1994


Warren Joseph Dorsa
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Effects of heat, lactic acid, and modified atmosphere packaging on *Listeria monocytogenes* on cooked crawfish tail meat

Dorsa, Warren Joseph, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1994
EFFECTS OF HEAT, LACTIC ACID, AND MODIFIED ATMOSPHERE PACKAGING ON LISTERIA MONOCYTOGENES ON COOKED CRAWFISH TAIL MEAT

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in

The Department of Food Science

by

Warren Joseph Dorsa
B.S., Nicholls State University, 1978
M.S., Mississippi State University, 1981
May 1994
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He wishes to thank his friends and fiance Jessica, for their constant support, love, and encouragement during this undertaking. Finally, he wishes to dedicate this work to his parents Ted and Nancy, for without their unflattering support, encouragement, and love throughout the years, his life would not be the joyous event it has become during the completion of this task.
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<td>pediocin (form of)</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>atm</td>
<td>atmosphere(s)</td>
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<tr>
<td>BPB</td>
<td>buterfield phosphate buffer</td>
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<tr>
<td>°C</td>
<td>degrees Celsius</td>
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<tr>
<td>CA</td>
<td>citric acid</td>
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<td>CC</td>
<td>cellulose casing method</td>
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<td>CFU</td>
<td>colony forming units</td>
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<td>CO₂</td>
<td>carbon dioxide</td>
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<td>kilograms</td>
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<tr>
<td>L</td>
<td>liter</td>
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<td>LA</td>
<td>lactic acid</td>
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<td>LOA</td>
<td>listeria oxoid agar</td>
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<td>MAP</td>
<td>modified atmosphere packaging</td>
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<tr>
<td>min</td>
<td>minute(s)</td>
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<tr>
<td>mL</td>
<td>milliliter(s)</td>
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<td>MPa</td>
<td>millibar per atmosphere</td>
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<td>P</td>
<td>probability</td>
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<td>PA-1</td>
<td>pediocin (form of)</td>
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<td>PB</td>
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<td>PB-a</td>
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<td>pH</td>
<td>(-) log of hydrogen ions</td>
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<td>PKa</td>
<td>dissociation constant</td>
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<td>PMF</td>
<td>proton motive force</td>
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<td>PS</td>
<td>potassium sorbate</td>
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<tr>
<td>sp</td>
<td>species (singular)</td>
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<tr>
<td>spp</td>
<td>species (plural)</td>
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<td>SOD</td>
<td>superoxide dismutase</td>
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<td>TDT</td>
<td>thermal death time</td>
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<td>TNRF</td>
<td>3 neck reaction flask method</td>
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<td>TSA</td>
<td>tryptic soy agar</td>
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<td>USDA</td>
<td>U.S. Department of Agriculture</td>
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<td>USFDA</td>
<td>U.S. Food and Drug Administration</td>
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<tr>
<td>wt</td>
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<td>w/w</td>
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The generation time of *L. monocytogenes* was calculated to be 72.2, 28.5, 17.0, and 6.9 h on crawfish tail meat held at 0, 4, 6, and 12°C, respectively. Potassium sorbate (PS) (0.3%) spray treatments on crawfish held at 4°C, extended the lag phase of the bacterium. However, once exponential growth began, a generation time not significantly different (P>0.05) than that of untreated crawfish was observed. Samples sprayed with 0.3% citric acid (CA) and held at 4°C supported growth of the bacterium equally well as that of untreated samples.

Thermal inactivation of *L. monocytogenes* using a three neck reactionary flask method yielded D55,60,65 values of 10.23, 1.98, and 0.19 min, respectively. A z value of 5.5°C was calculated. Polybag and cellulose casing methodology yielded significantly higher (P> 0.05) D60 values of 4.68 and 3.84 respectively, for the bacterium. D60 values for *L. monocytogenes* decreased as the percent lactic acid (LA) spray treatments levels were increased. Increasing sensitivity to heat with LA was not solely due to a reduction of pH. D60 values for the bacterium on samples packed in air, O2, CO2, or N2 were not significantly different (P>0.05), however, D60 of samples packed under O2 were lowest.
Generation times for heat damaged *L. monocytogenes* on crawfish meat stored at 4°C and packed under air, O₂, CO₂, or N₂ was determined. Twenty percent of the cell population was damaged when thermally treated at 60°C for 12.5 min. The bacterium consistently grew slowest in CO₂ and O₂. Samples receiving a treatment of 1% LA, packaging under either of the 4 different atmospheres, and storage at 4°C, were lethal to the bacterium.

These studies indicate that post-packaging heat treatments of 60°C or greater in the presence of CO₂ or O₂, and the addition of 1% LA sprays, would be detrimental to the survival of *L. monocytogenes* on crawfish meat.
INTRODUCTION

Increased public awareness and perception of food quality and safety has escalated government monitoring of seafood products. As a result, development of more safety focused processing techniques is critical. Employment of multi-barrier methodology through modification of presently used processes would be an economical way to accomplish this goal.

Outbreaks of foodborne listeriosis involving a variety of foods has established Listeria monocytogenes as an important foodborne pathogen (Fleming et al., 1985; Kvenberg, 1988; Linnan et al., 1988; Sclech et al., 1983). The association of L. monocytogenes with seafood products has also been established (McLauchlin, 1987). Frozen seafood products such as shrimp, crabmeat, lobster, langostinos, scallops, squid, and surimi have been shown to contain L. monocytogenes when samples were obtained from several different countries (Weagant et al., 1988). The study found that 26% of the products sampled contained L. monocytogenes. Farber (1991) found 13% of fish products at the wholesale level in the U.S.A., Britain, and Taiwan, to be positive for L. monocytogenes. He also found ready-to-eat shrimp, crab, and salmon to contain the bacterium. L. monocytogenes flourishes well in aquatic environments of
decaying vegetative matter (Gray and Killinger, 1966), which is the typical environment in a crawfish pond. As a result, *L. monocytogenes* can usually be found on live crawfish (crayfish) entering processing plants.

Crawfish tail meat is hand picked from parboiled whole crawfish and usually packed for retail sale in one pound, low oxygen permeable polybags, that are sometimes vacuum sealed. While boiling temperatures (100°C) are sufficient to kill *L. monocytogenes* (Fain et al., 1991), cross contamination from workers and food contact surfaces may occur. Postprocessing contamination with *L. monocytogenes* has been identified as a major source of contamination for many food products (Farber and Peterkin, 1991). Low numbers of *L. monocytogenes* have been observed in selected frozen crawfish tail meat (Dorsa and Marshall, 1993). One pound bags of crawfish tail meat are typically sold unfrozen on ice. Harrison et al. (1991) found that *L. monocytogenes* populations on vacuum packed iced shrimp did not increase. Despite this finding, it is important to consider that evenly distributed product in ice-packs or 0°C conditions are not always practical during product transportation or during retail display of packaged crawfish tail meat. As a result, it is possible for the product to be subjected to periods of warming, potentially resulting in proliferation of *L. monocytogenes*. 
*L. monocytogenes* has been shown to grow well on many food products stored under refrigeration. The generation times for the bacterium has been determined for red meats, vegetables, and seafood products (Berrang et al., 1989; Marshall et al., 1991; Shelef, 1989). The generation time for the bacterium on crawfish tail meat has not been determined, however.

Most information available for the heat resistance of *L. monocytogenes* in foods has concentrated on milk and red muscle products. *L. monocytogenes* has been shown to survive improper heat treatments in red meats and chicken (Farber, 1989; Harrison and Carpenter, 1989). Little is known about the thermal resistance of *L. monocytogenes* in seafoods, although D-values for some products such as crab and lobster have been determined (Budu-Amoako et al., 1992; Harrison & Huang, 1990). Presently, there are few crawfish processors that subject crawfish tail meat to post-packaging heat treatments. Since post-packaging heat treatments might be beneficial in reducing post-processing contamination and insure product safety, there is a need to study the heat resistance of *L. monocytogenes* on this product.

*L. monocytogenes* has been detected in 53% of vacuum packed processed meats obtained from retail stores (Grau and Vanderlinde 1992). Several studies with modified atmospheres indicate that the bacterium can survive low
temperature conditions in reduced oxygen or anaerobic conditions. Marshall et al. (1991, 1992) found that atmospheres lower in oxygen than air, reduced growth of the bacterium at low temperatures, but were not lethal. Manu-Tawiah et al. (1993) determined that atmospheres containing CO₂ concentrations normally used for commercial packaging of fresh red meats are unlikely to reduce the risk factor for listeriosis in ready-to-eat pork chops. Additional studies have determined that if *L. monocytogenes* is heat injured prior to incubation, exposure to oxygen will decrease its chances for recovery (Knabel et al., 1990). These findings agree with earlier researchers who theorized that severely heat injured cells acquire a strict anaerobic nature (McCord et al., 1971). Dallmier and Martin (1988) reported that catalase (CAT) and superoxide dismutase (SOD) are rapidly inactivated when the bacterium is heated. The subsequent buildup of toxic O₂ radicals due to inactivation of these enzymes converts *L. monocytogenes* into an obligate anaerobe (Knabel et al., 1990). Consequently, the use of various modified package atmospheres in combination with heat treatments to control or eliminate *L. monocytogenes* on crawfish tail meat warrants investigation.

Studies have shown that organic acids and their salts inhibit the growth of *L. monocytogenes*. Ahamad and Marth (1989) determined that citric and lactic acids, with large
dissociation constants, caused cell damage to *L. monocytogenes*. El-Shenawy and Marth (1988) observed that *L. monocytogenes* was susceptible to sorbic acid. Chung and Lee (1981) found that potassium sorbate on English sole delayed the onset of logarithmic growth of *Pseudomonas sp.* Organic acids are effective for controlling several bacteria in broth media and some food products. Continued investigation into the use of organic acids on other food products is warranted. The impact of combining organic acid treatments with other barriers, in an attempt to enhance their effectiveness in controlling *L. monocytogenes* on crawfish tail meat, remains unknown.

More specifically, Wei et al. (1991) determined that decreasing the pH of a *Listeria spp.* suspension for 2 h using 0.1 N HCl was not effective in killing the bacterium below detectible levels. Ita and Hutkins (1991) suggested that inhibition of *L. monocytogenes* by acids may not be caused by a decrease in the intracellular pH, but rather by some specific effect of undissociated acid species on metabolic or other physiological activities. The inhibitory effects of LA and other organic acids can be correlated with their dissociation constants or pKₐ values. In general, weak acids having higher pKₐ values are thought to be more inhibitory to *L. monocytogenes* at a given pH than strong acids at the same pH (Ita and Hutkins, 1991). These
studies allow the development of a working hypotheses where weak organic acids should be effective for controlling *L. monocytogenes* on crawfish tail meat.

The bacterial properties of lactic acid (LA) are well documented (Ingram et al., 1956; Van Netten and Mossel, 1980; Woolthuis and Smulders, 1985). Ockerman et al. (1974) found that spraying 12% LA on hot sheep carcasses reduced the surface aerobic colony counts by about $1 \log_{10}$. Similar reductions with only 1.0% LA on hot beef carcasses were achieved by Snijders et al. (1979). Woolthuis and Smulders (1985) determined that 1.25% LA solutions resulted in noticeable decontamination of calf carcasses. Ahamad and Marth (1989) observed that tryptose broth acidified with 0.3 or 0.5% LA was lethal to *L. monocytogenes* at 7, 13, 21, and 35°C. They also found the rate of inactivation to be much greater at 35°C than at lower temperatures. Considering the previous research relating to the use of LA to control *L. monocytogenes* on red meat carcasses, LA treatment on crawfish tail meat might prove effective for controlling the bacterium.

Roberts (1989) noted that except where one or two factors limit microbial growth, our understanding of the relative contributions of multiple factors to give safe and shelf-stable food products is surprisingly poor. As a result, there is an inadequate data base with which to
develop microbiologically stable and safe foods that have a long shelf life. Roberts (1989) stated that there is an urgent need for a better understanding of the effects that combinations of factors have on microbial growth and survival in foods. Chung et al. (1982) found that the presence of an organic acid magnified the effect of heat injury of several microorganisms isolated from seafood. Thus heat treatments in combination with an organic acid could form the basis of a multifactorial (multibarrier) processing technique to improve crawfish tail meat safety.

The overall objective of the present study was to determine the impact of heat, lactic acid, and various gasses when used independently and in combination against L. monocytogenes in cooked crawfish tail meat. A multibARRIER approach was postulated to more aggressively destroy L. monocytogenes; however, such methodology could not be considered independent of desirable sensory qualities of crawfish tail meat. The specific objectives of this work were:

1. To determine the generation time for L. monocytogenes on crawfish tail meat at 0, 4, 6, and 12°C.
2. To develop and evaluate the effect of several thermal destruction methods used for D value determination of L. monocytogenes on crawfish tail meat.
3. To determine the lethal effects of heat, lactic acid and modified atmosphere packaging (MAP) applied both separately and in combination on *L. monocytogenes* on crawfish tail meat.

4. To determine the effects resulting from process deviations of lactic acid in combination with MAP on survivability of heat injured *L. monocytogenes* at 4°C on crawfish tail meat.

5. To measure the sensory effect of lactic acid during refrigerated storage of crawfish tail meat.
General Microbiology of *L. monocytogenes*

*L. monocytogenes* is a foodborne pathogen that infects humans, domesticated animals, wildlife and various aquatic species. It is considered an environmental contaminant widely present in plant, soil, and surface water samples (Moore, 1988; Weis and Seeliger, 1975). It also has been found in silage, sewage, slaughterhouse solid waste, effluent of abattoirs and poultry packing plants, milk of normal and mastitic cows, pond reared rainbow trout, and human and animal feces (Gray and Killinger, 1966; McCarthy, 1990; Watkins and Sleath, 1981). Because of environmental preferences of the bacterium, both pond raised and wild stock crawfish (Crayfish, *Procambarus clarkii*) are natural hosts.

The bacterium was first described by Murray et al. (1926). The pathogenic form of *L. monocytogenes* is a smooth, small gram-positive, nonsporeforming, facultatively anaerobic, non-acid fast, diphtheroid-like rod with rounded ends measuring 1.0 to 2.0 microns by 0.5 microns (Gray and Killinger, 1966). It is catalase positive and oxidase negative and expresses a beta-hemolysin which produces zones of clearing on blood agar (Farber and Peterkin, 1991). The bacterium possesses peritrichous flagella, which give it a
characteristic tumbling motility, occurring only in a narrow temperature range. When grown at temperatures between 20 and 25°C, flagellin is both produced and assembled at the cell surface, but at 37°C flagellin production is markedly reduced (Peel et al., 1988). Colonies of the bacterium demonstrate a characteristic blue-green sheen under obliquely transmitted light (Henry, 1933).

Infection Characteristics

In humans, the greatest impact of *L. monocytogenes* is to the elderly, immunosuppressed, and to pregnant women and their fetuses. The symptoms of the resulting infection, listeriosis, include meningitis, central nervous system infection, stillbirths, abortions, premature labor and septicemia (Health and Welfare, 1990). There are, however, instances when apparently normal healthy individuals have become ill with listeriosis in both foodborne epidemics (Schultz, 1945) and sporadic cases (Azadian et al., 1989). Most cases of human listeriosis appear to be sporadic, although a portion of these cases may be previously unrecognized common-source clusters (Ciesielski et al., 1988).

Serovar 4b of *L. monocytogenes* is the most virulent in human and animal disease. This serovar is only rarely isolated from foodstuffs, in contrast to the more common
serovars 1/2a and 1/2c (Menudier et al., 1991). The existence of avirulent serovars 1/2a strains may account for the infrequent involvement of this serovar in human disease. Serovar 1/2c has been found to be less virulent than serovar 4b, which may help explain its relatively low involvement in human listeriosis (McLauchlin, 1990).

The infective route of orally ingested cells appears to be via the Peyer patches of the intestine and then into phagocytes, passing into the mesenteric lymph nodes by way of the lymphatic pathways (Farber and Peterkin, 1991). Mice infected orally have shown variable responses, with 50% infectious doses ranging from $1.7 \times 10^3$ to $9.9 \times 10^6$/g cells (Audurier et al., 1980; Golnazarian et al., 1989). Other studies on oral feeding of mice with *L. monocytogenes* have shown that very high ($\geq 2.5 \times 10^8$/g cells) numbers are required to cause invasion of the Peyer's patches or death in normal mice (MacDonald and Carter, 1980). Miller and Burns (1970) found that a dose of $\geq 4 \times 10^7$/g cells caused fetal death in 6 of 10 pregnant mice. Feeding trials in which nonhuman primates were used showed that only animals receiving a dose of $10^9$/g *L. monocytogenes* cells became noticeably ill. Smaller numbers of cells did not result in noticeable symptoms of disease (Farber et al., 1990). The minimum number of pathogenic *L. monocytogenes* that must be ingested by humans to cause illness in either normal or
susceptible individuals is not known. Farber & Peterkin (1991) compiled a list of cases in which the numbers causing illness were approximated for humans. In most of these cases the approximated number of cells that caused illness was $10^6/g$ of food.

**Foodborne Listeriosis**

Not until the late 1970's was foodborne listeriosis documented in North America and Europe. In 1986 the Council of State and Territorial Epidemiologists recommended that listeriosis become a reportable disease in the United States (Gellin and Broome, 1989). But as early as 1979, the first outbreak of listeriosis occurred in Boston. Lettuce, celery and tomatoes were implicated and 23 cases were reported with 5 fatalities (Ho et al., 1986). Coleslaw was the cause of a listeriosis outbreak in Nova Scotia in 1981 resulting in 18 deaths from 41 cases (Schlech et al., 1983). Pasteurized milk was implicated as the transmitter of a listeriosis outbreak in Massachusetts during 1983, where 49 cases were reported with 14 fatalities (Fleming et al., 1985). The largest outbreak occurred in southern California in 1985 where 142 cases were reported causing 48 deaths and was traced to Mexican style cheese (Linnan et al., 1988). Farber and Losos (1988) reported that all of these outbreaks were caused by *L. monocytogenes* serotype 4b.
In 1980 an epidemic of perinatal listeriosis in New Zealand suggested a possible link to the consumption of shellfish and raw fish, resulting in 22 reported cases with 5 deaths (Lennon et al., 1984). To date, there have been no reported cases of listeriosis from the consumption of crawfish. However, since crawfish are crustaceans that are grown in static pond water, typically high in detritus loads, the potential for contamination of hand picked crawfish tail meat is omnipresent.

Presence in Seafood

Listeria is considered an environmental contaminant and can be found in waters of rivers, lakes, ponds and canals (Moore, 1988; Watkins & Sleath, 1981). In 1966, Gray and Killinger reported L. monocytogenes to be present in pond raised rainbow trout. Bracket (1988) hypothesized a cycle of infection for Listeria indicating that contaminated water infects fish and shellfish which in turn infect humans through consumption of the contaminated food product. Since crawfish are boiled as part of the peeling process, the packaged tail meat is considered cooked. Cross contamination during peeling is a possible avenue of product inoculation with L. monocytogenes. It is conceivable that consumers might mistakenly consider crawfish tail meat
ready-to-eat from the retail package, resulting in a possible infection with the bacterium in consumers.

A Class I recall was made by the U.S. Food and Drug Administration (USFDA) on vacuum and air packed smoked shad because of the presence of L. monocytogenes (Anonymous, 1992). Several surveys have detected various levels of Listeria spp. in seafood products from retail markets. Jemmi (1990) detected L. monocytogenes in 12% of smoked and marinated fish from 377 samples tested. Frozen seafood products such as shrimp, crabmeat, lobster, langostinos, scallops, squid, and surimi have been shown to contain L. monocytogenes in samples obtained from several different countries (Weagant et al., 1988). The study found that 26% of the products sampled contained L. monocytogenes. Fuchs and Sirvas (1991) surveyed the incidence of Listeria in ceviche products bought from markets in Lima and Callao, Peru. Approximately, 75% of the samples they tested contained Listeria innocua and 9% contained L. monocytogenes.

Noah et al. (1991) isolated Listeria from frozen lobster tails, shrimp, prawn, breaded shrimp, whole fish, and fish fillets. Of 211 composites, 28% were positive for Listeria spp. Wong et al. (1990) conducted a local market survey in Taiwan and found L. monocytogenes in 10.5% of seafoods containing fish and squid. Farber (1991) found 13%
of ready-to-eat shrimp, crab, and salmon at the wholesale level in the U.S.A., Britain and Taiwan to be positive for *L. monocytogenes*. No surveys conducted to date have included peeled crawfish tail meat. Since live crawfish are raised in a potentially hazardous environment, it would warrant the inclusion of this product in future surveys for *L. monocytogenes*.

**Thermal Tolerance**

Following the 1983 Massachusetts outbreak of listeriosis from pasteurized milk, the thermal tolerance of *L. monocytogenes* was questioned (Fleming et al., 1985). Since this time, Bradshaw et al. (1985) determined the $D_{63.3}$ value to range from 13.4 to 28.4 s. Donnelly et al. (1987) did similar studies and calculated $D_{62}$ values to range between 6 and 24 s. The suggestion was made that an intracellular positioning of the bacterium provided a protective milieu (Bunning et al., 1988). However, after an extensive study involving sterile whole milk in which *L. monocytogenes* was either freely suspended or located within bovine milk phagocytes, Bunning et al. (1988) reported the intracellular position of the bacterium did not significantly increase thermal resistance.

Recent studies conducted on thermal resistance of *L. monocytogenes* in red meats have resulted in several
interesting observations. The addition of beef fat does not enhance the heat resistance of the bacterium, but the presence of curing salt substantially increases it (Farber et al., 1989b; Mackey et al., 1990). However, work by Fain et al. (1991) with *L. monocytogenes* in lean and fatty turkey meat indicated that the bacterium was more resistant to heat when in fatty meat than in lean turkey meat. Crawfish tail meat is typically packaged with fat remaining. Since the present state of knowledge is inconclusive, the presence of fat in crawfish tail meat should be a consideration when conducting any thermal studies with *L. monocytogenes* on crawfish tail meat.

Additionally, the heat shock phenomenon must be taken into account when heating solids that heat much slower than liquids. Heat shock in this case would be defined as any sublethal exposure to heat that alters the ability of a bacterium to function or survive normally. Liquids, which heat rapidly, do not allow bacteria acclimation time to temperature induced stresses as do solids, in which some limited survival may result from a bacterial adjustment to environmental changes. This is especially true for meats, which heat slowly to a final internal temperature (Farber and Brown, 1990) as is the case for 1 lb crawfish tail meat packages.
Little research has been performed to determine the thermal resistance of *L. monocytogenes* in seafoods. Thermal death times for *L. monocytogenes* in crab meat and lobster meat have been evaluated (Budu-Amoako et al., 1992; Harrison and Huang, 1990). No research has been conducted to determine the effect of heat on the bacterium on crawfish tail meat. Harrison and Huang (1990) determined D values at 55 and 60°C to be 12.00 and 2.61 min, respectively for crab meat. Budu-Amoako et al. (1992) determined the D$_{60}$ value on lobster for *L. monocytogenes* was 2.39 min. Interestingly, Harrison and Huang's (1990) D$_{55-60}$ values ranged from 40.43 to 2.61 min when using non selective tryptic soy agar (TSA) as the plating medium, but when using a selective medium, modified Vogel-Johnson agar, the D$_{55-60}$ values were reduced to 34.48 to 1.31 min. This implies that injured cells may have been inhibited on the selective medium.

McCarthy et al. (1990) tested the recovery of heat stressed *L. monocytogenes* from artificially and naturally contaminated shrimp. No *L. monocytogenes* were recovered from naturally contaminated shrimp boiled at 1, 3, or 5 min. However, *L. monocytogenes* were detected in experimentally internally-infected shrimp when boiled up to 5 min. This study also revealed the affects of freezing on heat stressed cells in frozen cooked shrimp. They found no *L. monocytogenes* cells survived the cook-freeze-thaw process.
This indicates that the combination effect of the multi-barriers, heat then cold, can be lethal to the bacterium. The increase in lethality observed by McCarthy et al. (1990) resulting from exposure of the bacterium to multi-barriers does not always hold for other combinations, however. Pickett and Murano (1993) determined that heat shock did not result in any significant difference in survival of *L. monocytogenes* when incubated at 37°C and exposed to the minimum inhibitory concentrations of chlorine-base, iodophor, phosphoric acid, or quaternary ammonium compounds or citric, lactic, or propionic acids. The addition of a third barrier, however, could possibly change the effect of any one barrier, a facet the present study will investigate.

**Affects of Organic Acids on *L. monocytogenes***

*L. monocytogenes* is more acid tolerant than most foodborne pathogens and can grow or survive in broth media with pH values as low as 4.3-5.2 (Ahamad and Marth, 1989; Conner et al. 1986; El-Shenaway and Marth, 1988; Farber et al., 1989a; Parish & Higgins, 1989). Studies by Ahamad and Marth (1989) and Farber et al. (1989) indicated that the effects of various organic acids differ. These studies determined that acetic acid is more detrimental to *Listeria spp.* than either lactic, citric or hydrochloric acid. Acetic acid acts mainly by lowering the pH of the
environment to the point where microorganisms can no longer grow (Liewen and Marth, 1985). Although the undissociated form of acetic acid has antimicrobial action, it is primarily the hydrogen ion and the resulting decrease in pH that inhibits microorganisms (Cowles, 1941; Levine & Fellers, 1939). Subsequent studies by Buchanan et al. (1993) support these findings at low pH values. However, researchers determined that at higher pH values LA was more effective than acetic acid. The pH of crawfish tail meat was determined in the present study to be 7.6, supporting the use of LA rather than acetic acid, as a controlling agent for L. monocytogenes. Also the rate at which lactic and acetic acids inactivate L. monocytogenes is dependent on the identity of the acid, the concentration of the acid, and the pH of the system.

The inhibitory effects of citric, lactic and sorbic acids can be correlated with their dissociation constants or pKₐ values (Ita and Hutkins, 1991). In general, weak acids having higher pKₐ values are thought to be more inhibitory to L. monocytogenes, at a given pH, than strong acids, at the same pH (Ita and Hutkins, 1991). The amount of the molecule in the undissociated form is determined by pH. As a result, this along with solubility properties, determines the foods in which these acids are most effective (Ray and Bullerman, 1982). For example the antimicrobial
effectiveness of sorbic acid increases as the pH value approaches its pKₐ value of 4.75, the upper pH limit for activity being 6.0-6.5.

Many weak acids, in their undissociated or protonated form, have the ability to penetrate the cell membrane and accumulate within the cell cytoplasm (Ita and Hutkins, 1991). If the interior of the cell is more alkaline than the pKₐ of the acid, more of the acid will dissociate, releasing a proton and acidifying the cytoplasm of the cell (Booth, 1985). These events are thought to result in a variety of detrimental effects (Kashket, 1987). As a defense, many bacteria possess proton pumps or proton/cation exchange systems to deal with the influx of protons and to maintain the cytoplasm near neutral. However, if these pH regulatory systems are unable to function sufficiently (i.e., if the proton concentration is too great), then the pH gradient (the difference between the intracellular and the extracellular pH) will collapse. Intracellular acidification will then result in loss of cell viability or cell destruction. The acidified cell is forced to consume a great deal of energy in an attempt to maintain pH homeostasis. It has been further suggested that the internal or cytoplasmic pH is the relevant pH which ultimately affects cell metabolic activities (Booth, 1985).
It is also established that undissociated and uncharged weak lipophilic acids, such as acetic acid, are permeable through the cell membrane (Kashket, 1987). In contrast, lactic and citric acids are generally unable to passively diffuse across cell membranes; uptake of these acids by cells is instead thought to be carrier mediated (Kashket, 1987). Ita and Hutkins (1991) determined that acetic acid ($pK_a = 4.76$) accumulated in the cells of $L.\ monocytogenes$ at a higher concentration than lactic ($pK_a = 3.86$) or citric ($pK_a = 3.13$) acid. Their work was consistent with other reports showing that $L.\ monocytogenes$ was most inhibited by acetic acid, followed by lactic then citric acid (Ahamad & Marth, 1989; Sorrells et al., 1989; Young & Foegeding, 1992). Since organic acids have been shown effective for inhibiting the growth of $L.\ monocytogenes$, studies that would evaluate their effectiveness as one part in a system of barriers to control the bacterium would be of interest.

**Citric Acid**

Citric acid (CA) (molecular wt 192.12) is generally recognized as safe (GRAS) for use in foods under FDA regulations (Food & Drug Admin., 1990). Thus no upper limits are imposed for foods not covered by Federal Standards of Identity. CA is presently used in the seafood
industry primarily for flavor enhancement and to prevent discoloration.

Because CA is a weak acid having a higher dissociation constant ($pK_a = 3.13$) than strong acids, its ability to inhibit the growth of $L. monocytogenes$ has been studied. Ahamad and Marth (1989) determined that citric and lactic acids, with smaller $pK_a$'s, were less detrimental to $L. monocytogenes$ than was acetic acid. In subsequent studies done by Ahamad and Marth (1990) it was found that even though acetic acid caused greater inactivation of $L. monocytogenes$, CA caused the greatest degree of injury followed by lactic and acetic acid. CA treated cells incurred more initial inactivation than the other two acids, however eventually acetic acid produced more total cell inactivation.

Ita and Hutkins (1991) observed that both citric and lactic acids were more effective in lowering intracellular pH of the pathogen than acetic acid. When citric and lactic acid-treated cells at pH values of 3.5, 4.0, and 4.5 were incubated for 24 h, both the pH gradient (intracellular pH-external pH) and the intracellular pH values decreased. They noted, however, that although the total amount of added LA reached 160 mM, the amount of undissociated acid was less than 50 mM ($pK_a$ of lactic acid = 3.86).
Lactic Acid

LA (molecular wt 90.08) is listed as GRAS in the United States (Food and Drug Admin., 1990). Similarly, in Europe it is considered a harmless constituent of food (Lueck, 1980). The bacterial properties of LA are well documented (Ingram et al., 1956; Van Netten and Mossel, 1980; Woolthuis and Smulders, 1985).

Ryser and Marth (1988) studied the effect of acetic and lactic acid on *L. monocytogenes* in cold-packed cheese. They observed the degree of dissociation related to the pKₐ of acidic (4.76) and lactic acid (3.86). They noted that at pH 5.0, 36.0 and 5.9% of acetic and lactic acid, respectively, will be undissociated. Therefore, the higher proportion of undissociated acetic as compared to LA in cheese food at pH 5.0 would most likely account for the increased capability of acetic acid to decrease a viable *Listeria* population. Buchanan, et al. (1993) determined that at pH levels ≤6.0 lactic acid was a more effective inactivator of the bacterium than acetic acid. Models produced from their studies also indicated that LA was more effective than acetic acid for inactivating the bacterium when expressed in terms of concentration of undissociated acid.

Ockerman et al. (1974) found that spraying 12% LA on hot sheep carcasses reduced the surface aerobic colony counts by about 1 log₁₀ CFU/cm² and did not bleach the meat.
Smijders et al. (1979) achieved similar reductions with only 1% LA on hot beef carcasses, however they reported that some bleaching of product did occur. Woolthuis and Smulders (1985) determined that LA solutions of 1.25% exhibited bactericidal qualities. When measured 24 h postmortem, aerobic colony counts (3 d, 30°C) were reduced as much as 1.3 log_{10} CFU/cm² on calf carcasses. Additionally, 1.25% LA solutions did not produce commercially unacceptable calf carcass discoloration.

Ahamad and Marth (1989) observed that in tryptose broth (TB) acidified with 0.05% LA, *L. monocytogenes* proliferated under a wide range of temperatures (7, 13, 21, 35°C). This also was the case when 0.1% LA was employed except at 7°C where lag times were longer. They further determined that strain V7 of *L. monocytogenes* showed more resistance to LA than strain CA. In addition, the same study indicated that while no growth of the bacterium occurred when acid concentration was 0.2%, it did survive. At 0.3 and 0.5%, LA was lethal to the bacterium. The rate of inactivation was much greater at 35°C than at lower temperatures. The D-value of 0.3% citric acid (49.5 h) was also determined to be higher than that of 0.3% LA (29.53 h) at 35°C. At 0.5% acid the D-value for citric was 32.47 h and lactic was 14.6 h at the same temperature.
Bruno and Montville (1993) determined the mechanisms by which bacteriocins from lactic acid bacteria inhibit *L. monocytogenes*. They found that the dissipation of the proton motive force (PMF) is accomplished by pediocin PA-1, AcH, leuconocin S and nisin. These antimicrobial proteins are classified as colicins and human defensins. They are characterized according to their energy-dependent or energy-independent mode of PMF depletion. They determined that pediocin PA-1, AcH and leuconocin S dissipated PMF in an energy-independent fashion, whereas action of nisin was energy-dependent. Morille et al. (1993) determined that nisin in a neutral-pH pudding product was limited as an antilisterial agent. However, in systems acidified to pH ≥5.0, nisin inhibited the growth of the bacterium at 6, 25, and 37°C.

**Sorbic Acid**

Sorbic acid (pKₐ = 4.75) is GRAS under regulations of the U.S. FDA (Food & Drug Admin., 1990). As a result no upper limits are imposed for foods not covered by Federal Standards of Identity. In natural cheese that has a standard of identity for example, the maximum quantity of sorbic acid may not exceed 0.3% by weight. The upper limits are normally set at 0.1 and 0.2% in the U.S. in foods.
covered by Federal Standards of Identity (Liewen & Marth, 1985).

Sorbic acid is among the most thoroughly studied food preservatives. Since sorbic acid is only slightly soluble in water it is commonly solubilized with sodium or potassium hydroxide. The most widely used forms are sorbic acid and its potassium salt. These compounds are collectively known as sorbates (Liewen and Marth, 1985). It has been found non-toxic in numerous acute, subchronic and chronic toxicity tests (Gaunt et al., 1975; Luck, 1976). Sorbic acid is metabolized in the body like other fatty acids and has a half-life of 40-110 min, depending on the dosage (Liewen & Marth, 1985). As a result, sorbic acid, which occurs naturally in mountain ash berries or can be chemically synthesized (Windholz et al., 1976), is used as an antimicrobial agent in a variety of foods (Sofos and Busta, 1981). The acid serves to extend the storage life of such products as butter, cheese, meats, cereals and bakery items (Anonymous, 1979; Kaul et al., 1979), some fruits, berry products, vegetable products and other foods (Anonymous, 1978; Moline et al., 1963). The effectiveness of sorbic acid against various microorganisms has been reviewed (Liewen & Marth, 1985; Sofos & Busta, 1981).

Work by Ryser and Marth (1988) showed that L. monocytogenes was inactivated more rapidly in cold-pack
cheese food containing 0.3% sorbic acid than when it was absent. El-Shenawy and Marth (1988) observed the effect of sorbic acid on *L. monocytogenes* in tryptose broth supplemented with potassium sorbate ranging from 0 to 0.3%. They adjusted the pH of the broth to 5.6 or 5.0 and incubated it at several temperatures ranging from 4 to 35°C. The bacterium grew in potassium sorbate-free controls under all conditions except 4°C and pH 5.0. In all treatment combinations, as percent potassium sorbate increased, inhibition of the bacterium increased. This indicates an inhibitory effect of sorbic acid toward the bacterium. The study also indicates that the behavior of the bacterium in the presence of different concentrations of potassium sorbate is affected by incubation temperature and pH of the substrate.

Chung and Lee (1981) noted that potassium sorbate delayed the onset of microbial logarithmic growth in English sole but did not alter the rate of microbial growth nor ultimate predominance by *Pseudomonas sp.* in fish stored at 1.1°C. Other microorganisms such as *Flavobacterium-Cytophaga sp.*, *Arthrobacter*, *Acinetobacter*, and *Staphylococcus* disappeared from samples treated with sorbic acid during 1.1°C storage. Chung and Lee (1982) determined the effect of potassium sorbate on sub-lethally injured bacteria found on English sole. They found that even though...
Pseudomonas sp. was relatively resistant to potassium sorbate and was not affected by 0.3% potassium sorbate after freeze-thaw treatment, it showed delayed onset of logarithmic growth for up to 20 h after heating at 50°C for 5 min.

The process by which sorbate inhibits microbial growth is not clear and a variety of mechanisms are probably involved in its antimicrobial action (Liewen and Marth, 1985). Melnick et al. (1954) proposed that sorbic acid inhibits certain dehydrogenase enzymes which are involved in the β-oxidation of fatty acids. York and Vaughn (1964) proposed that sorbic acid uncouples oxidative phosphorylation by inhibiting enzymes within the cell. Troller (1965) proposed that the inhibitory action of sorbate resulted from inhibiting catalase, which would cause an increase of toxic hydrogen peroxide within the cell. Freese et al. (1973) have proposed that sorbate prevents microbial growth by inhibiting the transport of substrate molecules into cells. Yousef and Marth (1983) concluded that sorbate inhibited aflatoxin biosynthesis in the same manner. Inhibition of transport of carbohydrates has also been suggested as the inhibitory mechanism of sorbic acid (Deak and Novak, 1972).

Several investigators have shown that CO₂ and sorbate in combination will effectively inhibit microorganisms.
Elliot et al. (1981, 1982) showed that CO₂ and sorbate acting synergistically inhibited *Salmonella enteriditis* and *Staphylococcus aureus*. Carbon dioxide and sorbate will inhibit mold spoilage of grains (Danzinger et al., 1973). Sorbate will delay the growth of psychrotrophic bacteria in vacuum-packed pork loins (Myers et al., 1983). Since sorbates have proven successful for controlling many different bacteria in a variety of foods, its effectiveness in controlling *L. monocytogenes* in crawfish tail meat should be evaluated.

**Modified Atmosphere Packaging (MAP)**

The use of MAP to extend shelf-life of foods and suppress the growth of bacteria has been studied extensively (Gill and Tan, 1979; Hintlian and Hotchkiss, 1986). Killeffer (1930) conducted some of the earliest studies with CO₂ and seafood products. His work with cod was later corroborated by Coyne (1932, 1933) using fish held under high levels of CO₂ and by Stansby and Griffiths (1935) who worked with haddock. Several subsequent studies have been conducted showing the beneficial effects of MAP to extend the shelf life of fresh fish (Gray et al., 1983; Mokhele et al. 1983; Molin et al., 1983). Przybylski et al. (1989) noted that while microbiological counts for catfish packaged in CO₂/air (80:20) atmosphere were significantly lower than
those packaged in 100% air, growth did progress. Other researchers have observed reductions in microbiological levels of muscle products packaged in various CO₂ atmospheres (Banks et al., 1980; Mokhele et al., 1983; Parkins et al., 1981; Wang & Ogrydziak, 1986).

Studies using CO₂ dissolved in refrigerated brines improved quality of rockfish and chum (Barnett et al., 1971), silver hake (Hiltz et al., 1976), and pink shrimp (Bullard and Collins, 1978; Barnett et al., 1978). Brown et al. (1980) demonstrated that rockfish and silver salmon stored at 4.5°C, under high levels of CO₂, had lower microbial counts and less undesirable sensory changes than controls. They also determined that levels of trimethylamine (TMA) and ammonia were markedly lower in samples held in atmospheres containing 20 or 40% CO₂. Gerdes et al. (1989) and Wang and Brown (1980) observed these same trends for TMA and ammonia in crawfish held under atmospheres of high CO₂.

Although MAP has been shown to suppress the growth of spoilage bacteria the subsequent increased potential for the growth of several pathogenic bacteria, such as *L. monocytogenes*, has been questioned (LaBell, 1986). Wei et al. (1991) determined that *L. monocytogenes* suspended in distilled water was completely killed after CO₂ treatments at 6.18 MPa (61.2 atm) and 35°C for 2 h. They observed a
similar affect of CO₂ on L. monocytogenes in spiked shrimp. Garcia et al. (1987) and Garcia and Genigeorgis (1987) determined that when salmon fillets were stored at 4°C, under several modified atmospheres (vacuum, 100% CO₂, and 70% CO₂+30% air), Clostridium botulinum did not produce detectable toxin for 60 d. Toxin detection coincided with spoilage at 30°C, but preceded spoilage at 8 and 12°C.

In studies done by Marshall et al. (1991, 1992), L. monocytogenes on chicken was moderately inhibited under refrigeration temperatures by MAP (76 and 80% CO₂) compared with storage in air. They also observed that growth was not completely retarded nor was the organism killed by the applied storage environments.

Kallander et al. (1991) determined that spiked cabbage stored at 25°C under 70% CO₂ yielded a slightly lower count (>1 log/g) of L. monocytogenes after 24 h of storage than did cabbage stored in normal atmosphere (>2 logs/g). They also observed that during subsequent storage at 25°C, L. monocytogenes counts decreased to undetectable levels (<20 CFU/g) at 6 d of storage under both atmospheres. The decline, however, was more dramatic for the cabbage in modified than in normal atmosphere between days 3 and 4 of storage.

Ingham (1990) working with crawfish determined under MAP (80% CO₂/balanced propriety) and refrigeration, growth
of *Aeromaonas hydrophila* and *Plesiomonas shigelloides* was effectively inhibited. Since researchers have used MAP to successfully control *L. monocytogenes* in various muscle foods and MAP has proved inhibitory to pathogens found on crawfish, its application to control *L. monocytogenes* on crawfish warrants investigation.
MATERIALS AND METHODS

Sample Preparation

For all studies conducted, frozen crawfish tail meat (*Procambarus clarkii*) was purchased from local suppliers, in one pound heat sealed retail packs. The frozen meat was stored in these retail packs at -10°C for less than 6 months until needed.

Before using, the frozen package was thawed under refrigeration for 24 h. After thawing, a mercury-in-glass thermometer was placed in the geometric center of the package through the top of the bag. The bag was then submerged into boiling water and heated to a core temperature of 90°C (approximately 10 min) to reduce background microflora. The package of crawfish meat was immediately removed and placed into an ice bath until a core temperature of approximately 20°C was achieved.

Preparation of Inocula

*L. monocytogenes* strains Scott A (Economics Laboratories, St. Paul, MN), F5069, and S4b (C. Donnelly, Univ. of Vermont, Burlington, VT) were maintained as stock cultures through monthly transfers on trypticase soy 0.6% yeast extract agar slants (TSYEa) (BBL Microbiology Systems, Cockeysville, MD) and stored at 4°C. All *L. monocytogenes*
cultures were verified to species using the API *Listeria* system (bioMérieux, La Balme-les-Grottes, France). This system consists of the following 10 tests to differentiate between *L. innocua* and *L. monocytogenes*: presence or absence of arylamidase (DIM test), hydrolysis of esculin, presence of α-mannosidase, and acid production from D-arabitol, D-xylose, L-rhamnose, α-methyl-D-glucoside, D-ribose, glucose-1-phosphate, and D-tagatose. A study conducted by Bille et al. (1992) determined that with this system, 97.7% (252 of 258) of the *L. monocytogenes* strains tested were correctly identified and differentiated from 99.4% (175 of 176) of the *L. innocua* strains tested. Other gram-positive genera included by Bille et al. (1992) were *Enterococcus* spp., *Lactobacillus* spp., *Carnobacterium* spp., *Erysipelothrix* spp., *Oerskovia* spp., *Corynebacterium* spp., and *Rhodococcus* spp. The 3 biochemical characteristics that are positive for *Listeria* isolates include: hydrolysis of esculin and acid production from D-arabitol and α-methyl-D-glucoside. These tests easily eliminate the above listed non-*Listeria* cultures.

Individual strains of *L. monocytogenes* were subcultured by quiescent incubation for 20-22 h in trypticase soy 0.6% yeast extract broth at 30°C. Ten mL of each subcultured strain were mixed into a sterile centrifuge tube. The mixed strain cells were harvested by centrifugation (3000 X g) for
10 min at 4°C (RC5C, Sorval Instruments, Norwalk, CT), washed twice in sterile Butterfield Phosphate Buffer (BPB), and resuspended to give desired target levels. Samples were spread plated on TSA to verify target levels. It is known that a particular species of bacterium grown strictly under the same conditions will always reach approximately the same maximum number of viable cells in a given time period (Nickerson and Sinskey, 1972). Using this established criterium, subsequent inoculation target levels for all experiments were successfully replicated under the same strict conditions.

Thermal Inactivation Studies

Three-neck Flask Method (TNF)

Sample preparation

The first method employed to determine the lethal effects of heat on *L. monocytogenes* was TNF, as described by the Food Processors Institute (1988). For this method 25 g of heat treated crawfish tail meat was placed into a sterile stomacher bag with approximately 225 mL of sterile BPB to make a 1:10 dilution. The sample was blended for 2 min using a stomacher (Model STO, Tekmar, Cincinnati, OH) and aseptically transferred to a 3 neck 1000 mL sterile reaction flask.
Thermal treatment

An initial uninoculated sample was taken from the flask to verify product sterility. The flask containing the sample was secured into a swirling water bath that was maintained at the targeted study temperature. Twenty-five mL of a mixed cell suspension of approximately $10^9$ cells per mL, were aseptically pipetted into the sample slurry. The flask was swirled in the water bath, allowing the sample slurry and inoculum to mix. When the sample reached the study temperature a zero time sample was taken. Sample temperature was monitored using a high precision mercury-in-glass thermometer (Sargent-Welch, Skokie, IL) placed from the top of the flask into the middle of the sample slurry. The sample slurry remained in motion and completely immersed during the heat treatment. The swirling motion was stopped for several seconds during each sampling period. Five mL aliquots from all 3 test temperatures (55, 60, and 65°C) were removed at designated time intervals and placed into sterile tubes. Tubes were rapidly cooled by swirling in an ice slurry, then iced for up to 1 h until analyzed.

Enumeration of L. monocytogenes

After cooling, 1-mL portions of iced samples were transferred to 9 mL sterile BPB diluent and mixed thoroughly. The resulting 1:10 dilution was further
serially diluted as required. Enumeration of the *L. monocytogenes* mix as conducted by spread plating 0.1 ml dilutions on Tryptic Soy Agar (TSA) modified with 0.6% yeast extract and 1.0% sodium pyruvate (Fain et al., 1991; Smith and Hunter, 1988). Plates were allowed to stand for 4 h at ambient temperature (approximately 25°C) after spread plating to enable recovery of injured cells (Smith, 1990). After this period, approximately 10 mL of Listeria Oxford Agar (LOA) (Unipath Limited, Hampshire, England) was used as an overlay. A study by Xavier and Ingham (1993) suggest that when determining the survival of facultatively anaerobic micro-organisms in foods that have been subjected to heat-shock, an anaerobic enumeration method produces higher counts. Their work showed that an overlay method could be used as an alternative to a total anaerobic enumeration method.

After being overlaid, plates were incubated at 27°C for 48 h before enumeration. *L. monocytogenes* were enumerated using standard microbial count methods (Messer et al., 1985).

*D value determination*

A thermal death time (TDT) plot was established by plotting log$_{10}$ number of survivors against time for each heating temperature (Nickerson and Sinskey, 1972). The time
required to bring about a 90% reduction in the number of survivors (i.e. the D value for each treatment) was calculated from the slope of the best fit line representing data on the TDT plot. The log_{10} of D value (min) was plotted versus temperature (°C) to determine the z value. Lotus Freelance 3.01 Software (Lotus Corp., Orem, UT) was used to determine slope, y intercept and goodness of fit values (e.g., R^2) for the best fit line by least squares linear regression of the thermal death time (TDT) plot.

**Polyethylene Bag Method (PB)**

*Sample inoculation*

The second method employed 3-mil polyethylene bags (PB) (Koch, Kansas City, MO) that have a gas transmission rate of 9 cc/m^2/24 h at 0°C. This type of PB is normally used to commercially pack crawfish meat. For this method, samples were inoculated by blending 1 mL of the mixed culture (10^9 cells per mL) with 100 g of crawfish tail meat, prepared as described previously, in a Waring blender to produce a homologous composite sample. Inoculation levels of approximately 10^7 CFU/g were consistently achieved. Two separate 100 g inoculated samples were aseptically transferred to a 4000 mL collection beaker then hand mixed under a laminar flow hood (Labconco, Kansas City, MO) for 5
min, using a sterile spatula. At least 2 10-g subsamples of this homogeneous composite sample had *L. monocytogenes* enumerated to verify beginning inoculation levels. Inoculated samples were either left untreated or spray treated with LA as described in the following procedure. Treatment combinations are given in Table (1).

*Preparation & application of lactic acid*

A stock solution of 10% LA was prepared and filter sterilized. Appropriate amounts of the stock solution to produce desired concentrations of 0.5, 0.75, or 1.0% w/w were sprayed onto inoculated crawfish using an autoclavable, forced air, clinical atomizer. These concentrations were chosen because they cover the common range of usage for this preservative. Additionally, a preliminary sensory study indicated that the highest concentration did not significantly alter the sensorial quality of crawfish tail meat. The pH of the crawfish tail meat was measured using an Orion model 420A pH meter (Orion Research Inc., Boston, MA). To determine if the effect of LA was attributable to a reduction in pH caused by the addition of LA, the pH of additional crawfish samples was adjusted using 10% HCl to values approximating that of 0.5 and 1% LA. Approximately 3.5 and 7.5 mL of HCl per 100 g of crawfish meat was required to achieve pH values of 6.5 and 5.4, respectively.
Table 1. Treatment combinations for inoculated crawfish samples

<table>
<thead>
<tr>
<th>GAS TYPE</th>
<th>TREATMENT °C</th>
<th>CONCENTRATION % LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIR</td>
<td>60</td>
<td>0.00</td>
</tr>
<tr>
<td>100% CO₂</td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>100% O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% N₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIR</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>100% CO₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% N₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Treated crawfish were segregated into 10-g samples and placed into polyethylene bags (Figure 1). The bags were then heat sealed using a standard industrial heat sealer, model LS (Rennco, Homer, MI) after forcing as much air from the head space as possible without applying a vacuum.

Introduction of modified atmospheres

The polyethylene bags with samples were vacuum packed using a Multivac Model A300/22 vacuum-packing machine (Multivac SEPP Haggenmuller K.G., Germany) and held under vacuum for approximately 15 min. Bags were then opened and resealed using several methods. For the heating trials studying LA levels alone, bags were heat sealed with no head space using a standard industrial heat sealer, Model LS (Rennco, Homer, MI). If the sample bags were to be given a headspace of 100% air, 100% O₂, 100% CO₂, or 100% N₂ they were backflushed with the appropriate gas using the Multivac vacuum-packing machine. Verification of O₂ and CO₂ levels in the packs was done before and after heat treatments using a Servomex Food Package Analyzer, Series 1400 (Servomex, Inc., Crororborough, Sussex, England) (Table 2).

Thermal treatment and enumeration

For all thermal treatments the sample bags were clipped to a stainless steel test tube rack and completely submerged in a 60°C swirling water bath. Sample temperature was
Figure 1. Inside dimensions of polybag used in both thermal and storage studies.
Table 2. Gas composition as determined immediately after introduction to sample packs and after 60°C heat treatment

<table>
<thead>
<tr>
<th>Package Gas</th>
<th>Before heat</th>
<th>After heat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$O_2$</td>
<td>$CO_2$</td>
</tr>
<tr>
<td>Air</td>
<td>21.6</td>
<td>0.6</td>
</tr>
<tr>
<td>$O_2$</td>
<td>99.0</td>
<td>0.1</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>0.0</td>
<td>99.6</td>
</tr>
<tr>
<td>$N_2$</td>
<td>0.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>
monitored and come up time recorded (Figure 2) using thermocouples placed in the geometric center of 2 uninoculated sample bags. Sample bags remained in motion and completely immersed during the entire heat treatment. Bags were removed at designated time intervals and agitated in an ice bath for rapid cooling until analysis.

After cooling, a zero time sample was immediately taken by aseptically removing it from the heat sealed bag and placing it into a stomacher bag to which a sufficient volume of sterile BPB was added to achieve an initial dilution of 1:10. The diluted sample was homogenized by stomaching (Tekmar) for 2 min and additional decimal platings were made by removing 0.1-mL from serially diluted samples. This procedure was repeated for all samples. Enumeration of \textit{L. monocytogenes} was done as previously described.

\textit{D value determination}

\textit{D} values (min) for the polybag method were determined as described previously for the TNF method. \textit{D} value means for thermal treatments of different LA concentrations and all atmospheres were subjected to ANOVA for determination of significant differences at the 5\% probability level using Minitab Statistical Software (Minitab, Inc., PA). Student's t-test was used to quantify significant (P<0.05) differences between means of each individual analysis.
Figure 2. Center temperature for come-up and cool-down times for 10 g samples of crawfish tail meat in polybags (PB) and polybags with head space (PB-a) and cellulose casings (CC).
Cellulose Casing Method (CC)

Sample inoculation

A third heat treating method was utilized to determine a $D_{60}$ value for *L. monocytogenes* in crawfish tail meat. This method utilized 18 mm EZ Peel Nojax cellulose casings (CC) (Viskase Corp., Chicago, IL). Cellophane and cellulose casings as a vessel for ground or emulsified food products inoculated with a variety of bacteria are used extensively for $D$ value determinations (Zaika et al., 1990).

For this method crawfish tail meat was prepared and inoculated as described for the PB method. Ten g of inoculated product was aseptically stuffed, using a sterilized glass funnel, into individual casings. Casings were then sealed by tying off before being secured to test tube racks and placed into the water bath for thermal treatment. Cell enumeration and $D$ value determination was accomplished as described for the PB method. No additional treatments other than heat were applied to the crawfish tail meat using this method.

$D_{60}$ value means for all three thermal treatment methods (TNF, PB, and CC) were subjected to ANOVA for determination of significant differences at the 5% probability level using Minitab Statistical Software (Minitab, Inc. PA).
Sensory Evaluation

A trained sensory panel of 15 judges was used to evaluate all LA treatment levels. A single training session that involved group sampling of crawfish tail meat treated with known levels of LA was conducted. During the training session open discussion of any observed sensory alterations due to LA on crawfish meat was discussed among panelist. All levels of LA (0.5, 0.75 and 1.0%) that would be tested for by the taste panel were introduced to the panelist during the training session. Since these levels were relatively low and difficult to detect, samples treated with 10% LA also were used to sensitize the panel to the taste of LA in crawfish tail meat.

Uninoculated crawfish tail meat samples were spray treated with 0.0, 0.5, 0.75, and 1.0% LA then sealed in heatable polyethylene bags (Koch, Kansas City, MO). They were heat treated at 60°C for 10 min in a swirling water bath as described previously. Samples were tested immediately after treatment then held at 4°C and tested after 2 and 5 days of storage. Immediately prior to being given to the sensory panel, the samples were placed in boiling water for 10 min then cooled to ambient temperature to assure product safety.

Samples were presented to panelists at room temperature under white fluorescent lighting. All panelist were
isolated from one another during sampling periods. For each LA treatment level, 2 segregated 20-g samples were used to evaluate four attributes. The samples were evaluated on a 10-point descriptive scale for odor (10=clean odor, 1=strong odor), color (10=bright excellent look, 1=graying dull look), taste (10=clean desirable flavor, 1=strong acid/undesirable flavor), and texture (10=tender, 1=tough).

Mean scores of each attribute for all LA treatment levels were subjected to ANOVA. Determination of significant differences was done at the 5% probability level using Minitab Statistical Software (Minitab, Inc., PA).

Storage Studies
Effect of Storage at 0, 6, & 12°C
Preparation of inoculated samples

One hundred g of heat treated crawfish tail meat was placed into a Waring blender (Waring Products, Co., Winsted, CT) prior to introduction of the inoculum. One mL of the diluted mixed *L. monocytogenes* culture (10^7 cells per mL) was added to the 100-g sample and blended to obtain a uniform distribution of cells. Each 100-g portion was aseptically transferred to a 4000 mL collection beaker after inoculation. The inoculation procedure was repeated 6 times in the same beaker until 600 g of crawfish tail meat was uniformly inoculated. The total inoculated 600 g of
crawfish meat in the collection beaker was then hand mixed
aseptically using a sterile spatula, under a laminar flow
hood (Labconco, Kansas City, MO) for 5 min. This
homogeneous composite sample contained approximately 10^5
CFU/g inoculum. Negative control samples were left
uninoculated to test for sterility.

After inoculating the crawfish tail meat, 25-g samples
were aseptically placed into sterile, 6-oz whirl pack bags
and randomly assigned to 3 storage treatments of 0, 6, and
12°C for 20 d. Additionally, negative controls of 25-g
portions were distributed to storage treatments.

Enumeration of bacteria

Two replicate experiments were conducted with each
replicate consisting of 3 storage temperatures (0, 6, and
12°C). Samplings were made at 2-d intervals for 0 and 6°C,
and at 1-d intervals for 12°C stored samples. For each
sampling period, 2 25-g samples were analyzed to enumerate
*L. monocytogenes*. In addition, a 25-g portion of
uninoculated meat was used to verify the sterility of the
initial uninoculated crawfish tail meat. Each sample was
aseptically removed from a "whirl pack" and placed
individually into a stomacher bag and stomached as
previously described.
Enumeration of *L. monocytogenes* was accomplished by duplicate surface platings of serially diluted samples made on LOA (Unipath). Counting of the bacterium was conducted using standard microbial count methods (Messer et al., 1985). Inoculated plates were incubated at 27°C for 48 h prior to counting. Mean values for each treatment were reported as the average of duplicate platings of all sampling points from a given time period. The number of bacteria present on the crawfish meat was determined for each sampling and expressed as \( \log_{10} \text{CFU/g} \).

**Calculation of Generation Time**

The generation time of *L. monocytogenes* in the crawfish tail meat was determined for each temperature using the following formula (Marshall and Schmidt, 1988). Two points on the logarithmic growth phase of each curve were used in the calculation. Generation times were reported as the time required for *L. monocytogenes* populations to double in number.

\[
\text{Generation Time} = \frac{(0.301(T_2 - T_1))}{(\log P_2 - \log P_1)}
\]

Where:
- \( T_1 \) = time of \( P_1 \)
- \( T_2 \) = time of \( P_2 \)
- \( P_1 \) = CFU/g at \( T_1 \)
- \( P_2 \) = CFU/g at \( T_2 \)
Effect of Citric Acid & Potassium Sorbate on Growth of L. monocytogenes

Preparation of inoculated samples

Initial storage studies were done with crawfish samples treated with potassium sorbate (PS) and citric acid (CA). Crawfish tail meat samples were stored and prepared for inoculation as described previously in "Crawfish tail meat sample preparation". Working cultures of L. monocytogenes also were subcultured as described previously in "Preparation of inocula". Samples were inoculated as described previously for thermal inactivation studies using the polyethylene bag method in "Sample inoculation". Mixed cultures were serially diluted before using, allowing inoculation levels of approximately 10^5 CFU/g. Six separate inoculated 100 g samples were aseptically transferred to a 4000 mL collection beaker then hand mixed as described previously.

PS or CA were prepared as individual 10% stock solutions and sterilized by filtration. Three ml of stock solution were sprayed onto 100 g of inoculated crawfish to achieve a 0.03 g/kg (0.3% w/w) concentration. The spraying procedure employed the same method described for thermal studies with LA. Typical use levels in foods for PS and CA range from 0.002 to 0.3% (Liewen and Marth, 1985). Treated and untreated samples were then segregated aseptically into
15-g sub-samples that were placed into the same type 3-mil polyethylene bags (Koch) used for thermal studies.

**Effect of Lactic Acid & Atmospheres on Growth of L. monocytogenes**

**Preparation of inoculated samples**

For storage studies done with LA and various gas atmospheres, 5 mL of each subcultured strain were transferred to a sterile centrifuge tube. The crawfish tail meat samples were then secured in a 60°C swirling water bath and heated for 12.5 min. This procedure yielded cell counts of approximately 10^6 CFU/g. Of this population, approximately 20% were heat damaged. The number of heat damaged cells was determined by the difference in number of CFU recovered on LOA divided by the number of CFU recovered on TSA.

Crawfish tail meat samples were inoculated as described previously for thermal inactivation studies using the polyethylene bag method in "Sample inoculation". Inoculation levels of approximately 10^4 CFU/g were consistently achieved. Nine separate 100 g inoculated samples were aseptically transferred to a 4000 mL collection beaker then hand mixed as described previously for thermal inactivation studies. One percent LA treated samples and untreated controls were prepared for storage as described above. Treatment combinations are given in Table (1). The
percentage of \( O_2 \) and \( CO_2 \) for all package atmospheres was determined using the Servomex Food Package Analyzer (Servomex) both before and after a 21 d storage period at 4°C (Table 3).

**Enumeration of bacteria**

Bacteria were enumerated using standard microbial count methods as described previously (Messer et al., 1985). At 2-d intervals, 15-g crawfish tail meat samples treated with PS or CA were used for *L. monocytogenes* enumeration. For samples treated with LA and various gas atmospheres, counts were made at 3-d intervals. Samples were prepared for enumeration as described previously for thermal inactivation studies of the polyethylene bag method. Enumeration of *L. monocytogenes* was accomplished by duplicate surface platings made on LOA (Unipath). Inoculated plates were incubated at 27°C for 48 h prior to counting.

**Experimental protocol**

Three replicate experiments were conducted at 4°C. At each sampling point, one randomly selected 15 or 25-g portion of crawfish tail meat was used to enumerate microbial populations for each replicate. In addition, a 15 or 25-g portion of uninoculated meat was used to verify the
Table 3. Gas content before and after 21 d storage at 4°C of package atmospheres and pH of crawfish tail meat treated with or without lactic acid (LA).

<table>
<thead>
<tr>
<th>Package Gas</th>
<th>Before storage pH</th>
<th>Before storage O₂</th>
<th>Before storage CO₂</th>
<th>After storage pH</th>
<th>After storage O₂</th>
<th>After storage CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>7.6</td>
<td>21.6</td>
<td>0.5</td>
<td>7.6</td>
<td>15.4</td>
<td>0.5</td>
</tr>
<tr>
<td>+1% LA</td>
<td>5.4</td>
<td>21.4</td>
<td>0.4</td>
<td>5.5</td>
<td>15.9</td>
<td>0.3</td>
</tr>
<tr>
<td>O₂</td>
<td>7.6</td>
<td>99.1</td>
<td>0.1</td>
<td>7.6</td>
<td>98.0</td>
<td>0.5</td>
</tr>
<tr>
<td>+1% LA</td>
<td>5.4</td>
<td>99.3</td>
<td>0.1</td>
<td>5.4</td>
<td>97.8</td>
<td>0.9</td>
</tr>
<tr>
<td>CO₂</td>
<td>7.6</td>
<td>0.0</td>
<td>99.7</td>
<td>6.8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>+1% LA</td>
<td>5.4</td>
<td>0.0</td>
<td>99.5</td>
<td>5.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>N₂</td>
<td>7.6</td>
<td>0.1</td>
<td>0.0</td>
<td>7.6</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>+1% LA</td>
<td>5.4</td>
<td>0.0</td>
<td>0.0</td>
<td>5.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

ND - Gas content of packages back filled with 100% CO₂ lost all head space during storage resulting in non-readable head space gas content.
sterility of the initial, uninoculated crawfish tail meat. Mean values for each treatment were reported as the average of duplicate platings of all sampling points of a given time period. The number of bacteria present on the crawfish meat was determined for each sampling and expressed as log_{10} CFU/g.

Generation time means for each treatment combination were subjected to ANOVA for determination of significant differences at the 5% probability level using Minitab Statistical Software (Minitab, Inc., PA). Lotus Freelance 3.01 software (Lotus Corp., Orem, UT) was used in determining slope, intercept, and goodness of fit values (e.g., R^2) of log CFU/g versus temperature and best fit line by least squares linear regression, of the exponential growth phase of *L. monocytogenes* (Dogra and Schaffner, 1993). Slopes resulting from the exponential growth phase of the bacterium for each treatment were also compared using ANOVA.
RESULTS AND DISCUSSION

Low Temperature Growth

Growth of *L. monocytogenes* on precooked crawfish tail meat stored at 0, 6, and 12°C, either to stationary phase or 20 d, are shown in Figure 4. The initial population of *L. monocytogenes* on inoculated crawfish samples held at 0 and 6°C was approximately $10^4$ CFU/g. At 0°C, less than 1 log$_{10}$ CFU/g growth was observed for the entire storage time of 20 d (Figure 3). Similar results were reported for shrimp held on ice where no increase in *L. monocytogenes* populations occurred for 21 d (Harrison et al., 1991). An initial decrease in the population of *L. monocytogenes* stored at 0°C was observed (Figure 3). Harrison et al. (1991) showed that shrimp samples held at -20°C had a decrease in *Listeria spp.* populations of less than 1 log when stored for 3 months. Any inhibitory environment that the bacterium is exposed to in cold storage seems to cause an initial decrease in population. If the bacterium can adjust to the 0°C environment, growth ensues.

Generation time of *L. monocytogenes* incubated at 0°C was approximately 72.2 h, dramatically longer than 17.0 or 6.9 h at 6 and 12°C, respectively. Consequently, as long as crawfish tail meat is constantly held at 0°C, *L. monocytogenes* will grow poorly.
Figure 3. Growth of *L. monocytogenes* on crawfish tail meat stored at 0, 4, 6, and 12°C.
Studies conducted by Glass and Doyle (1989) indicated that at 4.4°C the rate of growth for *L. monocytogenes* depended largely upon product type and pH. In the present storage study, crawfish tail meat inoculated with approximately 10⁴ CFU/g began exponential multiplication immediately with no observed lag phase at 6°C (Figure 3). A 1 log increase per 2-d period was observed until 10 d, at which time maximum population density for the bacterium on crawfish tail meat and stationary phase was reached. This temperature is important when determining the growth rates of *L. monocytogenes* in crawfish tail meat since 6°C (42.8°F) is close to the temperature of many retail display cases and home refrigerators. Because *L. monocytogenes* multiplies rapidly at this storage temperature, it may be beneficial to further heat process precooked crawfish tail meat prior to distribution.

At 12°C, the initial *L. monocytogenes* count of approximately 10⁵ CFU/g underwent rapid exponential multiplication, with no observed lag phase, for only 3 d before maximum population density for the bacterium on crawfish tail meat and stationary phase was reached (Figure 3). Even though 12°C is not considered proper refrigeration, it is possible to reach this abusive temperature if product mishandling occurs during transportation or storage. Crawfish tail meat, stored at
abusive temperatures even for short periods of time, could support rapid growth of *L. monocytogenes* and should be regarded as unsafe for consumption unless it undergoes an additional heat treatment.

In the seafood industry, a 7 to 10 d shelf life is typical for fresh, iced, products. Czuprynski et al. (1989) observed that *L. monocytogenes* at reduced temperature (4°C) increased in virulence in intravenously inoculated mice, although it did not appear to affect mice that had been infected orally. Thus, an increase in the virulence of the bacterium in refrigerated foods may be possible. The present storage studies indicated that *L. monocytogenes* can flourish on precooked packaged crawfish tail meat when stored under refrigeration temperatures for several days.

The generation time for *L. monocytogenes* on crawfish tail meat appears shorter than for other non-seafood products. Generation times for the bacterium at 4°C on beef (74.1 h), beef extract (50.6 h) (Shelef, 1989), and chicken (43.3 h) (Marshall et al., 1991) have been determined. On asparagus, broccoli, and cauliflower, the bacterium experienced good multiplication when stored at 4°C (Berrang et al., 1989). The generation time for the bacterium on seafood products, especially crustaceans, appears to be lower than on non-seafood products (Figure 4). Farber (1991) reported the generation time for the bacterium at 4°C
Generation times for *L. monocytogenes* in different food products at 4°C.

Figure 4.
on crustaceans such as crab, lobster, and shrimp, to be 16.9, 15.5 and 25.3h, respectively. Further research directed at determining why the bacterium grows so successfully on shellfish products, may unlock a key to controlling the bacterium on all food products.

**Storage Studies Using Citric Acid & Potassium Sorbate**

Chung and Lee (1981) observed that PS delayed onset of exponential growth but did not alter rate of microbial growth nor ultimate predominance of *Pseudomonas* *sp.* in English sole stored at 1.1°C. The present study notes the same effect when 0.03 g/kg PS was sprayed onto crawfish inoculated with *L. monocytogenes* and held at 4°C (Figure 5). The lag phase of the bacterium was extended by 2 d on treated crawfish. Once exponential growth began, however, the generation time was not significantly different (*P > 0.05*) than the generation time for untreated crawfish meat (Table 4). Additional studies by Chung and Lee (1982) evaluating the effectiveness of PS for controlling growth of several seafood bacterial isolates (*Arthrobacter sp.*, *Moraxella sp.*, and *Pseudomonas* *sp.*) found PS treatments ineffective unless amounts as high as 2.73% were used. Considering the findings of Chung and Lee (1981, 1982) and those of the present study, additional study of PS was not pursued.
Figure 5. Growth of *L. monocytogenes* on crawfish tail meat at 4°C and treated with 0.03 g/kg Citric Acid (CA) or Potassium Sorbate (PS).
Table 4. Generation times, least squares regression factors and goodness of fit for *Listeria monocytogenes* in cooked crawfish tail meat treated with citric acid (CA) or potassium sorbate (PS).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Generation Time (h)</th>
<th>Y-intercept (Log_{10} CFU)</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>28.5</td>
<td>5.45</td>
<td>0.239 ± 0.028</td>
<td>0.958</td>
</tr>
<tr>
<td>CA</td>
<td>29.5</td>
<td>5.47</td>
<td>0.229 ± 0.029</td>
<td>0.953</td>
</tr>
<tr>
<td>PS</td>
<td>28.1</td>
<td>4.30</td>
<td>0.261 ± 0.029</td>
<td>0.977</td>
</tr>
</tbody>
</table>
Samples sprayed with 0.03 g/kg CA supported growth of L. monocytogenes equally well as untreated samples (Figure 5). The generation time of the bacterium on CA-treated samples was not significantly different (P>0.05) than on untreated samples (Table 4). The present study indicated that 0.03 g/kg CA had no inhibitory effect against L. monocytogenes on cooked crawfish meat. These results indicate that post processing sprays of 0.03 g/kg PS or CA would have little or no benefit in adding any degree of safety against L. monocytogenes on crawfish tail meat. As a result, additional studies using mixed barriers and CA or PS were not perused. However, as discussed previously other researchers have had success controlling the growth of L. monocytogenes using CA at higher concentrations. Consequently, additional research directed at the use of CA as a barrier for controlling L. monocytogenes should not be overlooked in the future.

**Thermal Inactivation**

The inoculation levels (log_{10} CFU/ml) at time zero for each temperature tested were 9.6 (55°C), 9.3 (60°C), and 8.3 (65°C). The heat resistance of L. monocytogenes in crawfish tail meat, as designated by D values (min) determined using TNF methodology, is shown in Table 5. From these data, a TDT plot was constructed showing a z value of 5.5°C.
Table 5. Thermal death times, least squares regression factors and goodness of fit for D value determinations using TNF methodology for *L. monocytogenes* in crawfish tail meat.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>D values (min) ±s.d.</th>
<th>Y intercept (log₁₀)</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>10.23 ±0.32</td>
<td>9.56</td>
<td>-0.783</td>
<td>0.929</td>
</tr>
<tr>
<td>60</td>
<td>1.98 ±0.15</td>
<td>8.48</td>
<td>-0.550</td>
<td>0.952</td>
</tr>
<tr>
<td>65</td>
<td>0.19 ±0.01</td>
<td>7.91</td>
<td>-0.080</td>
<td>0.987</td>
</tr>
</tbody>
</table>
Regression analysis values for intercept, slope, and R² of the log₁₀ survivors versus temperature are shown in Table 5.

D value ranges for *L. monocytogenes* on crawfish tail meat determined using the TNF method were very similar to those determined previously on crab and lobster meat (Budu-Amoake et al., 1992; Harrison and Huang, 1990). Harrison and Huang (1990) determined D values for the bacterium on crab meat at 55 and 60°C to be 12.00 and 2.61 min, respectively. Budu-Amoake et al. (1992) determined a D value for *L. monocytogenes* on lobster to be 2.39 min at 60°C. They calculated a z value for the bacterium of 5°C. D values for crawfish meat obtained using the TNF method were slightly lower than in both of those studies. Crawfish tail meat is packed with adherent hepatopancreas and fat having a total lipid content of 0.939 g/100 g (USDA, 1987). Crab meat has a total lipid content of 1.188 g/100 g (USDA, 1987). The higher D value observed for crab meat at 60°C might be attributable to the protective characteristics of fat (Mackey et al., 1990). However, this did not appear to be the case when comparing the D values of crawfish tail meat to that of lobster which has a total lipid content of 0.358 g/100 g (USDA, 1987).

Other factors may have contributed to the lower values observed in the TNF thermal study. The previous studies
done with crab meat and lobster, both used non selective TSA to enumerate \textit{L. monocytogenes}. The present study used TSA as a base medium and allowed a 4 h recovery time to resuscitate injured cells. An overlay of selective LOA was then added. The presence of the antimicrobial agent, acriflavine, in this selective medium may have inhibited the growth of some heat damaged cells that were unable to recover completely before being overlaid. Inhibition would cause the recovery of fewer cells than had actually survived thermal treatment, thus yielding a lower D value than the previous two studies. When Harrison and Huang (1990) used Modified Vogel-Johnson agar, a more selective medium than TSA, to enumerate \textit{L. monocytogenes} from crab meat, fewer bacteria were recovered. The D values for 55 and 60°C using this medium were 9.18 and 1.31 min, respectively; both values are lower than those observed in the present study. Additionally, the use of a mixed strain inoculum in the present study, as opposed to strain Scott A singly, may have been a contributing factor producing the reported variable results.

\(D_{60}\) values for \textit{L. monocytogenes} differed (P<0.05) when various methods of determination were employed (Table 6). The TNF method required the crawfish samples to be suspended in an aqueous environment, which leaves the bacterium less protected by constituents of the crawfish meat. Therefore,
Table 6. Thermal death times, least squares regression factors and goodness of fit for $D_{60}$ values determinations of *L. monocytogenes* using various methods in crawfish tail meat.

<table>
<thead>
<tr>
<th>Method</th>
<th>D values (min) ±s.d.</th>
<th>Slope</th>
<th>Std. Err. of slope</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>1.98 ±0.15$^a$</td>
<td>-0.550</td>
<td>0.078</td>
<td>0.952</td>
</tr>
<tr>
<td>PB</td>
<td>4.68 ±0.56$^b$</td>
<td>-0.215</td>
<td>0.012</td>
<td>0.979</td>
</tr>
<tr>
<td>CC</td>
<td>3.84 ±0.23$^b$</td>
<td>-0.251</td>
<td>0.012</td>
<td>0.987</td>
</tr>
</tbody>
</table>

* D values with similar superscripts are not significantly different (P>0.05).
a lower D value can be expected when using the TNF method over the PB or CC methods. The $D_{60}$ values determined using the PB and CC methods did not differ ($P>0.05$) (Table 6).

Traditionally TDT tests to determine D values for bacteria in foods have utilized TDT tube, TDT can, or TNF methods. The use of CC method is one commonly used for meats. Since crawfish tail meat is typically packaged by the industry in polyethylene bags, the present study used this type of container and developed a new method (PB) for assessing thermal tolerance of bacteria. The CC and PB methods yielded similar D values ($P>0.05$) for *L. monocytogenes*. The D value determined using TNF method was significantly less than both the CC and PB method ($P<0.05$). Other D value determinations discussed later, done using PB method with head space (PB-a) yielded significantly ($P<0.05$) higher D values (10.58 min) than PB or CC without head space. Variations in D values observed with different methods indicates that methods used for the determination of D values for *L. monocytogenes* on crawfish tail meat are critical. Since researchers have used many different methods to determine D values for the bacterium, it is very difficult to accurately compare behavior of the bacterium in different foods.

When head space was added to the PB samples (PB-a), the $D_{60}$ values (10.58 CFU/g) increased significantly ($P<0.05$).
Heating profile of PB-a samples was about 0.5 to 1.0 min slower to reach 60°C than the PB samples (Figure 3). This may have been caused by an air insulation effect due to the headspace. The head space in PB-a crawfish tail meat samples forces product away from some walls of the polyethylene bags. This created a situation where less surface area of crawfish sample was in proximity to liquid heating medium (water), thus reducing the speed at which the core temperature reached 60°C. The reduction in come-up time would allow *L. monocytogenes* more time to acclimate to the rise in temperature and reduce the accumulation of lethality units (Nickerson and Sinskey, 1972). As a result, fewer bacteria were killed with the PB-a method than for CC and PB methods under similar time frames. It is also possible the head space may have allowed the thermocouples to shift slightly during agitation in the water bath. A slight shift from the geometric center of the samples may have caused the centers of the crawfish tail meat samples to remain at a slightly cooler temperature. This would happen because the thermocouple would have indicated that samples had reached the targeted treatment temperature premature to the actual occurrence. The resulting lower core temperature of the samples would reduce the effectiveness of the thermal treatment, yielding higher D values.
Thermal Inactivation with Lactic Acid

A $D_{60}$ value of 4.68 min was calculated for *L. monocytogenes* on untreated crawfish tail using the PB method. LA sprays were found to enhance the lethality of heat against *L. monocytogenes* (Table 7). Although a stepwise increase of lethality of heat against the bacterium was observed between the control, 0.5, and 0.75% LA, no statistically significant improvement (P<0.05) in lethality was noted until treatments reached 1.0% LA (Table 7). A 1.0% LA treatment increased lethality of heat during a 14-min thermal treatment by approximately 4 logs over the control (Figure 6).

$D_{60}$ values calculated for *L. monocytogenes* on crawfish tail meat that was pH adjusted using HCl to approximate the pH of 0.5 and 1.0% LA were 4.92 and 3.54 min, respectively. There was no significant difference between crawfish tail meat controls and samples adjusted with HCl to approximate the pH of 0.5 or 1% LA treatments (Table 7). Although the $D_{60}$ value (Table 7) and resulting linear regression of TDT slope (Figure 7) for *L. monocytogenes* on crawfish tail meat samples pH adjusted with HCl to approximate that of 1.0% LA was smaller than the $D_{60}$ for controls, it was significantly greater (P<0.05) than 1.0% LA treated samples. This indicates that while a reduction of pH may increase
Table 7. Least squares regression factors and goodness of fit for $D_{90}$ values for *L. monocytogenes* on crawfish tail meat treated with different levels of lactic acid or HCl.

<table>
<thead>
<tr>
<th>Acid</th>
<th>% Acid</th>
<th>pH</th>
<th>D-value</th>
<th>Slope</th>
<th>Std. Err. of Slope</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>0.00</td>
<td>7.6</td>
<td>4.68a</td>
<td>-0.215</td>
<td>0.013</td>
<td>0.979</td>
</tr>
<tr>
<td>LA</td>
<td>0.50</td>
<td>6.4</td>
<td>4.41a</td>
<td>-0.237</td>
<td>0.019</td>
<td>0.962</td>
</tr>
<tr>
<td>HCl</td>
<td>0.35</td>
<td>6.5</td>
<td>4.92a</td>
<td>-0.216</td>
<td>0.019</td>
<td>0.923</td>
</tr>
<tr>
<td>LA</td>
<td>0.75</td>
<td>6.3</td>
<td>3.46a</td>
<td>-0.289</td>
<td>0.013</td>
<td>0.989</td>
</tr>
<tr>
<td>LA</td>
<td>1.00</td>
<td>5.5</td>
<td>2.49b</td>
<td>-0.417</td>
<td>0.015</td>
<td>0.994</td>
</tr>
<tr>
<td>HCl</td>
<td>0.75</td>
<td>5.4</td>
<td>3.54a</td>
<td>-0.282</td>
<td>0.019</td>
<td>0.973</td>
</tr>
</tbody>
</table>

*a, b* D values with similar superscripts are not significantly different when compared to the control ($P>0.05$).
Figure 6. Thermal death curve for *L. monocytogenes* at 60°C using various levels of spray applied lactic acid.
Figure 7. Linear regression comparing the effect of 1% lactic acid and the equivalent HCl adjusted pH, on lethality of 60°C toward *L. monocytogenes* on crawfish tail meat.
lethality of heat towards *L. monocytogenes*, LA augments lethality independent of pH.

To date, the exact mechanism that makes environments containing weak acids like LA more lethal to *L. monocytogenes* than an equivalent low pH environment altered with strong acids has not been defined. It has, however, been established that weak acids in their undissociated or protonated form have the ability to penetrate the cell membrane and accumulate within the cell cytoplasm (Ita and Hutkins, 1991). It may be that LA simply is able to penetrate the cell more effectively than other acids. Once the acid penetrates the interior of the cell, it releases a proton and acidifies the cytoplasm of the cell (Booth, 1985). The inability to maintain a proper pH gradient via a proton/cation exchange system, coupled with the exhaustion of energy needed to maintain normal metabolic processes, results in the cells inability to survive (Kashket, 1987). Continued research directed at establishing the exact mechanism is certainly warranted.

**Sensory Evaluation**

Sensory analysis was conducted to determine the effect of study levels of LA on product acceptability. Although the sensory panel found no significant difference (P>0.05) for all characteristics tested between controls and any LA
treated samples (Table 8), the panel observed a slight toughening of the product when LA at any level was added. The panel also noted that the typical fishy odor and taste of crawfish was reduced with application of any level of LA. Additionally, LA treated crawfish appeared to be brighter in color. Many panelists found these characteristics to be a desirable change.

While crawfish tail meat was stored at 4°C under various gases, browning of the samples was visually observed in packages containing O₂ after 6 d, when compared to other samples packed without O₂. Paralleling this observation, Bentley et al. (1989) observed no surface discoloration on beef patties packaged under 100% N₂, 100% CO₂, or Vacuum and stored at 0, 4, or 8°C for up to 21 d. Of atmospheres evaluated, they determined that beef patties stored in a CO₂ environment produced the highest values for all sensory traits evaluated, including tenderness, juiciness, flavor, and overall desirability. When crawfish tail meat samples stored in 100% CO₂ were visually compared with samples packed in all other atmospheres, an increase of color brightness was observed. This occurrence in crawfish has been previously quantified by Gerdes et al. (1989). Using a HunterLab color difference meter (HunterLab Assoc., Richmond, VA), they documented an increase in redness color
Table 8. Taste panel results of crawfish samples packed under air and stored at 4°C. Values of 5.0 = standard quality, >5.0 = less favorable quality, and <5.0 = more favorable quality.

<table>
<thead>
<tr>
<th>%LA</th>
<th>DAY</th>
<th>ODOR</th>
<th>COLOR</th>
<th>TASTE</th>
<th>TEXTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0</td>
<td>4.3±1.3</td>
<td>4.3±1.2</td>
<td>4.4±1.4</td>
<td>4.3±1.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.7±1.4</td>
<td>4.4±1.2</td>
<td>4.8±1.5</td>
<td>4.5±1.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.0±1.5</td>
<td>4.5±1.2</td>
<td>4.5±1.3</td>
<td>4.8±1.3</td>
</tr>
<tr>
<td>0.50</td>
<td>0</td>
<td>4.8±1.7</td>
<td>4.4±1.5</td>
<td>4.6±1.8</td>
<td>4.2±1.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.6±1.4</td>
<td>4.3±1.2</td>
<td>4.5±1.4</td>
<td>4.7±1.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.7±1.1</td>
<td>4.4±0.9</td>
<td>4.4±1.1</td>
<td>4.8±1.2</td>
</tr>
<tr>
<td>0.75</td>
<td>0</td>
<td>4.6±1.0</td>
<td>4.4±1.0</td>
<td>5.0±1.2</td>
<td>4.7±1.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.2±1.1</td>
<td>4.2±1.2</td>
<td>4.0±1.4</td>
<td>4.3±1.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.1±1.5</td>
<td>4.6±1.0</td>
<td>5.4±1.4</td>
<td>5.3±1.2</td>
</tr>
<tr>
<td>1.00</td>
<td>0</td>
<td>4.6±1.5</td>
<td>4.5±1.0</td>
<td>4.8±1.4</td>
<td>4.5±1.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.6±1.1</td>
<td>4.4±1.4</td>
<td>4.7±1.2</td>
<td>5.0±1.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.1±1.1</td>
<td>4.6±1.2</td>
<td>5.0±1.5</td>
<td>4.9±1.1</td>
</tr>
</tbody>
</table>
values when crawfish samples were stored on ice in high CO₂ atmospheres for 21 d. Consumers may find this change desirable, however, additional consumer surveys would be required to determine this.

In general it appears that LA treatment levels used for the present study are acceptable sensorially. As a result the use of these levels on crawfish tail meat could be recommended.

Heat and Modified Atmospheres

During 60°C heat treatments of up to 20 min, gas measurements of package headspace revealed no meaningful changes in CO₂ or O₂ composition of any atmospheres studied (Table 2). The TDT curves for *L. monocytogenes* on crawfish tail meat are shown in Figure 8. There was no statistical difference in D₆₀ values for *L. monocytogenes* on crawfish meat packed in any of the 4 atmospheres (Table 9). D₆₀ values for *L. monocytogenes* on crawfish meat packed in atmospheres that contained O₂ were lower, however than for atmospheres which were void or limited of O₂ (Table 9).

Recent research conducted on *Escherichia coli* as well as *L. monocytogenes* may offer an explanation for the decreased thermal resistance of *L. monocytogenes* in atmospheres rich in O₂. Murano and Pierson (1993) determined D value of heat-shocked *E. coli* O157:H7 to be
Figure 8. Thermal death curve for *L. monocytogenes* on crawfish tail meat at 60°C packed under various atmospheres.
Table 9. Least squares regression factors and goodness of fit for D-value determinations after 60°C thermal treatment of crawfish tail meat treated with or without 1% LA and packed in different modified atmospheres with head space.

<table>
<thead>
<tr>
<th>Atmosphere Type</th>
<th>pH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>D-value</th>
<th>Slope</th>
<th>Std. Err. of Slope</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>7.6</td>
<td>10.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.097</td>
<td>0.003</td>
<td>0.995</td>
</tr>
<tr>
<td>+1% LA</td>
<td>5.4</td>
<td>3.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.360</td>
<td>0.031</td>
<td>0.986</td>
</tr>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.6</td>
<td>9.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.110</td>
<td>0.007</td>
<td>0.983</td>
</tr>
<tr>
<td>+1% LA</td>
<td>5.4</td>
<td>3.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.274</td>
<td>0.031</td>
<td>0.973</td>
</tr>
<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.6</td>
<td>11.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.088</td>
<td>0.007</td>
<td>0.975</td>
</tr>
<tr>
<td>+1% LA</td>
<td>5.4</td>
<td>3.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.313</td>
<td>0.037</td>
<td>0.973</td>
</tr>
<tr>
<td>N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.6</td>
<td>12.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.081</td>
<td>0.005</td>
<td>0.983</td>
</tr>
<tr>
<td>+1% LA</td>
<td>5.4</td>
<td>3.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.340</td>
<td>0.014</td>
<td>0.996</td>
</tr>
</tbody>
</table>

<sup>a</sup> pH of samples are after heating at 60°C

<sup>b,c</sup> D values with similar superscripts are not significantly different (P>0.05).
higher when cells were incubated anaerobically than when incubated aerobically. It has been theorized that severely heat-injured cells acquire a strict anaerobic nature (McCord et al., 1971). Thus, any cell damage due to oxidation reactions, which are normally enhanced by heat, are diminished in oxygen free environments. Additionally, Dallmier and Martin (1988) showed that after 10 min at 60°C, *L. monocytogenes* (Scott A) retained only 42% of its initial catalase (CAT) activity. It has been hypothesized that inactivation of enzymes like CAT and superoxide dismutase (SOD) during heating, would interrupt the cells ability to deactivate toxic oxygen radicals during subsequent recovery periods. The result would be a buildup of toxic oxygen radicals in an O₂ rich environment. Consequently, the inactivation of these enzymes converts *L. monocytogenes* into an obligate anaerobe (Knabel et al., 1990).

The addition of 1% LA to crawfish tail meat caused a significant decrease (P<0.05) in *D₆₀* values for *L. monocytogenes*, regardless of packaging atmosphere (Table 9). There was, however, no difference (P>0.05) between gas environments (Figure 9). Presence of LA masked the affect of an O₂-free environment on thermal resistance. *D₆₀* values determined for *L. monocytogenes* on crawfish tail meat using 1% LA and head space containing molecular O₂, were higher than *D₆₀* values determined from samples packed using 1% LA
Figure 9. Thermal death curve for *L. monocytogenes* on crawfish tail meat at 60°C spray treated with 1% lactic acid and packed under various atmospheres.
and no head space (Tables 5 & 9). This might be attributable to an inability of the bacterium to detoxify toxic products of molecular O₂, the superoxide radical (O₂⁻) and hydrogen peroxide (H₂O₂), after thermal destruction of the enzymes CA and SOD.

4°C Storage Under Various Atmospheres & 1% LA

Generation times at 4°C for heat shocked *L. monocytogenes* on crawfish tail meat packed under Air, 100% O₂, 100% CO₂, and 100% N₂ were 26.4, 33.6, 105.1, and 26.9 h, respectively. The most inhibitive package environment was 100% CO₂, exhibiting significantly (P<0.05) larger generation times than all other gas treatments (Figure 10). Carbon dioxide penetrates cells very easily (Daniels et al., 1985) and this may facilitate its chemical effects on the internal metabolic processes of *L. monocytogenes*. It has been long established that increased inhibition of bacteria at lower temperatures is correlated to increased solubility of the gas in the water phase (Golding, 1945). Carbon dioxide was rapidly absorbed by crawfish tail meat in the present study. Approximately 30 min after packing crawfish tail meat samples in 100% CO₂ with 4:1 head space (gas to product) no head space remained in the packs. This rapid absorption enables conversion of CO₂ to carbonic acid. Carbonic acid produces a rapid acidification of bacterial
Figure 10. Growth of *L. monocytogenes* stored under 4 different atmospheres at 4°C for 21 d.
cell internal pH leading to altered metabolic activities (Daniels et al., 1985). During the present study, pH of crawfish tail meat dropped approximately unit during the 21 d storage period when packed under CO\(_2\) (Table 3).

These findings contribute support to a hypothesis that as with heat, CO\(_2\) appears to exert an effect on certain enzyme systems affecting growth of the bacterium (Foster and Davis, 1949; Fenestil et al., 1963; King and Nagel, 1975). Several mechanisms have been hypothesized as explanations for the reduction of growth of a bacterium in the presence of CO\(_2\). Stimulated mitochondrial ATPase activity and the uncoupling effect on oxidative phosphorylation resulting in a decreased level of energy available to the bacterium for metabolism and growth is one (Fenestil et al., 1963). King and Nagel (1975) postulated that CO\(_2\) concentrations above 50% inhibit the activity of isocitrate dehydrogenase, malate dehydrogenase and inhibits certain decarboxylation enzymes through a mass action effect.

The present study found that thermally treated *L. monocytogenes* tended to recover more effectively on crawfish tail meat packaged in N\(_2\) and air, which were free or limited in O\(_2\), than in an environment of 100% O\(_2\) (Figure 10). Knabel et al. (1990) reported that after heat shock in milk, anaerobic recovery of *L. monocytogenes* resulted in higher numbers when compared with recovery under aerobic
conditions. Murano and Pierson (1993) found the same to be true for *Escherichia coli* O157:H7 after heat shock. The present study determined that 20% of the cells were injured when thermally treated at 60°C for 12.5 min. Dallmier and Martin (1988) reported that CAT and SOD were rapidly inactivated when *L. monocytogenes* was heated at temperatures of 60°C. Consequently this inactivation of CAT and SOD during the 60°C thermal treatment and subsequent build up of oxygen radicals in an oxygen rich environment may have caused the bacterium to grow at a slower rate when compared to air and N₂. However, since a majority of surviving cells were not injured by the thermal treatment, no significant difference (P>0.05) was observed in growth inhibition between 100% O₂, air and N₂ atmospheres.

One percent LA added to crawfish tail meat followed by packaging under air, 100% O₂, 100% CO₂ and 100% N₂ atmospheres prevented growth of *L. monocytogenes* (Figures 11 & 12). The most effective atmosphere plus LA combination appears to be O₂ + 1% LA (Figure 13). This combination was significantly (P<0.05) more lethal to the bacterium than the N₂ + 1% LA combination and consistently more lethal than the CO₂ + 1% LA or Air + 1% LA combinations (Figure 13). The destructive element of this treatment combination is probably largely a result of build up of oxygen radicals by the bacterium. It appears that when this outcome is
Figure 11. Growth of *L. monocytogenes* on crawfish tail meat stored under Air and N₂ alone and in combination with 1% LA at 4°C for 21 d.
Figure 12. Growth of *L. monocytogenes* stored under CO₂ and O₂ alone and in combination with 1% LA at 4°C for 21 d.
Figure 13. Destruction of *L. monocytogenes* stored under Air, *N₂*, *CO₂*, and *O₂* in combination with 1% LA at 4°C for 21 d.
combined with the effects of LA, a package environment of 100% O₂ + 1% LA becomes the most lethal of the treatment combinations studied.

As discussed earlier, when LA was absent, CO₂ was the most restrictive packaging atmosphere to growth of *L. monocytogenes* on crawfish tail meat. However, any effect of pH reduction resulting from carbonic acid production experienced with introduction of CO₂ was completely masked by the presence of 1% LA. This was evidenced by the significant difference (P<0.05) between CO₂ and the other 3 atmospheres without LA present (Figure 10) versus the lack of significant difference (P>0.05) between CO₂ and other atmospheres when 1% LA was added as a treatment (Figure 13).
SUMMARY AND CONCLUSIONS

Heat treatments of approximately 4 min at 60°C will reduce \textit{L. monocytogenes} by 90% on packaged crawfish tail meat. The addition of LA spray treatments increases lethality of heat to the bacterium. The enhanced lethality of heat when LA was introduced was not strictly due to a simple pH effect. It appears incorporation of two barriers, heat and LA, in a process could contribute a significant degree of safety to the consumer in regards to crawfish tail meat.

When \textit{L. monocytogenes} was heat treated in an O\textsubscript{2}-free environment \(D_{60}\) values were consistently higher than in treatments done in the presence of O\textsubscript{2}. This observation supports the hypothesis that absence of O\textsubscript{2} in the environment used during heat treatments of \textit{L. monocytogenes} acts to protect the bacterium from heat injury. To encourage greater lethal heat processes against the bacterium, inclusion of sufficient amounts of O\textsubscript{2} in packaging environments should be considered.

Results from storage studies further strengthen the argument that packaging of crawfish tail meat and any subsequent heat treatment should be done in the presence of O\textsubscript{2}. As mentioned above, heat treatments administered under a 100% O\textsubscript{2} packing environment were the most destructive to
*L. monocytogenes*, compared with air, CO₂, and N₂. Storage at 4°C of 1% LA treated crawfish tail meat packed under 100% O₂ proved to be the most lethal barrier combination to heat injured *L. monocytogenes*.

It would appear that methodologies employed in the determination of thermal death times for *L. monocytogenes* in various food products need careful consideration. The present study indicates that methods which use no head space, thus allowing the bacterium little or no access to molecular O₂, may yield higher D values for a given product than similar methods which include a head space containing some molecular O₂. Therefore, application of heat treatments in a low-oxygen environment and subsequent storage of a product in a similar environment may increase, instead of decrease the probability of listeriosis being contracted from a given food product.

While maximum kill of the bacterium seems to occur when it is heated in the presence of O₂, minimum recovery is realized. Tightly packed TDT tubes and cellulose casings that allow very little O₂ to remain during heating trials, may yield higher D values for *L. monocytogenes*. Because of the threat of toxin production from *Clostridium botulinum* in an anaerobic environment, many industrial packaging methods allow inclusion of O₂. Consequently, laboratory methods for determining D values of *L. monocytogenes* should be designed
to allow the presence of $O_2$. However, since complete recovery of heat injured *L. monocytogenes* in an aerobic environment appears impossible, media overlays which exclude $O_2$ or pre-reduced media should be employed for enumeration of the bacterium.

Commercially processed crawfish are boiled for 5 to 10 min before hand peeling. Based on heat resistance of *L. monocytogenes* demonstrated in the present studies, this process would provide sufficient destruction of the bacterium before hand peeling and packaging. Thus, occurrence of the bacterium in packaged crawfish tail meat would be mainly due to cross contamination from plant workers or equipment. The adoption of strict in-plant sanitation programs to prevent post-boil cross contamination is paramount. Because of the possibility of process deviations, post-packaging heat treatments in the presence of $O_2$ and addition of 1% LA sprays should be implemented. While more extensive investigations into the mechanisms which cause interactions of these barriers in crawfish tail meat and other food products is needed, the addition of these steps in a crawfish packing plant would increase safety of the product.
REFERENCES


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VITA

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