is present in healthy animals (Vizcaino et al., 1974; Gronstol, 1979; Ralovich,

Table 3. Animal Sources of Listeria monocytogenes

Mammals	cattle[a], sheep[a], goats[a], swine[a], horses[a], dogs[a], cats[a] deer[b], raccoons[b], rabbits[c], voles[c], guinea pigs[b], lemmings[b], skunks[b], gerbils[c], chinchillas[a].
Birds	canaries[b], chaffinches[f], chickens[b], cranes[b], doves[b], ducks[b], eagles[b], geese[b], hawks[f], lorikeets[b], parrots[b], partridges[b], pheasants[b], pigeons[b], seagulls[g], sparrows[f], turkeys[b], white grouse[b], whitethroats[b], woodgrouse[b]
Frogs[d]	
Fish[b]	
Crustaceans[b]	

[a]Gitter (1985), [b]Gray and Kilinger (1966), [c]Bacon and Miller (1958), [d]Botzler and Cowan (1985), [f]Weis and Seeliger (1975), [g]Fenlon (1985).

1984). For example, Cottin et al. (1985) found 30 of 487 healthy cattle surveyed in France to be asymptomatic carriers of the organism.

Characteristics of Listeriosis

It has been estimated that approximately 5% of humans are asymptomatic carriers of L. monocytogenes (Braza, 1985). The number of organisms necessary to induce symptoms of listeriosis and the susceptibility of the population to the infection depend upon the age, sex, health and immune status. The most susceptible hosts are pregnant women and their unborn children, alcoholics, diabetics, patients receiving treatment that alters their natural immunity and the elderly (WHO, 1988).

Upon ingestion, L. monocytogenes enters the body through the intestine. Virulent strains of L. monocytogenes, once in the blood stream, are engulfed in the macrophages where they multiply and destroy them. Septicemia occurs when the organism invades the body through the blood stream, reaching the central nervous system, the heart, the eyes and other organs. Depending upon the stage of pregnancy, abortion, stillbirth, or neonatal sepsis may occur. Signs of listeric infection in pregnant women include fever, chills, headache, backache

and discolored urine (Seeliger, 1961). The fetus, once infected, may be destroyed by bacteremia.

There are some 30-50% neonatal fatalities due to listeriosis in the United States every year (Anonymous, 1988). The syndromes of listeric infection in the newborn include respiratory distress, heart failure, difficult respiration, refusal of food and drink, vomiting, and convulsions. There is a change in the blood constituents leading to increased levels of leukocytes and monocytes (monocytosis).

Other syndromes of listeriosis include meningitis, encephalitis, flu-like symptoms, nausea, conjunctivitis, and rhinitis. Listeriosis may result in death if the patient is not subjected to appropriate antibiotic therapy (Anonymous, 1988; Brackett, 1988).

Foodborne Listeriosis Outbreaks

Listeria monocytogenes has been recognized as the etiological agent of listeriosis since the 1920's. Data concerning major outbreaks are reported in table 4. The first well documented foodborne listeriosis in humans occurred between March 1 and September 1, 1981 in Nova Scotia, Canada. Among 41 people infected, 18 died (Schlech et al., 1983). *Listeria monocytogenes* Scott A type 4b was

Table 4. Major Human Listeriosis Outbreaks

Location	Year	Cases	Vehicles
[a]Halle (Germany)	1966	279	Unknown
[b]Anjou (France)	1976	162	Unknown
[c]Boston (USA)	1979	20	Raw vegetables/milk?
[d]Auckland (New Zealand)	1980	21	Seafood?
[e]Nova Scotia (Canada)	1981	41	Coleslaw
[f]Massachusetts (USA)	1983	49	Milk
[g]Lausanne (Switzerland)	1983-84	25	Unknown
[h]California(USA)	1985	314	Soft Cheese

[a]Ortel (1968), [b]Carbonnelle et al (1978), [c]Ho et al (1986), [d]Lennon et al (1984), [e]Schlech et al (1983), [f]Fleming et al (1985), [g]Malinverni et al (1986), [h]James et al (1985).

isolated from the patients and the suspected coleslaw. The source of the infection was traced to contaminated cabbage that had been fertilized with manure from sheep infected with Listeria.

Between June 30 and August 30, 1983, 49 people infected with L. monocytogenes were hospitalized in Massachusetts for meningitis and sepsis. Seven of the cases were fetus or infant-related and 42 were immunocompromised adults. Fourteen of the victims died during this outbreak caused by pasteurized milk. Listeria monocytogenes serotypes 1a, 3b, 4ab, and 4b, were isolated from the farms that supplied the raw milk (Fleming et al., 1985).

In 1985, 314 cases of listeriosis occurred in Los Angeles and Orange County, California. Among the victims were 181 pregnant women and/or their children and 133 immunocompromised persons. There were 105 deaths. Mexican style cheese made with unpasteurized milk was implicated in the outbreak (James et al., 1985).

Though vegetable and dairy products have been largely implicated in significant outbreaks, raw and processed meat were recently reported to be potential health hazards if

consumed raw (Schwartz et al., 1988). This report was confirmed by the death of a woman due to listeriosis after consumption of infected turkey franks (Anonymous, 1989).

A recent survey showed that about 800 people are infected every year in the United States. The overall mortality rate was estimated to be 19.1% (Ciesielski et al., 1988).

Recovery of Listeria monocytogenes in Food Processing Environments and Food Products

The growing importance of L. monocytogenes in the food industry has led to several studies on the prevalence of the organism in food processing environments and foods for human consumption. Surveys of food manufacturing environments and food products have revealed that neither are L. monocytogenes-free.

Elischerova et al. (1974) found the pathogen at varying levels in meat processing plants as well as on the hands and gloves of workers. It was shown that approximately 12% of the workers were carriers of L. monocytogenes. This study agreed with previous surveys in which 10% was the estimated level of carriers working in meat processing plants (Bojsen-Moeller, 1972). A recent report by Cox et al. (1989) has confirmed previous findings

of Elischerova and Bojsen-Moeller. They found 30% of 12 meat plants surveyed were contaminated with the organism. They took swab or liquid samples from different locations of every plant. They reported that 54, 46, 45, 33, 19, and 17% of drains, condensate and stagnant water, floors, food residues, processing equipment and miscellaneous areas, respectively, were contaminated with L. monocytogenes. Other biological studies in a poultry plant, have recovered the pathogen from 18.8, 12.5 and 37.5% of samples from feather pickers, chiller water overflow and recycled water for cleaning gutters, respectively (Genigeorgis et al., 1989).

Since L. monocytogenes is an environmental contaminant in meat processing plants, it is not surprising that many meat products, ready for human consumption, have been found to harbor the organism. Fifty-seven-percent of fresh and frozen poultry samples surveyed by Kwantes et al. (1971) were contaminated. Other reports have shown an incidence rate of 14.7% in "oven ready" poultry from 26 supermarkets (England) (Gitter, 1976). Elischerova et al. (1974) reported contamination rates of 70.2, 19 and 17.2% of poultry, beef and pork, respectively in Czechoslovakia. Bailey et al. (1989) reported that in the Southeastern United States, 38% of broiler carcasses analyzed were

contaminated by L. monocytogenes. According to a recent report by the World Health Organization (WHO, 1988), 15 to 80% of poultry and 30% of meat products are contaminated with L. monocytogenes. Farber et al. (1989) reported incidence levels of L. monocytogenes in 56.3% of chicken legs, 86.4% ground meats and 20% fermented sausages. Other products such as chopped beef, dry and fresh sausages, beef patties, minced pork, beef, lamb and pork carcasses have also been reported to be sources of the pathogen (Nicolas, 1985; Johnson et al., 1988).

Thus far, dairy products have been the primary sources of major foodborne listeriosis outbreaks (Fleming et al., 1985; James et al., 1985). Listeria monocytogenes has been recovered from raw and pasteurized milk, cheeses and ice cream (FDA, 1986; Fernandez et al., 1986, 1987; James et al., 1985; Lovett et al., 1987; Gilbert et al., 1988; Stone, 1987).

Among vegetable products, cabbage (Schlech et al., 1983), lettuce, celery, and tomatoes (Ho et al., 1986) have been shown to be sources of L. monocytogenes. Seafoods also have been reported to contain the pathogen (Lennon, 1984).

Cox et al. (1989) recovered L. monocytogenes from 20% of 35 household kitchens. Clothes, waste receptacles and

refrigerators were the primary sources. They concluded from their study that, humans were in contact with L. monocytogenes on a daily basis.

Control of Listeria monocytogenes

Since this organism is found everywhere in the environment, it is obvious that its control in the food industry is not an easy task. This is especially true in the meat industry where most processing steps are open to the environment (Anonymous, 1987). Increased awareness of the plant personnel involved in food production as to the ecology of L. monocytogenes should be the first consideration in designing a safety program to limit contamination. A Hazard Analysis Critical Control Point (HACCP) program should be developed to determine locations in the food processing operation at which control of L. monocytogenes would be most efficient.

Guidelines for control L. monocytogenes in the food industry start with the quality production of raw materials (Lovett, 1988). To limit the contamination of raw materials, it has been suggested that recyclable human and animal waste not be used to produce crops, especially those eaten raw or used as ingredients in other foods. The containers used in food transportation should be regularly

cleaned and sanitized (Lovett, 1989). Lopes (1986) found that food plant sanitizers such as quaternary ammonium compounds were effective against Listeria.

At the plant, raw materials should be segregated from processing and packaging areas. Workers from different locations should be kept apart during production. Proper processing procedures should be applied and continuously monitored. Doyle et al. (1987) suggested that HTST temperatures of 76.4-77.8°C (169.5-172°F) for 15.4 seconds are required to eliminate viable L. monocytogenes from milk. Other authors demonstrated that processing frankfurters for 70 min to an internal temperature of 160°F should kill L. monocytogenes (log 3 CFU/g or less) in raw meat (Zaik et al., 1990). Raw materials, finished product, equipment and the processing plant should be monitored constantly for microbiological contamination.

Listeria monocytogenes has been shown to be present in domestic environments (Cox et al., 1989; Anonymous 1989). Thus, it is essential that domestic hygiene be taken seriously in order to prevent further listeriosis outbreaks. Food products should not be abused nor should meat products be eaten raw. There is strong evidence that raw and undercooked meat products constitute potential sources of L. monocytogenes (Schwartz et al., 1988).

Parabens

The alkyl esters of p-hydroxybenzoic acid are known as parabens. They were first synthesized by Sabalitschka in the 1920's as replacements for benzoic and salicylic acids which are ineffective at neutral pH (Rahn et al., 1944). A few years later, Sabalitschka suggested these compounds had use as food and pharmaceutical preservatives (Lueck, 1980). The solubility of parabens in water is low and decreases as the alkyl chain length increases. At room temperature, the aqueous solubilities (w/w) of the methyl, ethyl, propyl and heptyl esters are 0.25g/100g, 0.17g/100g, 0.02g/100g and 0.0015g/100g, respectively. This is one difficulty encountered in the use of parabens as food preservatives. The compounds are soluble in fats and oils (Lueck, 1980), with solubility increasing as alkyl chain length increases.

Among the parabens, only the methyl, propyl and heptyl esters are approved for use in foods in the United States (Code of Federal Regulations, 1988). Methyl and propyl parabens are recognized as GRAS at levels not to exceed 0.1% (CFR 21.184.1490 and 21.184.1670). Their use depends on pH, solubility and their contribution to organoleptic properties (Lueck, 1980). Heptyl paraben may be used in

fermented malt beverages at < 12 ppm and in non-carbonated soft drinks and fruit-based beverages at < 20 ppm when standards of identity do not preclude such use (CFR 21.172.145). Heptyl paraben is normally dissolved in alkali or propylene glycol prior to use. Combinations of methyl and propyl parabens (2-3:1) at 0.06-0.1% are allowed in pharmaceutical and cosmetic fat emulsions to prevent growth of spoilage bacteria. A mixture of 5-15% methyl and propyl parabens in 5-15% alcohol is used to prevent mold growth in hard cheese in certain countries. Methyl and propyl parabens have been applied as antimicrobials at 0.05-0.1% in meat and sausage coatings containing gelatin (Lueck, 1980).

The antimicrobial effect of parabens increases with length of alkyl chain. Their inhibitory effects on various microorganisms have been demonstrated by a number of researchers (Table 5). Jurd et al. (1971) demonstrated that 0.04% propyl and heptyl parabens inactivated both gram positive and gram negative bacteria at pH 7.0 and 28°C, in plate count agar. Propyl paraben (500 ppm) was reported to reduce by approximately 3 logs a population of Staphylococcus aureus at pH 7.0 in trypticase soy broth in 48 hr at 35°C (Pierson et al., 1980). A previous study by the same author revealed that 200 ppm of propyl paraben in

Table 5. Antimicrobial Spectrum of Methyl, Propyl and Heptyl Esters of p-Hydroxybenzoic acid Against Selected Bacteria, Yeasts and Molds

Microorganism	Concentration for Total Inhibition* Methyl	Propyl	Heptyl
Bacteria			
Aeromonas hydrophila	--	>200	--
Bacillus cereus	2000	125-400	12
Bacillus subtilis	2000	250	--
Clostridium botulinum Type A	1000-1200	200-400	--
Enterobacter aerogenes	2000	1000	--
Escherichia coli	2000	400-1000	--
Klebsiella pneumoniae	1000	250	--
Lactococcus lactis	--	400	12
Listeria monocytogenes	>512	512	--
Pseudomonas aeruginosa	4000	8000	--
Pseudomonas fluorescens	1310	670	--
Pseudomonas fragi	--	4000	--
Salmonella typhosa	2000	1000	--
Salmonella typhyimurium	--	>300	--
Staphylococcus aureus	4000	350-500	12
Vibrio parahemolyticus	--	50-100	--
Fungi			
Aspergillus flavus	--	200	--
Aspergillus niger	1000	200-250	--
Byssochlamys fulva	--	200	--
Candida albicans	1000	125-250	--
Penicillium chrysogenum	500	125-200	--
Rhizopus nigricans	500	125	--
Saccharomyces bayanus	930	220	--
Saccharomyces cerevisiae	1000	125-200	100

*Minimum Inhibitory Concentration in ug/ml (ppm)

Sources: Aalto et al. (1953); Bargiota et al. (1987); Davidson (1983); Dymicky and Huhtanen (1979); Jurd et al. (1971); Lee (1973); Marwan and Nagel (1986); Moustafa and Collins (1969); Payne et al. (1989); Pierson et al. (1980); Robach and Pierson (1978); Sokol (1952); Venugopal et al. (1984)

a thiotone-yeast extract-glucose (TYG) prevented the germination of Clostridium botulinum 10755A and its toxin production for up to 120 hr at 37°C (Robach et al., 1978). Propyl paraben at 1200 ppm completely prevented and methyl paraben at 1000 ppm delayed germination and toxin production by the bacterium. The antimicrobial activity of parabens was reviewed by Davidson and Juneja (1990).

The acute toxicity of parabens generally decreases with decreasing length of the alkyl chain. The LD_{50}'s of methyl, ethyl, propyl and butyl parabens in mice (by mouth) were respectively 8g/kg, 6.3g/kg, 6.0g/kg and 13.2g/kg body weight (Sokol, 1972; Matthews et al., 1956; Sodo et al., 1973), while their sodium salts had LD_{50}'s of 2g/kg (methyl) and 3.7g/kg (propyl) (Matthews et al., 1956). Humans tolerated 2 g methyl and propyl parabens per day over one month. The parabens are absorbed from the gastrointestinal tract, hydrolyzed to p-hydroxybenzoic acid and excreted in the urine (Lueck, 1980).

Sodium Chloride

Sodium chloride is one of the oldest and most important food additives used by humans (Jensen, 1954; Marsden, 1980). When man first started using salt is not known, however the compound was well known in ancient

civilizations such as Egypt and Rome. Sodium chloride has various functions in the production and preparation of different types of foods. It may serve as a flavor constituent, a preservative or modify functional properties of some food components (Sofos, 1983).

As an antimicrobial, sodium chloride is used in the production of butter and margarine at 0.3-2%. It is an important ingredient in cheese production for flavor and partial preservation. It helps create a selective environment for the desirable organisms and suppresses the growth of spoilage and pathogenic organisms (Niven, 1980).

Sodium chloride has been used for many years in the manufacture of meat products. It is an important ingredient of pickling brines and serves as an adjunct to various preservation processes such as refrigeration, drying and smoking (Sofos, 1983). It plays an important role in the manufacture of emulsion type meat products. In addition to its contribution to flavor, sodium chloride helps extract salt soluble proteins (myofibrillar proteins) which form a matrix. This matrix, during heat coagulation binds fat, water, meat and other ingredients to yield a new product (Schmidt et al., 1981).

Sodium chloride is also useful in the preservation of seafoods (Hauschild et al., 1979). Fish such as herring,

anchovies, sprats, cod and salmon are preserved with sodium chloride. It is used in dry form or in solution at varying levels, depending on the type of fish product to prevent microbial attack. For instance, caviar is treated with 3-10% salt while suitable salt levels for anchovies and fish pastes are up to 20% (Lueck, 1980). Dipping fish fillets in 5% sodium chloride solution improved shelflife when fillets were packed in CO_2 (Mistsuda et al., 1980). Sodium chloride is normally supplemented with other agents (Sofos, 1983; Davidson et al., 1983).

Prior to processing, vegetables such as asparagus, beans, cabbage, carrots, turnips, onions, and olives are preserved in 15-25% sodium chloride solutions. Low salt concentrations (2-2.5%) are used in vegetable pickling. In sauerkraut or olive production, salt enhances the growth of lactic acid bacteria which produces lactic and acetic acids and CO_2, preserving the product (Pederson, 1979).

Sodium chloride owes its antimicrobial activity to its ability to reduce water activity (a_w). The a_w is a critical factor affecting microbial growth. This reduction of the availability of water in the growth medium can induce osmotic stress. Exposure of the bacterial cell to high concentrations of sodium chloride allows water to escape from the cell, resulting in plasmolysis of the

microorganism (Shahamat et al., 1980; Davidson and Juneja, 1990). Other possible mechanisms of inhibition include limiting oxygen solubility, alteration of pH, toxicity of sodium and chloride ions, and loss of magnesium ions (Banwart, 1979).

High concentrations of sodium chloride may have direct antimicrobial effects. It has been reported that 3-5% sodium chloride is necessary to inhibit most bacteria (Ayres et al., 1980). Toxin production of certain organisms may be effected by salt in growth media. Barbut (1986) showed that production of toxin by Clostridium botulinum could be retarded in presence of 1-3% salt. Sodium chloride at > 2% and pH < 6.5 were reported to inhibit Aeromonas hydrophila enterotoxin formation while 1% salt reduced biological effectiveness of toxins (Fehlhaber et al., 1987).

Some bacteria, including Listeria monocytogenes, are relatively resistant to sodium chloride. Murray (1955) found that Listeria monocytogenes was able to survive and remain fully virulent in 20% salt at 4°C for 8 weeks. Shahamat et al. (1980) reported that the organism was able to survive more than 18 weeks in 25.5% sodium chloride at 4°C in trypticase soy broth (pH 7.4). They also showed that the survival of this pathogen in sodium chloride

solution was effected by temperature. For example, the survival of the bacteria was lengthened from 5 days at 37°C to 32 days at 22°C and more than 132 days at 4°C (Shahamat et al., 1980). Thus, the lower the temperature, the longer the organism survived. Other studies revealed that L. monocytogenes grew in cabbage juice containing 0-5% sodium chloride at 30°C but the number of viable cells decreased when concentrations were > 2% (Conner et al., 1986). At 5% NaCl in cabbage juice, the viable population was reduced 90% in 70 days at 5°C. Listeria monocytogenes strain CA was inactivated within 85 days in skim milk with 12% sodium chloride or 110 days in whey with 12% sodium chloride (Papageorgiou et al., 1989). In contrast, L. monocytogenes Scott A survived for 130 days in both 12% salted skim milk and whey. From these studies, it is apparent that the resistance of Listeria to salt depends upon strain, temperature and food product.

Lactic Acid and Sodium Lactate

Lactic acid is naturally produced in many food products during fermentation by lactic acid bacteria (Pederson, 1979). Products such as cheese, cultured buttermilk, sour cream, sauerkraut, pickles and olives owe their flavor and keeping quality to lactic acid. The

antimicrobial activity of this acid depends on pH and the extent of its dissociation (Ingram et al., 1956). Lactic acid reduces the pH of food below the optimum for most spoilage and pathogenic microorganisms. The undissociated form of the acid has been demonstrated to be more active than the dissociated form (Eklund, 1983). This is explained by the fact the the undissociated form can penetrate the bacteria cell by diffusion then dissociates to produce acidification of the cell interior (Hunter et al., 1973). Lactic acid at 6-8 uM has been reported to inhibit sporeforming bacteria at pH 5.0, while yeasts and molds were found to be more resistant (Woolford, 1975).

Several research studies have been done in an attempt to decontaminate the surface of animal carcasses following slaughter with lactic acid (Smulders et al., 1986; Snijders et al., 1985). Lactic acid (1%, v/v) was found to reduce the initial microbial population of animal offal (hearts, tongues, livers) from log 4.0-5.0 CFU/cm^2 to log 1.0-2.0 CFU/cm^2 (Patterson et al., 1979). Woolthuis et al. (1984) were able to reduce the total aerobic counts, Enterobacteriaceae counts and Lactobacillaceae counts on vacuum packed porcine liver treated with 0.2% (v/v) lactic acid for 5 min and stored at 3°C for 4-5 days. Lactic acid has been proven useful in decontaminating veal tongues.

When 2% (v/v) lactic acid was applied to vacuum-packed tongues subsequently stored at 3°C, the mesophilic aerobic counts decreased from log 5.6 to log 2.7 CFU/cm² and Enterobacteriaceae counts were reduced from log 6.5 to log 4.0 CFU/cm² (Visser et al., 1988).

Sodium lactate has been used to improve the microbiological quality of many meat products. A recent report (Anonymous, 1989) suggested that products such as chicken rolls, beef rolls, hams, frankfurters, roast beef, pork sausage, fresh and smoked salmon and catfish fillets prepared with 3% (v/v) sodium lactate solution (60%) had an extended shelflife. There are very few reports concerning the action of lactic acid and sodium lactate on Listeria. Ahamad et al. (1989), recently showed that 0.1% sodium lactate inhibited L. monocytogenes in tryptose broth at 7°C. The report also suggested that the pathogen was inactivated at 7, 13, 21 and 35°C, when the growth medium (TB) was acidified with 0.3% lactic acid (Ahamad et al., 1989).

CHAPTER III

MATERIALS AND METHODS

MATERIALS

Strains of Listeria monocytogenes

Two strains of L. monocytogenes were used in this study. The first, Scott A (serotype 4b), was originally isolated by the Centers for Disease Control (CDC), Atlanta, GA, and was obtained from Dr. C.W. Donnelly, The University of Vermont, Burlington. The second strain, designated ERM, was isolated from commercial hot dogs. Both strains were stored on tryptose phosphate agar (TPA; Difco, Detroit, MI) slants at 4°C after incubation at 35°C for 24 hr. Prior to all analyses, the bacteria were incubated in tryptose phosphate broth (TPB; Difco, Detroit, MI) at 35°C for 18 hr.

Microbiological media

Tryptose phosphate agar was used for enumeration of viable cells of L. monocytogenes in growth studies involving chill brine and model meat suspensions. For

studies in which the microorganism was inoculated onto hot dogs, Modified Oxford Agar (MOX; Lee, 1989) was used for enumeration.

Antimicrobial agents

Reagent-grade methyl and propyl esters of p-hydroxybenzoic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Ethanol (95%) solutions of propyl paraben (PP) or methyl and propyl paraben (1:1, MPP) were prepared so as to have a final concentration in the test systems of 0.1% (w/v). For growth in meat suspensions, 1% ethanol served as a control. Food-grade sodium lactate (SL; ADM, Co., Decatur, IL) was added directly to test systems at a concentration of 2% (w/v).

METHODOLOGY

Resistance of Listeria monocytogenes in Chill Brine

A filter-sterilized (0.45 um membrane filter) 15% (w/v) brine solution was prepared using NaCl and 100 ml added to each of two sets of 4 sterile flasks. The first flask served as the control brine solution. In the remaining flasks, one of the following additives was added: SL, PP or MPP (1:1). The final pH of each solution was

determined using a pH meter (Fisher Accumet, Model 600, Springfield, NJ). The pH of each solution was as follows: 5.1, control; 5.3, PP; 5.0, MPP; and 4.6 SL. One set of flasks was inoculated with L. monocytogenes Scott A and the second with L. monocytogenes ERM. The initial number of viable cells was adjusted to log 3.0-3.5 CFU/ml. Flasks were incubated for 24 hr at 4°C. The contents of the flasks were serially diluted in sterile 0.1% peptone buffer and viable cells were enumerated by the pour plate method with TPA at 4, 8, 12 and 24 hr. Plates were made in duplicate and incubated at 35°C for 36-48 hr. Two replications were performed.

Chemical Analysis of Commercial Brine

Commercial chill brine which had been used to cool hot dogs after cooking was obtained at three intervals during a process day (beginning of process, after resalting and at the end of the process). Samples were taken at 7:00 A.M., 10:00 A.M. (after resalting) and 3:00 P.M. on 3 different days (7 day intervals). The samples were transported to the Department of Food Technology and Science at the University of Tennessee and stored at 4°C prior to analysis.

The commercial brine samples were analyzed for color, pH and proximate composition. Color was determined using a Hunterlab Tristimulus Colorimeter Model D25M-2 (Hunter Associates Laboratory, Inc., Reston, VA). The pH of the brine samples was measured using a pH meter (Fisher). The salt content of the samples were estimated by salometer at 60°F. The percentage salt was calculated according to the following equation:

Salt (%) = S° x 25/100 (S° = salometer reading at 60°F).

Moisture and total solids were determined by drying 100 ml brine samples at 80-100°C in an oven overnight. Ash content was determined by heating 2 ml samples at 600°C for 6 hr AOAC (1984). Protein content was determined using a 2 ml sample in the Kjeltech System 1015 Digestor and 1026 Distilling Unit (Tecator System, Haganas, Sweden). Total fat in a 100 ml sample of brine was determined by the chloroform-methanol method (Melton et al, 1979; Melton, 1990). All analyses were repeated three times.

Viability of Listeria monocytogenes in Commercial Brine

For viability studies, commercial brines collected at 10:00 A.M. were pasteurized at approximately 150°F for 30

min and cooled to 4°C. Samples were prepared to contain 0.1% PP, 0.1% MPP and 2% SL. The various brine samples were then inoculated with approximately log 3.0-3.5 CFU/ml of each strain of L. monocytogenes and treated as described above for laboratory-prepared brine. The brine samples were serially diluted in sterile 0.1% peptone buffer and viable cells were determined by the pour plate method with TPA at 3 hr intervals up to 12 hr and at 24 hr. The entire experiment was repeated two times.

Resistance of Listeria monocytogenes in Hot Dog and Chicken Suspensions

Hot dogs and chicken breasts were obtained from a commercial processor and a retail outlet, respectively, and stored at -20°C prior to testing. A 10% (w/v) suspension of each product was prepared by blending 25 g of product with 225 ml of 0.1% peptone diluent in a Stomacher Lab Blender (Model 400, Dynatech Lab. Inc., Alexandria, VA). A homogenous hot dog suspension was facilitated by adding 0.25% Tween 80 (sorbitan mono-oleate polyoxyethylene) (Fisher Scientific Co., Chemical Division, Fair Lawn, NJ). Visible fat was removed from chicken breasts prior to blending. Eight flasks of the suspension were then prepared to contain approximately 100 ml from each product.

The flasks and their contents were sterilized at 121°C for 15 min.

Ethanol (1% v/v; control), 0.1% PP, 0.1% MPP (1:1) or 2% SL were added to two sets of each sterile suspension. One set of flasks was inoculated with approximately log 3.5 CFU/ml of L. monocytogenes Scott A, while the other set was inoculated with the same initial number of L. monocytogenes ERM. All flasks were incubated at 35°C for 24 hr. The flasks contents were serially diluted in sterile 0.1% peptone buffer and viable L. monocytogenes were enumerated by the pour plate method with TPA every 3 hr up to 12 hr and a final sample at 24 hr. Plates were made in duplicate then incubated at 35°C for 36-48 hr. Two replications were performed.

Resistance of Listeria monocytogenes on Commercial Hot Dogs

Commercial hot dogs formulated with chicken, pork and beef were obtained from a local meat packer (Knoxville, TN) the same day of production and stored at -20°C until use. The hot dogs were inoculated by dipping in sterile water containing approximately log 6.0-7.0 CFU/ml of each strain of L. monocytogenes. Samples were then placed into a closed container for 45-60 min at room temperature to allow

for adequate attachment of L. monocytogenes cells. This treatment resulted in an initial inoculum of log 5.0-6.0 L. monocytogenes CFU/g.

The following sterile brine solutions were prepared: 15% (w/v) NaCl (control), 0.1% PP, 0.1% MPP (1:1), or 2% SL. The inoculated hot dogs were dipped into one of the brines for 5 minutes at 21°C and then placed in sterile plastic bags (Whirl-Pak, Nasco, Fort Atkinson, WI). One set of the samples was stored at 24°C for 24 hr, while the second set was stored at 4°C for 21 days. For hot dogs stored at 24°C, the number of viable L. monocytogenes were determined at 4, 8, 12 and 24 hr. Samples were blended in a Stomacher Lab Blender (Model 400, Dynatech Lab. Inc., Alexandria, VA) for 2 min, serially diluted in sterile 0.1% peptone buffer and dispensed into petri plates with MOX Agar. Plates were done in duplicate and incubated at 35°C for 20-24 hr. The same procedure was followed for samples stored at 4°C with the exception that samples were analyzed at 7, 14 and 21 days. Both experiments were repeated.

Statistical analysis

The General Linear Models procedure (SAS, 1985) was used for this analysis, where the antimicrobial treatments, chemical components, color characteristics, pH and

replication were the dependent variables. When significant differences among treatments occurred, the Student-Newman-Keuls (Lyman, 1988) means separation was used to detect differences among means.

CHAPTER IV

RESULTS AND DISCUSSION

VIABILITY OF *LISTERIA MONOCYTOGENES* IN CHILL BRINE

Viability in 15% Salt Brine

The ability of two strains of *Listeria monocytogenes* to survive in 15% salt brine, with and without antimicrobials, was determined (Fig. 1). For *L. monocytogenes* Scott A in 15% brine control, the number of viable cells remained essentially constant at the initial level of log 3.0 CFU/ml throughout the 24 hr test period (Fig. 1a). The addition of 0.1% PP or 2% SL also had no effect on the viability of this *L. monocytogenes* strain. No significant difference was detected between the number of *L. monocytogenes* in the PP or SL and the control ($p>0.05$) (Table 6). The presence of a 0.1% MPP however, resulted in a significant decrease ($p<0.05$) in the viable *L. monocytogenes* cells (Fig. 1a, Table 6). The viable count was reduced by approximately 99% after 4 hr and >99% at 12 hr. The antimicrobial did not completely eliminate *L. monocytogenes*, as viable cells were recovered after 24 hr.

Strain ERM demonstrated a slightly different response in the various brines (Fig. 1b). In the control brine, the number of viable cells remained generally constant at log 3.0 CFU/ml for 8 hr, increased slightly at 12 hr followed by a decrease to near the initial level at 24 hr. Propyl paraben caused a slight, but continuous, reduction in viable cells throughout the 24 hr test period. The final percentage inhibition caused by the PP was approximately 95%. Inhibition caused by the PP was significant ($p<0.05$) compared to the control (Table 6). Sodium lactate caused a slight reduction in viable L. monocytogenes in the first 12 hr, but showed no inhibitory effect ($p>0.05$) compared to the control over the 24 hr test period (Table 6). The most effective treatment in 15 % brine was the MPP combination. There was a significant reduction in viable L. monocytogenes counts between MPP and the control ($p<0.05$) treatment (Table 6). This treatment resulted in a 97% reduction at 4 hr and the microorganism was undetectable after 12 hr (Fig. 1b).

This study showed that L. monocytogenes survived in 15% salt brine for at least 24 hr at 4°C. Survival of L. monocytogenes was also shown by Wramby (1944) and Murray (1955). They found that this organism could survive for eight weeks in 20% NaCl at 4°C. Some authors have claimed

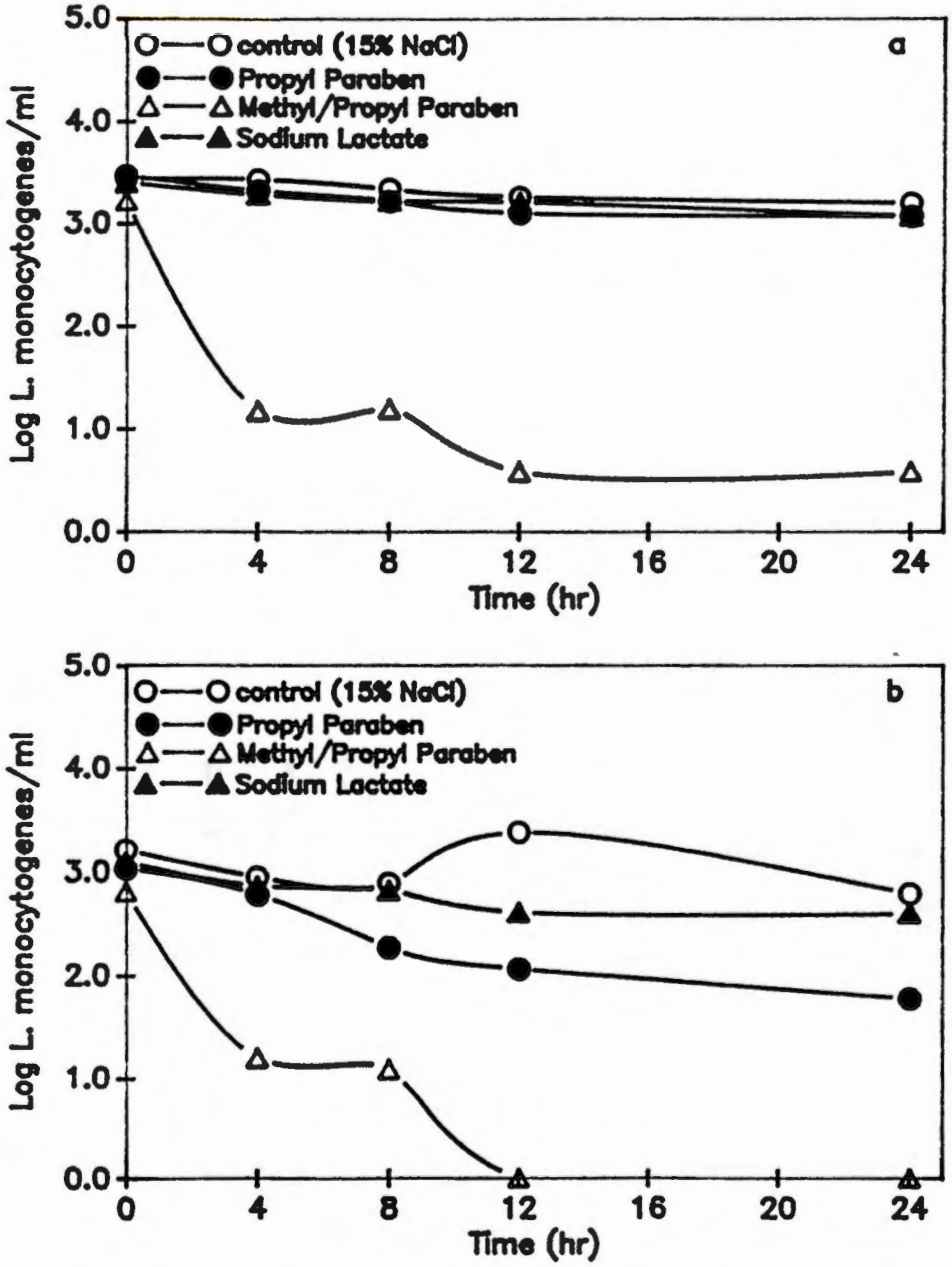

Figure 1. Survival of Listeria monocytogenes Scott A (a) and ERM (b) in 15% Salt Brine, With and Without Antimicrobials, at 4°C

Table 6. Mean Viable[a] *Listeria monocytogenes* Scott A and ERM in 15% Brine, With and Without Antimicrobials, at 4°C.

Antimicrobial	Log CFU/ml
L. monocytogenes Scott A	
Control (15% NaCl)	3.34[b]
Propyl Paraben (0.1%)	3.24[b]
Sodium lactate (2.0%)	3.24[b]
Methyl-propyl paraben (0.1%)	1.37[c]
L. monocytogenes ERM	
Control (15% NaCl)	3.01[b]
Propyl Paraben (0.1%)	2.35[c]
Sodium lactate (2.0%)	2.80[b]
Methyl-propyl paraben (0.1%)	0.86[d]

[a]Mean log count over 24 hr sampling period (n=10); Initial counts were log 3.44 CFU/ml for *L. monocytogenes* Scott A and log 3.20 CFU/ml for *L. monocytogenes* ERM

[b,c,d]Means with different superscripts are significantly different at P=0.05.

that L. monocytogenes grew in presence of 20% NaCl within one day (Kontnick et al., 1977). Shahamat et al. (1980) showed that L. monocytogenes was able to survive in 25.5% NaCl for more than 18 wk at 4°C and that survival was a function of temperature. The temperature effect on L. monocytogenes in cabbage juice containing 0 to 5% NaCl was shown by Conner et al. (1986). At 30°C, the organism could grow with up to 1.5% NaCl in cabbage juice, while there was a decrease in viable cell count at 3.5-5% NaCl. In contrast to most studies, Beganovic et al. (1971) showed that L. monocytogenes died after 15 to 30 days at 4°C when exposed to 10% salt.

Chemical Analysis of Commercial Brine

The total solids, salt content, ash, protein, fat and pH of commercial brine varied considerably depending upon the time of sampling (Table 7). Total solids, salt and ash contents of the brine increased 3.0-3.7% from the first to the second sampling periods. The concentration of the three components decreased at the last sampling period. The concentration of total solids and ash should be related directly to the salt content, as it is the primary component in the brine. The reason for the increase in salt (and therefore, total solids and ash) at the second

Table 7. Chemical Composition of Commercial Brine Samples as a Function of Time of Sampling.

Sampling Time	Moisture	TS[a]	Salt	Ash	Fat	Protein	pH
	Percentage						
7:00 A.M.	92.87[b]	7.13[b]	7.12[b]	7.38[b]	0.07[b]	0.09[b]	6.00[b]
10:00 A.M.	89.25[b]	10.75[b]	10.16[b]	10.60[b]	0.82[b]	0.13[b]	5.36[b]
3:00 P.M.	91.11[b]	8.89[b]	9.33[b]	8.96[b]	0.89[b]	0.12[b]	5.50[b]

[a]TS=Total Solids.
[b]Means with same superscripts are not significantly different ($p>0.05$)

sampling period is that the brine is usually resalted from 10:00-10:30 AM. This prevents the brine from freezing. The purpose of the salt in the commercial cooling brine is to lower the freezing point of the water to around 0°C. Such a temperature is needed to cool the hot dogs after they are cooked and assists in controlling microorganisms which might survive the process.

The concentration of protein and fat in the brine increased slightly from the first to second sampling times (Table 7). The presence of small amounts of proteins and fat in the brine may be the result of melted lipids and collagens from cooked products. The build up of these organic residues may have reduced the pH of the brine from an initial mean of 6.0 to 5.36-5.5 (Table 7).

The brine was found to change from a colorless to an orange-red solution as it was used in the cooling process (Table 8). The change in color is due to leaching of color pigments from the casing. Since the same brine is used the entire day, the accumulation of pigments enhances the color change throughout the process.

Growth of Listeria monocytogenes in Commercial Brine

Pasteurized resalted brine (10:00 A.M. sample) was used for evaluation of the survival of L. monocytogenes.

Table 8. Color Analysis of Commercial Brine Samples.

Sample Time	L[a]	a[b]	b[c]
	----------Hunterlab Values ----------		
7:00 A.M.	+15.00[d]	-0.20[e]	+0.80[e]
10:00 A.M.	+13.36[d]	+1.46[d,e]	+5.83[d]
3:00 P.M.	+10.93[e]	+5.46[d]	+5.93[d]

[a]L=Brightness (0=black to 100=white)
[b]a=Redness if positive, gray=0, greenness if negative
[c]b=Yellowness if positive, gray=0, blueness if negative
[d,e]Means that different superscripts are significantly different at p=0.05

Results showed that the initial log 3.50 CFU/ml L. monocytogenes Scott A remained constant for 12 hr at 4°C, then increased slightly to log 4.25 CFU/ml at 24 hr. The addition of all antimicrobials maintained the number of viable cells at or near the initial level throughout the 24 h (Fig. 2a). While slight differences were shown in viable counts, no significant difference was detected between any antimicrobial treatment and the control ($p>0.05$) (Table 9).

For brine inoculated with L. monocytogenes ERM, viable cells were found to decrease slightly throughout the incubation period at 4°C (Fig. 2b). The addition of PP or MPP had no significant ($p>0.05$) effect on the survival of L. monocytogenes ERM (Table 9). The SL treatment caused a significant ($p<0.05$) reduction in viable cells compared to the control, however this difference was of little practical value (Table 9).

Salt brine is used to cool hot dogs after thermal processing (150°F). The present study shows that the brine used currently in the sausage industry may not completely prevent growth of L. monocytogenes on hot dogs. Further, chill brine, which is not sterile (Rico-Munoz, 1989), may be a source of contamination for the surface of these products. It must be pointed out that the increase in viable L. monocytogenes Scott A cells took place only after

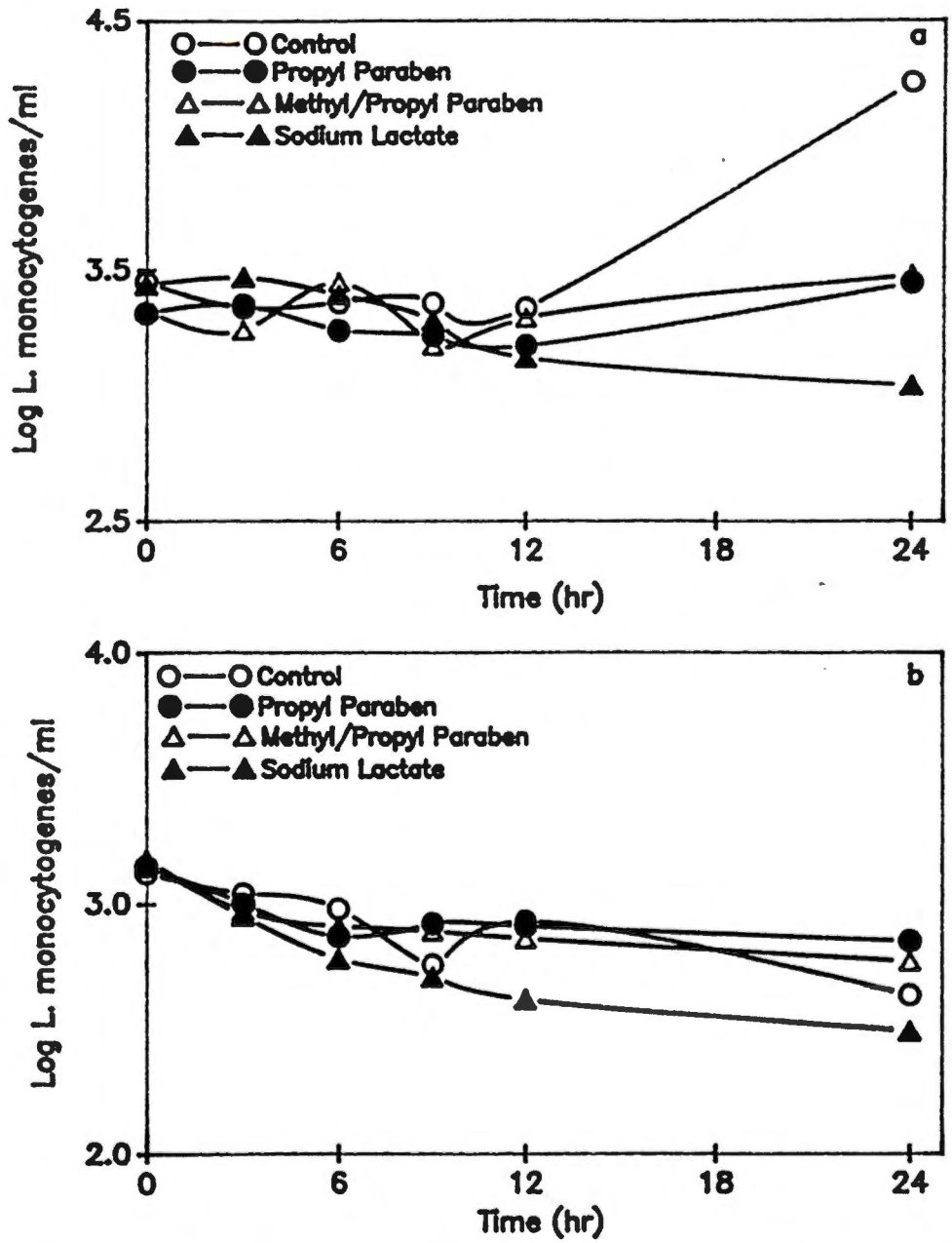

Figure 2. Survival of Listeria monocytogenes Scott A (a) and ERM (b) in Commercial Brine, With and Without Antimicrobials, at 4°C

Table 9. Mean Viable[a] *Listeria monocytogenes* Scott A and ERM in Commercial Brine, With and Without Antimicrobials, at 4°C.

Antimicrobial	Log CFU/ml
L. monocytogenes Scott A	
Control (15% NaCl)	3.53[b]
Propyl Paraben (0.1%)	3.32[b]
Sodium lactate (2.0%)	3.31[b]
Methyl-propyl paraben (0.1%)	3.34[b]
L. monocytogenes ERM	
Control (15% NaCl)	2.98[b]
Propyl Paraben (0.1%)	2.95[b]
Sodium lactate (2.0%)	2.79[c]
Methyl-propyl paraben (0.1%)	2.93[b]

[a]Mean log count over 24 hr sampling period (n=12); Initial counts were log 3.45 CFU/ml for *L. monocytogenes* Scott A and log 3.12 CFU/ml for *L. monocytogenes* ERM

[b-c]Means with different superscripts are significantly different at P=0.05.

a 24 hr incubation, which is much longer than the brine would normally be used.

To be used successfully, an antimicrobial would have to reduce the viability of L. monocytogenes in this system. None of the antimicrobials used was effective in reducing the viability of either L. monocytogenes Scott A or ERM. It is unclear as to why the MPP treatment was less effective in the commercial brine compared to the laboratory brine. Possible reasons could have been a reduced salt content in the commercial brine and the presence of potential binding components such as protein and fat.

VIABILITY OF LISTERIA MONOCYTOGENES IN MEAT

Viability of Listeria monocytogenes in Chicken and Hot Dog Suspensions

Listeria was found to grow very well in both 10% chicken and hot dog suspensions (Fig. 3). Listeria monocytogenes Scott A increased from the initial log 3.30 CFU/ml in the control chicken to a final count of log 7.91 CFU/ml after 24 hr at 35°C (Fig. 3a). The addition of 0.1% PP or MPP to the suspension maintained the initial inoculum at a nearly constant level until 12 hr with PP or 9 hr with

MPP. The final viable cell count increased to log 4.45 CFU/ml after 24 hr for PP, and log 5.31 CFU/ml for MPP. Both the PP and MPP treatments caused a significant ($p<0.05$) reduction in L. monocytogenes Scott A growth compared to the control (Table 10). The addition of 2% SL had no effect on the viable cell count throughout the incubation period (Fig. 3a, Table 10).

When L. monocytogenes ERM was inoculated onto the slurry, similar results were obtained, with the exception of PP (Fig. 3b). With PP, there was no increase in the viable cell count at any time. Instead, the initial number of viable cells was found to decrease slightly from log 2.82 CFU/ml to a final count of log 2.69 CFU/ml (Fig. 3b). Again, the PP and MPP treatments were significantly ($p<0.05$) more effective than the control and PP was more effective than MPP (Table 10). There was no significant difference between the SL treatment and the control ($p>0.05$) (Table 10).

The growth of both L. monocytogenes strains showed a similar pattern in the 10% hot dog suspension (Fig. 4). The number of viable L. monocytogenes Scott A cells in the control increased gradually from log 3.63 CFU/ml to log 8.86 CFU/ml at the end of the 24 hr incubation period at 35°C. The addition of 0.1% PP or MPP to the suspension

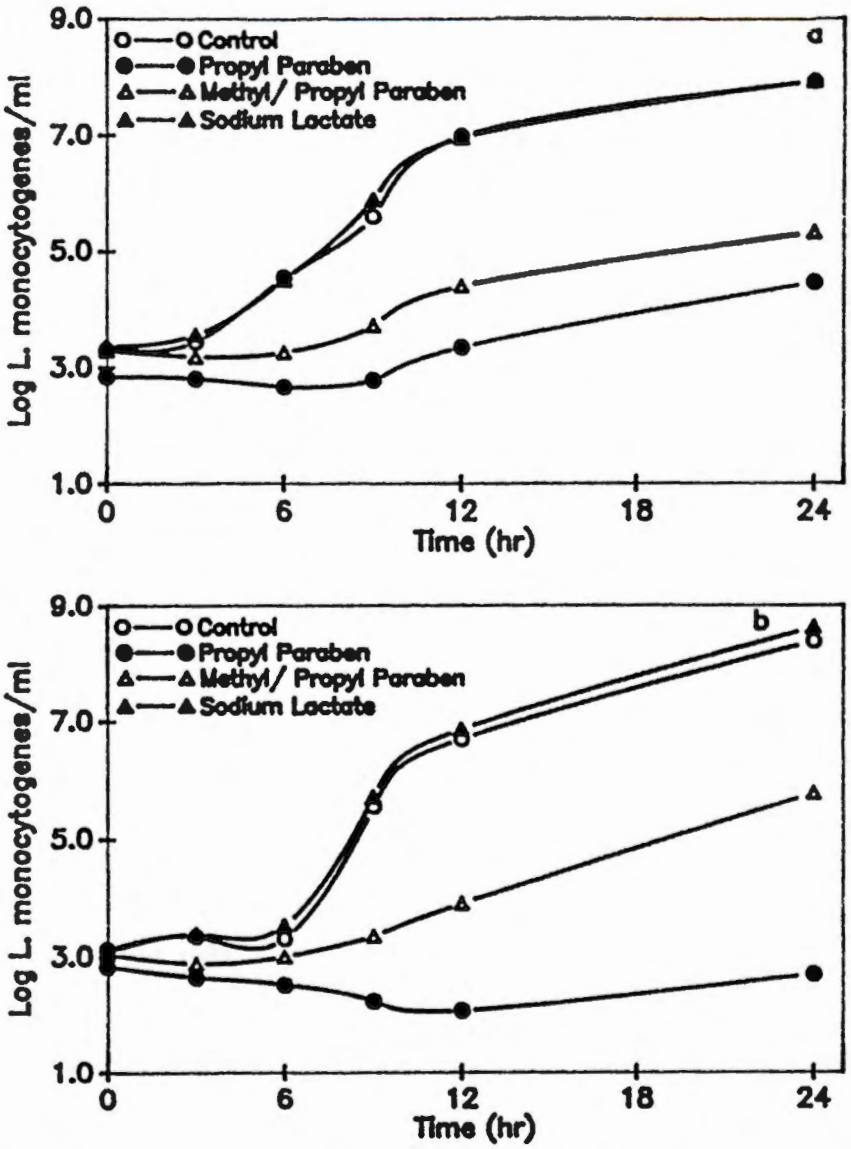

Figure 3. Growth of Listeria monocytogenes Scott A (a) and ERM (b) in 10% Chicken Suspension, With and Without Antimicrobials, at 35°C

Table 10. Mean viable[a] *L. monocytogenes* Scott A and ERM in 10% Chicken Suspension, With and Without Antimicrobials, at 35°C.

Antimicrobial	Log CFU/ml
L. monocytogenes Scott A	
Control (Ethanol)	5.29[b]
Propyl Paraben (0.1%)	3.14[d]
Sodium lactate (2.0%)	5.36[b]
Methyl-propyl paraben (0.1%)	3.86[c]
L. monocytogenes ERM	
Control (Ethanol)	5.07[b]
Propyl Paraben (0.1%)	2.49[d]
Sodium lactate (2.0%)	5.17[b]
Methyl-propyl paraben (0.1%)	3.65[c]

[a]Mean log count over 24 hr sampling period (n=12); Initial counts were log 3.30 CFU/ml for *L. monocytogenes* Scott A and log 3.12 CFU/ml for *L. monocytogenes* ERM

[b-d]Means with different superscripts are significantly different at P=0.05.

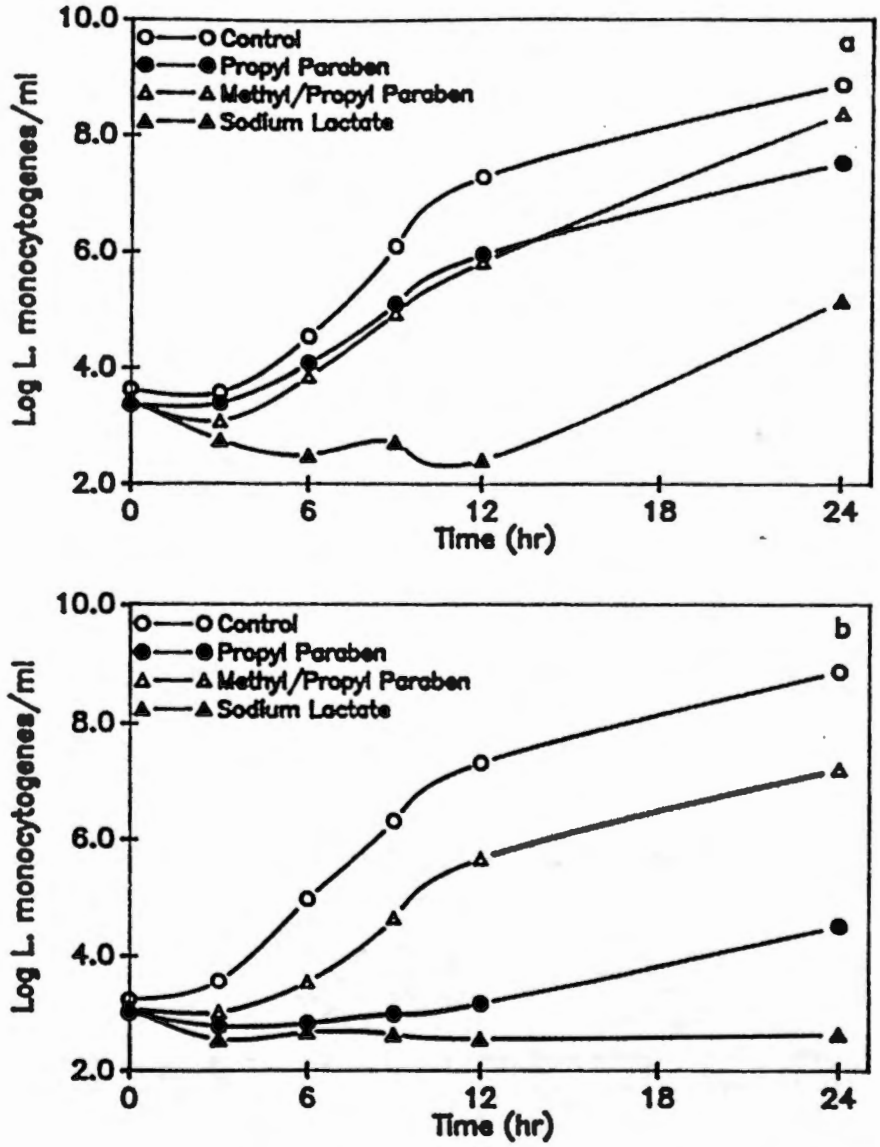

Figure 4. Growth of Listeria monocytogenes Scott A (a) and ERM (b) in 10% Hot Dog Suspension, With and Without Antimicrobials, at 35°C

extended the lag phase slightly. Final counts were log 6.74 CFU/ml for PP and log 8.36 CFU/ml for MPP. Both PP and MPP caused a significant ($p<0.05$) reduction in L. monocytogenes Scott A growth (Table 11). The addition of 2% SL resulted in a gradual reduction of the viable cell counts until 12 hr. The microorganism then increased to log 5.14 after 24 hr (Fig. 4a). The SL treatment was the most effective ($p<0.05$) in reducing L. monocytogenes growth (Table 11).

For L. monocytogenes ERM in the slurry (Fig. 4b), similar results were obtained with MPP. In contrast, the PP treatment was slightly more effective than for strain Scott A (Table 11). Addition of SL resulted in a slight reduction in viable cell count throughout the incubation period. The initial count of log 3.08 CFU/ml was decreased to log 2.62 CFU/ml at 24 hr (Fig. 4b). There was a significant difference between the SL treatment and the control ($p<0.05$).

The activity of the antimicrobials against L. monocytogenes Scott A and ERM in chicken and hot dog suspensions suggested that it might be used to control the growth of the organism on commercial hot dogs. As was shown previously, L. monocytogenes is capable of surviving in commercial brine and could contaminate product which was

Table 11. Mean viable[a] L. monocytogenes Scott A and ERM in 10% Hot Dog Suspension, With and Without Antimicrobials, at 35°C.

Antimicrobial	Log CFU/ml
L. monocytogenes Scott A	
Control (Ethanol)	5.69[b]
Propyl Paraben (0.1%)	4.92[c]
Sodium lactate (2.0%)	3.16[d]
Methyl-propyl paraben (0.1%)	4.91[c]
L. monocytogenes ERM	
Control (Ethanol)	5.71[b]
Propyl Paraben (0.1%)	3.20[d]
Sodium lactate (2.0%)	2.67[d]
Methyl-propyl paraben (0.1%)	4.55[c]

[a]Mean log count over 24 hr sampling period (n=12); Initial counts were log 3.38 CFU/ml for L. monocytogenes Scott A and log 3.23/ml for L. monocytogenes ERM

[b,c,d]Means with different superscripts are significantly different at P=0.05.

previously free of the organism. Therefore, the same compounds were evaluated in brine against L. monocytogenes inoculated on hot dogs.

Viability of Listeria monocytogenes on Commercial Hot Dogs

It was first determined whether L. monocytogenes inoculated onto the surface of hot dogs and exposed to 15% brine were capable of growth. It was found that the initial inoculum of log 5.7 CFU/g L. monocytogenes Scott A increased to approximately log 7.3 CFU/g after 24 hr at 24°C (Fig. 5a). Hot dogs inoculated with L. monocytogenes ERM showed similar results (Fig. 5b). This strain showed an increase from the initial inoculum of log 5.0 CFU/g to log 7.5 CFU/g following incubation at 24°C.

Three antimicrobial compounds were added to the salt brine dip to determine their effect on the survival and/or growth of L. monocytogenes. The addition of PP, MPP or SL had little effect on the growth of L. monocytogenes Scott A on hot dogs at 24°C (Fig. 5a). In all cases, the viable cell counts were similar to the control at each time interval. No significant difference was obtained between the SL and PP treatments and the control ($p>0.05$) (Table 12). While a significant difference was found between MPP treatment and the control ($p<0.05$), it was of little

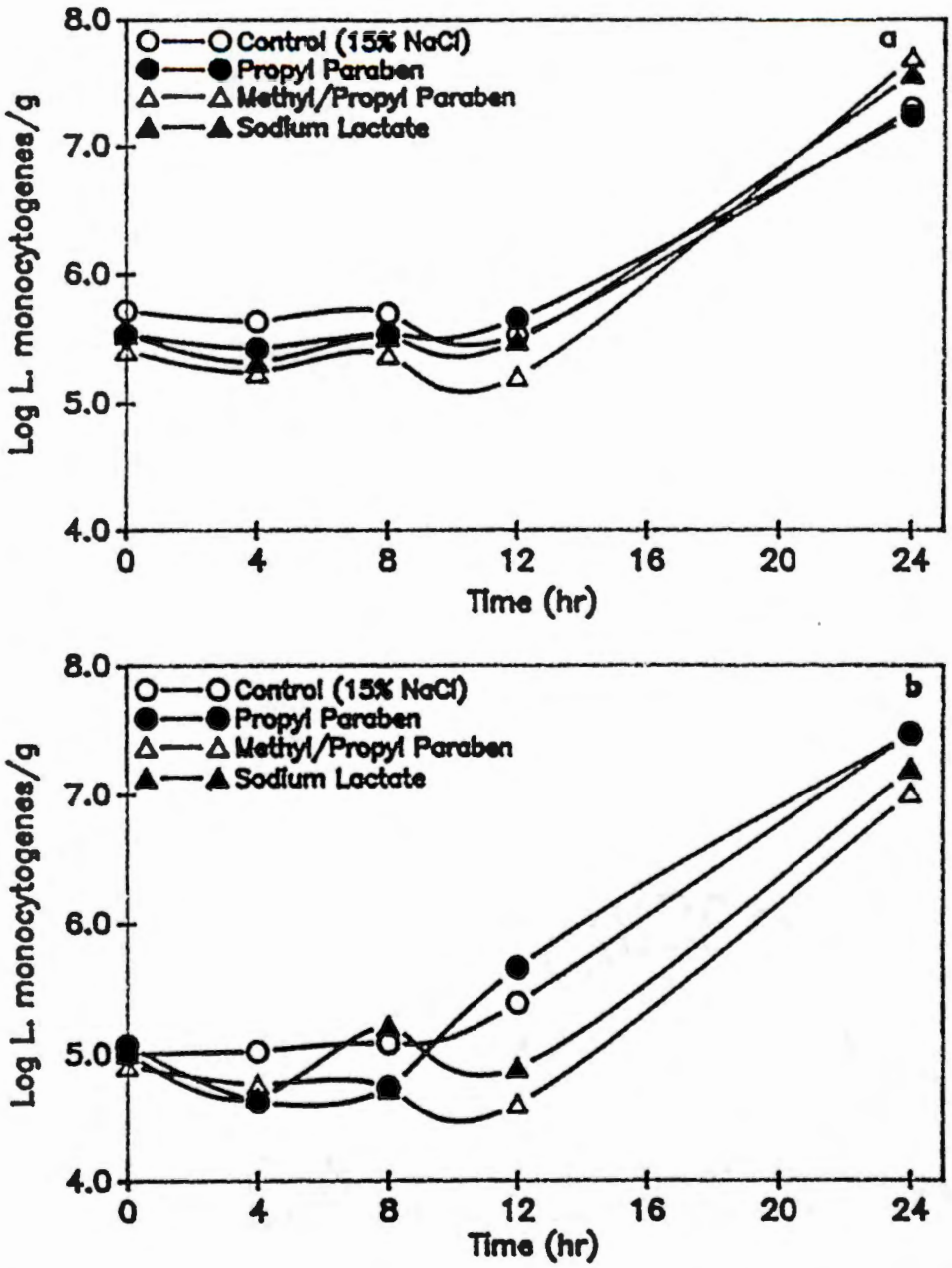

Figure 5. Growth of Listeria monocytogenes Scott A (a) and ERM (b) on Hot Dogs Dipped in Brine, With and Without Antimicrobials, at 24°C

Table 12. Mean viable[a] *L. monocytogenes* Scott A and ERM on Hot Dogs Dipped in 15% Brine, With and Without Antimicrobials, at 24°C.

Antimicrobial	Log CFU/g
L. monocytogenes Scott A	
Control (15% NaCl)	5.97[b]
Propyl Paraben (0.1%)	5.81[bc]
Sodium lactate (2.0%)	5.83[bc]
Methyl-propyl paraben (0.1%)	5.73[c]
L. monocytogenes ERM	
Control (15% NaCl)	5.60[b]
Propyl Paraben (0.1%)	5.52[b]
Sodium lactate (2.0%)	5.40[b]
Methyl-propyl paraben (0.1%)	5.20[b]

[a]Mean log count over 24 hr sampling period (n=10); Initial counts were log 5.72 CFU/g for *L. monocytogenes* Scott A and log 5.72 CFU/g for *L. monocytogenes* ERM

[bc]Means with different superscripts are significantly different at P=0.05.

practical value (Table 12). Hot dogs inoculated with L. monocytogenes ERM showed similar results (Fig. 5b) (Table 12). Propyl paraben and SL had no significant effect ($p>0.05$) on the growth of L. monocytogenes ERM on hot dogs other than a slight increase in the lag phase caused by SL. The only evidence of inhibition with MPP was an increase in the lag phase. No significant inhibition was demonstrated at 24 hr by the compounds (Fig. 5b).

On inoculated hot dogs stored at 4°C, the ability of L. monocytogenes to proliferate in foods at refrigeration temperatures was demonstrated. In the control, the initial inoculum of about log 5.72 CFU/g L. monocytogenes Scott A remained constant until day 7, then increased to approximately log 6.89 CFU/g (Fig. 6a). The final count was log 7.73 CFU/g. The only significant difference between antimicrobial treatments and the control ($p<0.05$) was for MPP (Table 13). The difference was most likely due to the 7 day extension of L. monocytogenes Scott A lag phase (Fig. 6a).

Listeria monocytogenes ERM showed the same growth pattern on the control hot dogs as was found for strain Scott A (Fig. 4b). The addition of antimicrobials to the brine dip however, had less effect on L. monocytogenes ERM. No significant difference was found between the PP

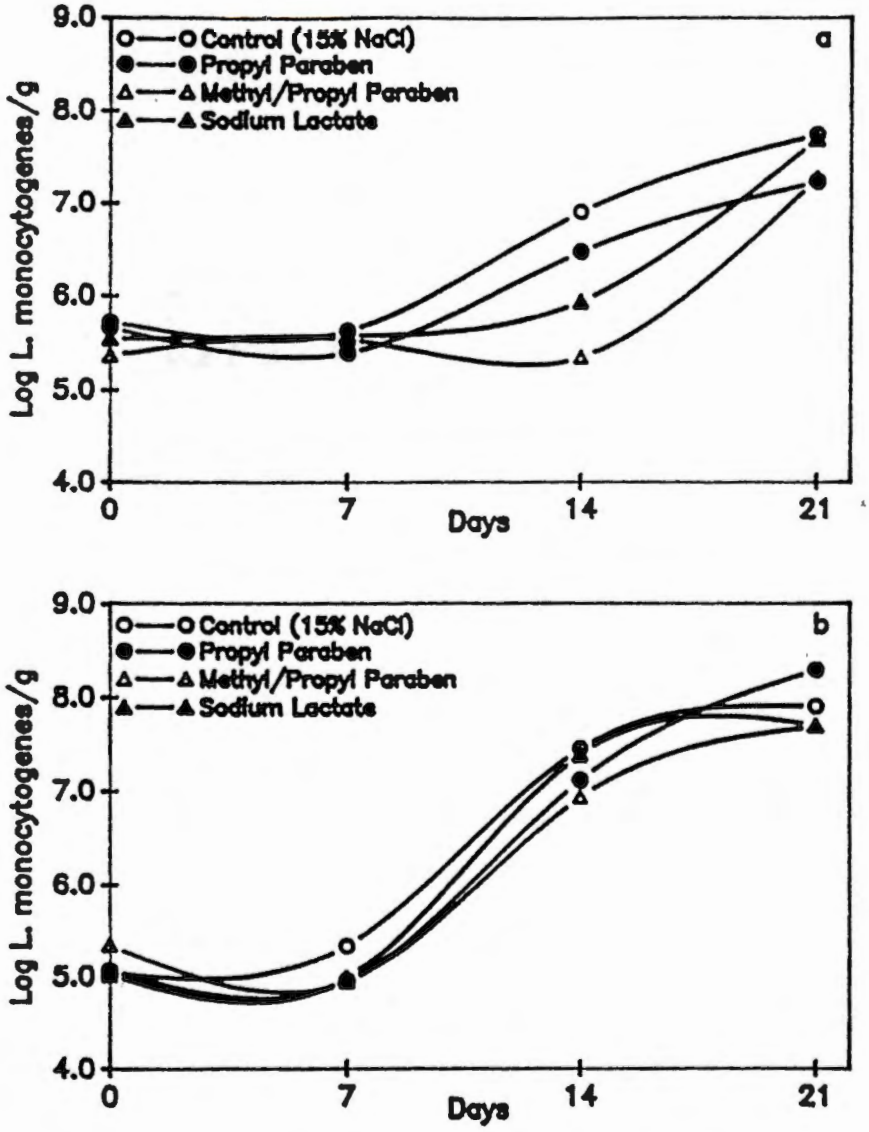

Figure 6. Growth of Listeria monocytogenes Scott A (a) and ERM (b) on Hot Dogs Dipped in Brine, With and Without Antimicrobials, at 4°C

Table 13. Mean viable[a] *Listeria monocytogenes* Scott A and ERM on Hot Dogs Dipped in 15% Brine, With and Without Antimicrobials, at 4°C.

Antimicrobial	Log CFU/g
L. monocytogenes Scott A	
Control (15% NaCl)	5.77[b]
Propyl Paraben (0.1%)	6.19[b]
Sodium lactate (2.0%)	6.18[b]
Methyl-propyl paraben (0.1%)	5.88[c]
L. monocytogenes ERM	
Control (15% NaCl)	6.43[b]
Propyl Paraben (0.1%)	6.36[bc]
Sodium lactate (2.0%)	6.27[c]
Methyl-propyl paraben (0.1%)	6.23[c]

[a]Mean log count over 21 d sampling period (n=8); Initial counts were log 5.0 CFU/g for *L. monocytogenes* Scott A and log 5.03 CFU/g for *L. monocytogenes* ERM

[bc]Means with different superscripts are significantly different at P=0.05.

treatment and the control ($p>0.05$) (Table 13). While treatments with MPP and SL were significantly different from the control ($p<0.05$), there was little practical value since no effect on the lag phase or final growth level was detected (Table 13).

It was demonstrated in the study that L. monocytogenes is capable of growth in meat (hot dogs and chicken) suspensions at 35°C and on commercial hot dogs at both abuse temperatures (24°C) and refrigeration (4°C). These results are in agreement with others who have shown the ability of Listeria to grow in both raw and processed foods (Elischerova, 1974; Kwantes et al., 1971; WHO, 1988; Ho et al., 1986). While growth of L. monocytogenes at low temperatures is slow, it is obvious that refrigeration alone cannot be assumed to maintain food in a safe condition. These results demonstrate the importance of proper monitoring of processing environments to reduce the chances of L. monocytogenes contamination. They also suggested that a safeguard is needed to slow or prevent the growth of L. monocytogenes on hot dogs which might be contaminated during manufacture.

The experiments with antimicrobials showed that L. monocytogenes could be inhibited in the 10% model meat systems with PP, MPP and SL. In contrast, these

antimicrobials had little effect on the two strains of L. monocytogenes on commercial hot dogs. There are several possible reasons for lack of effectiveness by the compounds. The solubility of the parabens in lipid may be one reason for their limited effect. Since they are hydrophobic (methyl 0.25g/100g water, propyl 0.02g/100g water), they may have solubilized in the lipid of the product and been less available for inhibition of the L. monocytogenes. Another factor which may have reduced inhibition of L. monocytogenes by all antimicrobials was the short time of exposure. Any cell destruction would have had to take place during the brine dip, as there would most likely be little carry-over to the product.

REFERENCES

REFERENCES

Aalto, T.R., Firman, M.C. and Rigler, N.E. 1953. p-Hydroxybenzoic acid esters as preservatives. I. Uses, antibacterial and antifungal studies, properties and determination. J. Am. Pharm. Assoc. (Sci. Ed.) 42:449-458.

Ahamad, N. and Marth, E.H. 1989. Behavior of Listeria monocytogenes at 7, 13, 21 and 35°C in tryptose broth acidified with acetic, citric, or lactic acid. J. Food Prot. 52:688-695.

Al-Ghazali, M.R. and Al-Azawi, S.K. 1986. Detection and enumeration of Listeria monocytogenes in sewage treatment plant in Iraq. J. Appl. Bacteriol. 60:251-254.

Anonymous. 1987. Meat industry research shows Listeria widespread, control difficult. Food Chem. News 29(17):27-28.

Anonymous. 1989. Listeriosis associated with consumption of turkey franks. MMWR. 38(15):267.

Anonymous. 1988. FSIS reports Listeria in 6.9% of raw beef samples. Food Chem. News 3(17):36.

Anonymous. 1989. Arlac S. Sodium lactate. Effects on shelf stability of various meat, poultry and seafood products. ADM Co., Decatur, IL.

AOAC. 1984. "Official Methods For Analysis." 14th ed. Association of Official Analytical Chemists. Washington, D.C.

Ayres, J.C., Mundt, J.O., and Sandine, W.E. 1980. Microbiology of foods. W.H. Freeman and Co. San Francisco, California. P. 132.

Bacon, M. and Miller, N.G. 1958. Two strains of Listeria monocytogenes (Pirie) isolated from feral sources in Washington. Northwest Sci. 32:132-139.

Bailey, J.S., Fletcher, D.L. and Cox, N.A. 1989. Recovery and serotype distribution of L. monocytogenes from broiler chickens in the Southeastern United States. J. Food Prot. 52:148-150.

Banwart, G.J. 1979. "Basic Food Microbiology". AVI Publ., Westport, Conn.

Barbut, S., Tanaka, N., Cassens, R.G., and Maurer, A.J. 1986. Effects of sodium chloride reduction and polyphosphate addition on Clostridium botulinum toxin production in turkey frankfurters. J. Food Sci. 51:1136-1138.

Bargiota, E., Rico-Munoz, E. and Davidson, P.M. 1987. Lethal effect of methyl and propyl paraben as related to Staphylococcus aureus lipid composition. Int. J. Food Microbiol. 4:257-266.

Beganovic, H., Milanovic, A. and Forsek, Z. 1971. The viability of Listeria monocytogenes in NaCl solution and pickle. Veterinaria (Sarajevo) 20:73-80.

Bojensen-Moller, J. 1972. Human listeriosis: Diagnostic, epidemiological and clinical studies. Acta Pathol. Microbiol. Scand. (B). 229:1-157.

Botzler, R.G., Cowan, A.B. and Wetzler, T.F. 1974. Survival of Listeria monocytogenes in soil and Water. J. Wildlife Dis. 10:204-212.

Botzler, R.G. and Cowan, A.B. 1985. Transfer of Listeria monocytogenes between frogs. J. Wildlife Dis. 21:173-174.

Brackett, R.E. 1988. Presence and persistence of Listeria monocytogenes in food and waters. Food Technol. 42:162-164.

Braza, M. 1985. Listeriosis and milk. N. Engl. J. Med. 312:438-440.

Breed, R.S., Murray, E.G.D., and Smith, N.R. 1957. "Bergey's Manual of Determinative Bacteriology", 7th ed. Williams and Wilkins Co, Baltimore.

Buchanan, R.E. and Gibbons, N.E. 1974. "Bergey's Manual of Determinative Bacteriology", 8th ed. Williams and Wilkins Co, Baltimore.

Carbonnelle, B., Cottin, J., Parvery, F., Chambreuil, G., Kouyoumdjian, S., Lirzin, M. and Vincent, F. 1978. Epidemie de listeriose dans l'oust de la France (1975-1976). Rev. Epidemiol. Sante Publique (Paris) 26:451-467.

Ciesielski, C.A., Hightower, A.W., Parsons, S.K. and Broome, C.V. 1988. Listeriosis in the United States: 1980-1982. Arch. Intern. Med. 148:1416-1419.

Code of Federal Regulations. 1988. Title 21. Food and drugs. Parts 100-199. Off. Fed. Reg., Natl. Arch. and Records Admin., Washington.

Conner, D.E., Brackett, R. E. and Beuchat, L.R. 1986. Effect of temperature, sodium chloride, and pH growth of Listeria monocytogenes in Cabbage Juice. Appl. Environ. Microbiol. 52:59-63.

Cottin, J., Genthon, H., Bizon, C. and Carbonnelle, B. 1985. Listeria monocytogenes in meat from 514 cattle. Sci. Aliments. 5:145-149.

Cox, L.J., Kleiss, T., Cordier, J.L., Cordellana, C., Konkel, P., Pedrazzini, C., Beumer, R. and Siebenga, A. 1989. Listeria spp. in food processing, non-food and domestic environments. Food Microbiol. 6:49-61.

Davis, G.H.G., Fomin, L., Wilson, E. and Newton, K.G. 1969. Numerical taxonomy of Listeria, Streptococci and possibly related bacteria. J. Gen. Microbiol. 57:333-348.

Davidson, P.M. 1983. Phenolic compounds. p. 37. In "Antimicrobials in Foods". Branen, A.L., and Davidson, P.M. (eds). Marcel Dekker, Inc. New York.

Davidson, P.M. and Juneja, V.K. 1990. Antimicrobial agents. p. 83. In "Food Additives". Branen, A.L., Davidson, P.M. and Salminen, S. (ed.). Marcel Dekker, Inc. New York.

Dijkstra, R.G. 1975. Recent experiences of the survival times of Listeria bacteria in suspension of brain, tissue, silage, faeces and in milk. p. 71. In "Problems of Listeriosis," ed. Woodbine, M. Leicester Univ. Press, Surrey, U.K.

Dijkstra, R.G. 1982. The occurrence of Listeria monocytogenes in surface water canals and lakes, in ditches of one big polder and in the effluents of canals of a sewage treatment plant. Zentrabl. Bakteriol. Hyg. 176:202-205.

Doyle, M.P., Glass, K.A., Beery, J.T., Garcia, J.A., Pollard, D.J. and Schulz, R.D. 1987. Survival of Listeria monocytogenes in milk during high-temperature, short-time pasteurization. Appl. Environ. Microbiol. 53:1433-1438.

Dymicky, M. and Huhtanen, C.N. 1979. Inhibition of of Clostridium botulinum by p-hydroxybenzoic acid n-alkyl esters. Antimicrob. Agents Chemother. 15:798.

Eklund, T. 1980. Inhibition of growth and uptake processes in bacteria by some chemical food preservatives. J. Appl. Bacteriol. 48:423-427.

Eklund, T. 1983. Antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J. Appl. Bacteriol. 54:383.

Elischerova, K., Stupalova, S. and Stepanek, J. 1974. Some ecological aspects of Listeria monocytogenes in meat industry. p. 148. In I. Ivanov (ed), Proc. 7th Inter. Symp. of Problems of listeriosis. National Agroindustrial Union, Sofia.

Farber, J.M., Sanders, G.W. and Johnston, M.A. 1989. A survey of various foods for the presence of Listeria species. J. Food Prot. 52:456-458.

Fehlhaber, K. and Sheibner, G. 1987. Studies on properties of Aeromonas hydrophila enterotoxins relevance to food hygiene. Arch. Exper. Vet. 41:121-126.

Fernandez-Garayzabal, J.F., Dominguez, L., Vazquez-Boland, J.A., Blanco Cancelo, L. and Suarez-Fernandez, G. 1986. Listeria monocytogenes dans le lait pasteurise. Can. J. Microbiol. 32:149-150.

Fenlon, D.R. 1985. Wild birds and silage as reservoirs of Listeria in the agricultural environment. J. Appl. Bacteriol. 59:537-543.

Fenlon, D.R. 1986. Rapid quantitative assessment of the distribution of Listeria in silage implicated in a suspected outbreak of listeriosis in calves. Vet. Rec. 118:240-242.

Fleming, D.W., Cochi, S.L., MacDonald, K.L., Brondum, J., Hays, P.S., Plikaytis, B.D., Holmes, M.B., Audurier, A., Broome, C.V. and Reingold, A.L. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 312:404-407.

Food and Drug Administration. 1986. Dairy products safety initiatives. Preliminary status. U.S. Food and Drug Admin. Washington D.C.

Genigeorgis, C.A., Dutulescu, D. and Fernandez-Garayzabal, J. 1989. Prevalence of Listeria spp. in poultry meat at the supermarket and slaughterhouse level. J. Food Prot. 52:618-624.

Gilbert, R.J., and Pini, P.N. 1988. Listeriosis and foodborne transmission. Lancet I:472-473.

Gitter, M. 1976. Listeria monocytogenes in "oven-ready" poultry. Vet. Rec. 99:336.

Gitter, M. 1985. Listeriosis in farm animals in Great Britain. p. 191. In "Isolation and Identification of Microorganisms of Medical and Veterinary Importance," Soc. for Appl. Bacteriol. Tech. Series No. 21, Collins, C.H. and Grange, J.M. (ed.). Academic Press. London.

Gray, M.L. 1960. Isolation of Listeria monocytogenes from oat silage. Sci. 132:1767-1768.

Gray, M.L. and Killinger, A.H. 1966. Listeria monocytogenes and listeric infections. Bacteriol. Rev. 30:309-382.

Gregorio, S.B. and Eveland, W.C. 1975. Isolation of Listeria monocytogenes from inapparent sources in Michigan. p. 83. In "Problems of Listeriosis". Woodbine, M. (ed.), Leicester University Press.

Gronstol, H. 1979. Listeriosis in sheep. Isolation of Listeria monocytogenes from grass silage. Acta Vet. Scand. 20:492-497.

Hauschild, A.H.W. and Hilsheimer, R. 1979. Effect of salt content and pH on toxigenesis by Clostridium botulinum in caviar. J. Food Prot. 42:245-248.

Ho, J.L., Shands, K.N., Friedland, G., Eckind, P. and Fraser, D.W. 1986. An outbreak of type 4b Listeria monocytogenes infection involving patients from eight Boston hospitals. Arch. Intern. Med. 146:520-524.

Hunter, D. and Segel, I.H. 1973. Effect of weak acids on amino acid transport by Penicillium chrysogenum. Evidence for a proton or charge gradient as the driving force. J. Bacteriol. 113:1184-1192.

Ingram, M., Ottoway, F.J.H. and Coppock, J.B.M. 1956. The preservative action of acid substances in food. Chem. Ind. (London) 42:1154.

James, S.M., Fannin, S.L., Agree, B.A., Hall, B., Parker, E., Vogt, J., Run, G., Williams, J. and Lieb, L. 1985. Listeriosis outbreak associated with Mexican-style cheese. Calif. Morb. Mort. Weekly Rep. 34:357-359.

Jay, J.M. 1986. "Modern Food Microbiology", 3rd ed. p. 272. Van Nostrand Reinhold Co., New York.

Jensen, L.B. 1954. "Microbiology of Meats", 3rd ed. The Garrard Press, Champaign, IL.

Johnson, J., Doyle, M.P. and Cassens, R.G. 1988. Survival of Listeria monocytogenes in ground beef. Int. J. Food Microbiol. 6:243-247.

Johnson, J.L., Doyle, M.P., Cassens, R.G. and Schoeni, J.L. 1988. Fate of Listeria monocytogenes in tissues of experimentally infected cattle and in hard salami. Appl. Environ. Microbiol. 54:497-501.

Jones, D. 1975. A numerical taxonomic study of coryneform and related bacteria. J. Gen. Microbiol. 87:52-96.

Jurd, L., King, A. D., Mihara, K. and Stanely, W.L. 1971. Antimicrobial properties of natural phenols and related compounds. 2. Obtusastyrene. Appl. Microbiol. 21:507-510.

Karches, H. and Teufel, P. 1988. Listeria monocytogenes, its occurrence in minced meat and behaviour in fresh onion mettwurst. Fleischwirtschaft 68:1388-1392

Kwantes, W. and Isaac, M. 1971. Listeriosis. Brit. Med. J. 4:296-297.

Lee, J.S. 1973. What seafood processors should know about Vibrio parahaemolyticus. J. Milk Food Technol. 36:405-408.

Lee, W.H. 1989. Personal communication.

Lennon, D., Lewis, B., Mantell, C., Becroft, D., Dove, B., Farmer, K., Tonkin, S., Yeates, N., Stamp, R. and Mickleson, K. 1984. Epidemic perinatal listeriosis. Pediat. Dis. 3:30-34.

Lopes, J.A. 1986. Evaluation of dairy and food plant sanitizers against Salmonella typhimurium and Listeria monocytogenes. J. Dairy Sci. 69:2791-2796.

Lovett, J.L., Francis, D.W. and Hunt, J.M. 1987. Listeria monocytogenes in raw milk: Detection, incidence, and pathogenicity. J. Food Prot. 50:188-192.

Lovett, J.L. and Twedt, R.M. 1988. Listeria. Food Technol. 42:188-190.

Lovett, J.L. 1989. Listeria monocytogenes. p. 288. In "Foodborne Bacterial Pathogens". Doyle, M.P. (ed.). Marcel Dekker, Inc. New York.

Lueck, E. 1980. "Antimicrobial Food Additives". Springer-Verlag, Berlin.

Lyman, O. 1988. An introduction to statistical methods and data analysis. 3rd ed. PWS-Kent Publishing Co. Boston.

Malinverni, R., Glauser, M.P., Bille, J. and Rocourt, J. 1986. Unusual clinical features of an epidemic of listeriosis associated with a particular phage type. Eur. J. Clin. Microbiol. 5:169-171.

Marsden, J.L. 1980. The importance of sodium in processed meats. p. 77. In Proc. Meat Ind. Res. Conf. Am. Meat Inst., Washington, D.C.

Marwan, A.G. and Nagel, C.W. 1986. Quantitative determination of infinite inhibition concentrations of antimicrobial agents. Appl. Environ. Microbiol. 51:559-561.

Matthews, C., Davidson, J., Bauer, E., Morrison, J.G. and Richardson, A.P. 1956. p-Hydroxybenzoic acid esters as preservatives. II. Acute and chronic toxicity in dogs, rats, and mice. J. Am. Pharm. Assoc. 45:260-267.

Mclauchlin, J. 1987. Listeria monocytogenes, recent advances in the taxonomy and epidemiology of listeriosis in humans. J. Appl. Bacteriol. 63:1-11.

Melton, S.L., Moyers, R.E. and Playford, G.G. 1979. Lipid extraction from soy products by different procedures. J. Am. Oil Chem. Soc. 56:489.

Melton, S.L. 1990. Personal communication.

Mitsuda, H., Nakajima, K., Mizuno, H. and Kawai, F. 1980. Use of NaCl solution and CO_2 for extending shelflife of fish fillets. J. Food Sci. 45:661-666.

Moore, W.E.C., Cato, E.P. and Moore, L.V.H. 1985. Index of the bacterial and yeast nomenclatural changes published in the International Journal of Systematic Bacteriology since the 1980 approved lists of bacterial names (1 January 1980 to 1 January 1985). Int. J. Syst. Bacteriol. 35:382-407.

Moustafa, H.H.and Collins, E.B. 1969. Effects of selected food additives on growth of Pseudomonas fragi. J. Dairy Sci. 52:335-340.

Murray, E.G.D., Webb, R.A. and Swann, M.B.R. 1926. A disease of rabbits characterized by large mononuclear leucocytosis, caused by a hitherto undescribed Bacterium monocytogenes. J. Path. Bacteriol. 29:407-439.

Murray, E.G.D. 1955. A characterization of listeriosis in man and other animals. Can. Med. Assoc. J. 72:99-103.

Nicolas, J. A. 1985. Contamination of meat and meat products with Listeria monocytogenes in Haute-Vienne, France. Sci. Aliments 5:175-179.

Niven, C.F. 1980. Technology of sodium in processed foods: General bacteriological principles with emphasis on canned fruits and vegetables, and dairy foods. p. 45. In "Sodium and Potassium in Foods and Drugs", White, P.C. and Croco, S.C. (eds.). Conf. Proc. Am. Med. Assoc., Chicago.

Nyfeldt, A. 1929. Etiologie de la mononucleose infectieuse. Compt. Rend. Soc. Biol. 101:590-591.

Ortel, S. 1968. Bakteriologische und epidemiologische untersuchungen wahrend einer listerios-epidemie. Deutsche Gesundheitswesen. 23:753-759.

Papageorgiou, D.K. and Marth, E.H. 1989. Behavior of Listeria monocytogenes at 4 and 22°C in whey and skim milk containing 6 or 12% sodium chloride. J. Food Prot. 52:625-630.

Patterson, J.T. and Gibbs, P.A. 1979. Vacuum packaging of bovine edible offal. Meat Sci. 3:209-222.

Payne, K.D., Rico-Munoz, E. and Davidson, P.M. 1989. The antimicrobial activity of phenolic compounds against Listeria monocytogenes and their effectiveness in a model milk system. J. Food Prot. 52:151-153.

Pederson, C.S. 1979. "Microbiology of Food Fermentations", 2nd ed. AVI Publ. Co., Westport.

Pierson, M.D., Smoot, L.A., and Vantassell, K.R. 1980. Inhibition of Salmonella typhimurium and Staphylococcus aureus by butylated hydroxyanisole and the propyl ester of p-hydroxybenzoic acid. J. Food Prot. 43:191.

Pirie, J.H.H. 1940. Listeria: Change of name for a genus of bacteria. Nature. 145:264.

Rahn, O. and Conn, J.E. 1944. Effect of increase in acidity on antiseptic efficiency. Ind. Eng. Chem. 36:185.

Ralovich, B. 1984. "Listeriosis Research. Present situation and prospective." Akademiai Kiado, Budapest.

Rico-Munoz, E. 1989. Personal communication.

Robach, M.C. and Pierson, M.D. 1978. Influence of para-hydroxybenzoic acid esters on the growth and toxin production of Clostridium botulinum 10755A. J. Food Sci. 43:787-789.

Sado, I. 1973. Synergistic toxicity of official permissible preservative food additives. Nippon Eiseigaku Zasshi 28:463-476.

SAS. 1985. SAS User's Guide: Statistics, Version 5 ed. SAS Institute Inc, Cary, NC.

Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S. and Broome, C.V. 1983. Epidemic listeriosis: Evidence for transmission by food. N. Engl. J. Med. 308:203-206.

Schmidt, G.R., Mawson, R.F. and Siegel, D.G. 1981. Functionality of protein matrix in comminuted meat products. Food Technol. 35:235-237.

Schwartz, B., Ciesielski, C.A., Broome, C.V., Gaventa, S., Brown, G.R., Gellin, B.G., Hightower, A.W. and Mascola, L. 1988. Association of sporadic listeriosis with consumption of uncooked hot dogs and undercooked chicken. Lancet. 1:779-782.

Seeliger, H.P.R. 1961. "Listeriosis." Hafner Pub. Co., New York.

Seeliger, H.P.R. and Finger, H. 1985. Listeriosis. p. 264. In "Infectious Diseases of the Fetus and Newborn Infant,". Remington, J.S. and Klein, J.O. (2nd ed.) Sanders, W.B. Co., Philadelphia.

Seeliger, H.P.R. and Jones, D. 1986. Listeria. p. 1235. In "Bergey's Manual of Systematic Bacteriology." vol. 2. Sneath, P.H.A. (ed.) William and Wilkins, Baltimore, MD.

Shahamat, M., Seaman, A. and Woodbine, M. 1980. Influence of sodium chloride, pH and temperature on the inhibitory activity of sodium on Listeria monocytogenes. p. 227. In "Microbial Growth and Survival in Extremes of Environment". Gould, G.W. and Corry, J.E.L. (ed.) Academic Press, London.

Shahamat, M., Seaman, A. and Woodbine, M. 1980. Survival of Listeria monocytogenes in high salt concentrations. Zentrabl. Bakteriol. Hyg. 246:506-511.

Skerman, V.B.D., McGowan, V. and Sneath, P.H.A. 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30:225-420.

Smulders, F.J.M., Barendsen, P., Van Logtestijn, J.G., Mossel, D.A.A. and Van Der Marel, G.M. 1986. Review: lactic acid: Considerations in favour of its acceptance as a meat decontaminant. J. Food Technol. 21:419-436.

Snijders, J.M.A., Van Logtestijn, J.G., Mossel, D.A.A. and Smulders, F.J.M. 1985. Lactic acid as a decontaminant in slaughter and processing procedures. Vet. Quart. 7:277-283.

Sofos, J. N. 1983. Antimicrobial effects of sodium and other ions in foods: A review. J. Food Safety 6:45-78.

Sokol, H. 1952. Recent developments in the preservation of pharmaceuticals. Drug Stand. 20:89.

Stone, D.L. 1987. A survey of raw whole milk for Campylobacter jejuni, Listeria monocytogenes and Yersinia enterocolitica. N.Z. J. Dairy Sci. Technol. 22:257-264.

Venugopal, V., Pansare, A.C. and Lewis, N.F. 1984. Inhibitory effect of food preservatives on protease secretion by Aeromonas hydrophila. J. Food Sci. 49:1078-1081.

Visser, I.J.R., Koolmees, P.A. and Bijker, P.G.H. 1988. Microbiological conditions and keeping quality of veal tongues as affected by lactic acid decontamination and vacuum packaging. J. Food Prot. 51:208-213.

Vizcaino, L.L. and Garcia, M.A. 1974. A note on Listeria milk excretion in sero-positive apparently healthy cows. p. 74. In "Problems of Listeriosis". Woodbine, M. (ed.). Leicester University Press, Leicester.

Watkins, J. and Sleath, K.P. 1981. Isolation and enumeration of Listeria monocytogenes from sewage, sewage sludge and river water. J. Appl. Bacteriol. 50:1-9.

Weis, J. and Seeliger, H.P.R. 1975. Incidence of Listeria monocytogenes in nature. Appl. Microbiol. 30:29-32.

Welshimer, H.J. 1968. Isolation of Listeria monocytogenes from vegetation. J. Bacteriol. 95:300-303.

Welshimer, H.J. and Donker-Voet, J. 1971. Listeria monocytogenes in nature. Appl. Microbiol. 21:516-519.

WHO. 1988. Report of the informal working group on foodborne listeriosis. Bulletin of WHO. No. WHO/EHE/FOS/88.5. 66:421-428.

Woolford, M.K. 1975. Microbiological screening of food preservatives, cold sterilants and specific antimicrobial agents as potential silage additives. J. Sci. Food Agric. 26:229.

Woolthuis, C.H.J., Mossel, D.A.A., Van Logtestijn, J.G., De Kruijf, J.M. and Smulders, F.J.M. 1984. Microbial decontamination of porcine liver with lactic acid and hot water. J. Food Prot. 47:220-226.

Wramby, G.O. 1944. Om Listeria monocytogenes bakteriologi och om orekomst av Listerella-infektion nos djur. Skand. Vet. Tidskr. 34:277-290.

Zaik, L.L., Palumbo, S.A., Smith, J.L., Del Corral, F., Bhaduri, S., Jones, C.O. and Kim, A.H. 1990. Destruction of Listeria monocytogenes during frankfurter processing. J. Food Prot. 53:18-21.

VITA

Dje, Yobouet was born in 1961 (Abli) Toumodi, Cote d'Ivoire. He graduated from Lycee (high school) Moderne de Koumassi (Cote d'Ivoire) in June, 1982. He earned his high school diploma "Baccalaureate D" with options in Mathematics, Biology and Geology. He was granted a scholarship by the Ivorian Government in October, 1982 for his college work at the University of Abidjan in the College of Science and Technique, Department CBG (Chemistry, Biology and Geology). He graduated in October, 1986 with the B.S. (Maitrise) in Biochemistry. Then he was granted a scholarship by the French government for Doctoral work in Food Technology. He also received a scholarship from the Ivorian government for graduate work in Food Technology and Science in The United States. In January, 1988, he enrolled in the Department of Food Technology and Science at the University of Tennessee, Knoxville. He was granted a Master of Science Degree in Food Technology and Science in May 1990.

The author was the recipient of the "Houphouet Boigny" in 1974 for the top elementary school graduate. He was the top high school graduate of Lycee Moderne de Koumassi in 1982 and an honor student in genetics and organic chemistry in 1986.