

INTRODUCTION

Listeria monocytogenes in ready-to-eat food products presents serious food safety concerns to food processors, regulatory agencies, consumers, and others. The food industry in the United States is currently under a “zero tolerance” policy for *L. monocytogenes* in ready-to-eat foods by both USDA/FSIS and CFSAN, because the infective dose is unknown. *Listeria monocytogenes* is ubiquitous in nature and can grow in many different processing environments, which causes it to be very difficult to eliminate from a food production facility. The organism’s optimum growth temperature is 30-37°C; however, it is known to grow at temperatures as low as 1°C and as high as 45°C (Hitchins, 1998). The ability of *L. monocytogenes* to grow at refrigeration temperatures, while competing organisms cannot, provides easy survival and proliferation of the organism. This growth of the organism on refrigerated, ready-to-eat food products causes a serious potential food safety hazard. Although *L. monocytogenes* can be destroyed if heated to a high enough temperature, there may be cross-contamination of the food product after the heat treatment and subsequent growth (Buncic *et al.*, 1990; Zaika *et al.*, 1989). Due to the risk of cross-contamination, post-processing treatments are needed to inhibit growth of *L. monocytogenes* due to contamination of ready-to-eat food products, such as frankfurters.

Turkey frankfurters are of particular interest for food safety due to the recent outbreaks of *L. monocytogenes* in this product, and because turkey frankfurters are sometimes eaten without re-heating to an adequate temperature (CDC, 1989; CDC, 1998). Additionally, due to the ubiquitous nature of *L. monocytogenes*, frankfurters provide an adequate pH, Aw, and nutrients for growth of the organism. The majority of contamination on frankfurters by *L. monocytogenes* occurs through cross-contamination during the peeling step, immediately before packaging (Wenger *et al.*, 1990). Additional steps prior to packaging could help to minimize growth of *L. monocytogenes* in ready-to-eat food products, like turkey frankfurters.

Modified atmosphere packaging (MAP) and the addition of antimicrobials have become a means of extending the shelf-life of ready-to-eat food products (Zeitoun and Debevere, 1991; Nilsson *et al.*, 1997; Pothuri, *et al.*, 1995; Barakat and Harris, 1999). MAP is achieved by placing a food product in a high gas-barrier package, removing air

from the package, flushing with a gas or combination of gases, and sealing the package. Carbon dioxide (CO₂) is primarily used in MAP for its ability to increase the shelf life of refrigerated food products, and inhibit spoilage and pathogenic microorganisms. High levels of CO₂ (>50%) have been shown to be inhibitory to the growth of *L. monocytogenes*, with increasing concentration, decreasing temperature, and decreasing pH, increasing the inhibitory affect (Daniels *et al.*, 1984; Hart *et al.*, 1991; Farber and Daly, 1994). Little data is available involving the combined effects of CO₂ with other processing treatments on the inhibition of *L. monocytogenes*. Combinations of CO₂ with sodium lactate, sodium acetate, or sodium diacetate are a few possible treatments that could be useful in reducing growth of *L. monocytogenes* in ready-to-eat meat products.

There has been recent interest in using organic acids and the salts of these acids to inhibit bacterial growth. Although the use of organic acids is more successful at inhibiting growth of *L. monocytogenes* than the salts of these acids, higher concentration of these salts can be used without damaging the quality of the food product (Buncic *et al.*, 1995). Sodium lactate, sodium acetate, and sodium diacetate have all been shown to inhibit the growth of *L. monocytogenes*. Sodium lactate (2-6%), sodium acetate (0.25-0.5%), and sodium diacetate (0.2-0.5%) have all prevented growth at refrigeration temperatures, with increasing concentration, decreasing temperature, and decreasing pH, causing increased inhibition (Miller *et al.*, 1992; McMahon *et al.*, 1999; Schlyter *et al.*, 1993(a), Schlyter *et al.*, 1993(b); Wederquist *et al.*, 1994).

The objective of this research was to determine the combined and individual affects of carbon dioxide with sodium lactate (2.4 and 4.8%), sodium acetate (0.25 and 0.50%), and sodium diacetate (0.25 and 0.50%) on the inhibition of *L. monocytogenes* on turkey frankfurters. Carbon dioxide, sodium lactate, sodium acetate, and sodium diacetate have all individually shown inhibitory effects on *L. monocytogenes*, but information on the combined effect is non-existent. This research is designed to determine whether these surface treatments would be effective in preventing growth of *L. monocytogenes* on ready-to-eat processed.

LITERATURE REVIEW

A. *Listeria monocytogenes*

1. Organism Characteristics

Listeria monocytogenes is a well-known food-borne pathogen that is widespread in nature. It can be found in plants, soil, water, the stools of 1-10% of humans, and in at least 37 mammalian and 17 bird species (CFSAN, 2000). *L. monocytogenes* is a short, Gram-positive, non-sporeforming rod, with tumbling end-over-end motility at 22°C. It is catalase positive, oxidase negative, and has slight β -hemolysis on blood agar. On Modified Oxford (MOX, Oxford Medium Base plus Modified Antimicrobial Supplement) Agar, the organism produces flat, dimpled colonies and causes a black halo indicating esculin hydrolysis. *Listeria monocytogenes* does not require CO₂ for growth, but growth is enhanced with 5-10% CO₂. There are 13 known antigenic types of the organism with serotypes 4B, 1/2A, and 1/2B providing 90% of the isolates (CFSAN, 2000). *Listeria monocytogenes* is known to survive refrigeration, freezing, heating, and drying, which provides difficulties for the food industry (CFSAN, 2000). In addition, this organism has been associated with outbreaks in raw milk, ice cream, raw meats, and ready-to-eat meat and cheese products.

2. Disease Characteristics

Listeria monocytogenes is estimated to cause 2500 illnesses and 500 deaths in the United States each year (CDC, 2000). Most healthy adults may have few or no symptoms at all, yet the risk of acquiring meningitis, septicemia, and encephalitis increases in persons with compromised immune systems. Such individuals include those ≥ 65 years, cancer patients, diabetics, AIDS patients, or persons with kidney disease (CDC, 2000). Listeriosis may be preceded by fever and “flu-like” symptoms including, nausea, vomiting, and diarrhea (CFSAN, 2000). The onset of these symptoms may occur a few days up to three weeks after ingestion. If pregnant women become infected, they may experience mild “flu-like” symptoms or perhaps no symptoms at all. Although only mild symptoms occur in the mothers, infection during pregnancy may lead to

spontaneous abortion of the fetus, stillbirth, or various other infections within the newborn, including pneumonia, meningitis, or septicemia. In general, mortality rates for listeriosis may be as high as 80% for neonatal infections, and 50-70% for meningitis and septicemia patients (CFSAN, 2000). Patients with listeriosis may be treated successfully with penicillin or ampicillin (CFSAN, 2000). The minimum infectious dose of *L. monocytogenes* is currently unknown, due to differing virulence among strains and its dependence on host factors such as age, health, and exposure to certain foods (NACMCF, 1991).

3. Foodborne Outbreaks

Although *L. monocytogenes* can be inactivated if heated to a high enough temperature (70°C for meat products), post-heating contamination of *L. monocytogenes* has recently become a concern in ready-to-eat food products. Most cases of listeriosis occur as sporadic incidents; however, there have been many significant outbreaks of the illness. Mexican-style cheese was associated with at least 86 illnesses and 29 deaths due to listeriosis in Los Angeles and Orange Counties, CA in 1985 (CDC, 1985). In August 1998, 40 illnesses due to high environmental levels of *L. monocytogenes* in the production facility were linked to deli meats and frankfurters (CDC, 1998). Deli turkey meat was also discovered to be responsible for 29 illnesses due to this organism in 10 states between May 17-November 26, 2000 (CDC, 2000). An outbreak of listeriosis in November 2000, was associated with homemade Mexican-style cheese, causing 12 illnesses in North Carolina (CDC, 2001). This outbreak was due to contaminated raw milk used in the production of the cheese.

4. Factors Affecting Growth or Survival in Ready-To-Eat Meat Products

a. Temperature

Temperature is an important factor that may allow growth of *L. monocytogenes* in meat products. The optimum growth temperature for *L. monocytogenes* is 30-37°C. This microorganism has been found to grow at temperatures as low as 1°C and as high as 45°C, while also being able to survive freezing (Hitchins, 2000). Since most ready-to-eat meat products are stored frozen or at refrigeration temperatures, the ability of *L.*

monocytogenes to survive and grow at low temperatures provides opportunity for proliferation in contaminated products. For this reason, strict temperature control is necessary to prevent or minimize *L. monocytogenes* growth. Any temperature abuse of the food product can enhance growth rate of *L. monocytogenes*. Glass and Doyle (1989) demonstrated that *L. monocytogenes* could grow on a variety of processed meat and poultry products including ham, bologna, wieners, and sliced turkey when held aerobically at 4.4°C. A significant increase in growth was also seen in processed chicken held aerobically for four weeks at 4°C and 10°C (Carpenter and Harrison, 1989). An additional study reported approximately 65.5% of sampled vacuum packaged retail poultry wieners held at 5°C supported growth of *L. monocytogenes* (McKellar, et al., 1994). A 7-log increase in growth of the organism was also observed on vacuum packaged turkey bologna stored at 4°C for 63 days (Wederquist, et al., 1994). The ability of this organism to grow at refrigeration temperatures, while competing organisms cannot, allows for easy survival and proliferation (Pearson and Marth, 1989). This survival and proliferation of *L. monocytogenes* at refrigeration temperatures causes a serious food safety hazard in ready-to-eat food products, such as deli meats, cheeses, and frankfurters.

b. pH

An additional factor for growth and survival of *L. monocytogenes* is the organism's ability to grow over a wide pH range. The optimum pH range for growth is 6-8, with possible growth between pH 4.1-9.6 (Pearson and Marth, 1989; Tienungoon, *et al.*, 2000). The minimum pH capable of supporting the growth of *L. monocytogenes* is dependent upon temperature, water activity (Aw), and the composition of the food product. The pH range for meat products is approximately 5.1-6.2 for ground beef, 5.9-6.1 for ham, 6.0 for veal, and 6.2-6.4 for chicken (Jay, 2000). Since the pH range of meat products is well within the growth range of *L. monocytogenes*, contamination is an important concern in ready-to-eat meat products. At 10°C, the minimum pH for growth was reported in one study to be 4.4 (Colburn *et al.*, 1990), while no growth occurred at or below pH 5.13 at 5°C. In an additional study, growth was observed above pH 5.0 at 10°C, but only above pH 6.0 at 5°C (McClure et al., 1991). These studies show that the

minimum growth pH of *L. monocytogenes* increases with decreasing temperature. This indicates that a combination of a low pH with a low temperature may be helpful in preventing *L. monocytogenes* growth in contaminated food products.

c. Water Activity (Aw)

In addition to temperature and pH, water activity (Aw) also affects the growth and survival of *L. monocytogenes*. As previously stated, *L. monocytogenes* is known for its ability to grow in environments that many other pathogens cannot. This organism has been shown to survive and grow at unusually low Aw. In one study, Petran and Zottola reported growth of the pathogen in a 39.4% sucrose solution with a Aw of 0.92 (Petran and Zottola, 1989). *Listeria monocytogenes* did not grow, but was observed to survive at 4°C in hard salami at a Aw of 0.79-0.86 (Johnson et al., 1988). The Aw of most fresh meat products is above 0.99 (Jay, 2000). Due to the ability of *L. monocytogenes* to grow at extremely low Aw, ready-to-eat meat products provide an exceptional environment for survival and proliferation. The combination of a low Aw with low temperature, high CO₂ atmosphere, chemical preservatives, and a low pH may be helpful in reducing these food safety hazards.

d. Microbial Competition

The growth and survival of *L. monocytogenes* in meat products may also be affected by microbial competition of normal spoilage organisms. Ready-to-eat meat products may contain psychrotrophic and lactic acid bacteria that compete with *L. monocytogenes* for available nutrients. Gram-negative bacteria (*Pseudomonas* and related bacteria), are the main spoilage organisms in meat products that are stored aerobically and can be inhibited if stored in vacuum or within a high CO₂ atmosphere (Ingram, 1962). When the growth of these organisms is suppressed, growth of lactic acid bacteria (lactobacilli, pediococci, leuconostocs, and streptococci) may proliferate (Brody, 1989).

Lactic acid bacteria are able to grow anaerobically, at low temperatures, and high salt concentrations. These factors may be responsible for souring, slime formation, and off-color and off-flavor production in meat products. The use of lactic acid bacteria

producing hydrogen peroxide, lactic acid, and bacteriocins to inhibit the growth of *L. monocytogenes* in food products have recently been reported (Juven et al., 1997). *L. monocytogenes* was inhibited by the pediocin AcH producing *Pediococcus acidilactici* JBL1095 in vacuum-packaged beef frankfurters at 25°C for 8 days (Degnan et al., 1991). *Lactobacillus. alimentarius* FloraCarn L-2 was shown to reduce the population of *L. monocytogenes* in vacuum packaged ground beef from 7.4 log₁₀ CFU/g to 4.3 log₁₀ CFU/g at 4°C (Juven et al., 1997). In addition, a 2 log₁₀ CFU/g reduction of *L. monocytogenes* by a bacteriocin-producing *Pediococcus* was also observed in fermented semidry sausage (Berry et al., 1989).

e. Atmosphere

Listeria monocytogenes has been observed to grow aerobically or anaerobically, and growth of the organism is enhanced in an atmosphere containing 5-10% CO₂. Due to the large number of food products that are currently being vacuum packaged, growth of *L. monocytogenes* in temperature-abused, vacuum-packaged ready-to-eat meat products has recently become a major concern in the food industry. Although conflicting research exists, in an anaerobic atmosphere, minimal growth of *L. monocytogenes* is expected at temperatures at or below 4°C. One report showed an increase of 5x10² to 2.1x10⁵ CFU/g in vacuum-packaged frankfurters during 20 days of storage at 4°C (Buncic *et al* (1990). After 63 days of storage, a 7-log increase of *L. monocytogenes* was also observed in vacuum packaged cooked turkey bologna stored at 4°C (Wederquist, *et al.*, 1994). However, an increase of only 1 log₁₀ CFU/g of *L. monocytogenes* was seen in vacuum packaged ground beef held at 4°C for 9 weeks of storage (Juven et al., 1997). Another study reported a lack of growth of *L. monocytogenes* in vacuum packaged meat stored at 5°C (Tsigarida *et al.*, 2000). A lack of *L. monocytogenes* growth was also seen in vacuum packaged Canadian retail wieners during 28 days of storage at 5°C (McKellar *et al.*, 1994). Due to the growing concern with this pathogen in vacuum-packaged, temperature-abused, ready-to-eat food products, other modified atmosphere packaging methods have been researched. These include carbon dioxide (CO₂) and nitrogen (N₂) particularly for their ability to increase shelf life while inhibiting the growth of pathogenic microorganisms.

A modified high CO₂ packaging atmosphere has recently been shown to provide decreased growth of *L. monocytogenes* in food products. The lag time and generation time of this pathogen was reported to increase with an increasing CO₂ concentration, compounded with a pH and temperature decrease (Farber et al., 1996). A 100% CO₂ atmosphere provided an 8 day lag phase, while *L. monocytogenes* reached almost a 5-log increase in 8 days at 5°C on vacuum-packaged cold-smoked salmon (Nilsson et al., 1997). Growth of the organism was inhibited by 100% CO₂ on chicken breast meat at 6°C (Hart et al., 1991). Farber and Daley also discovered inhibition of *L. monocytogenes* in turkey roll slices packaged under an atmosphere containing 70% CO₂ and 30% N₂ (Farber and Daley, 1994).

f. **Chemical Preservatives**

Sodium nitrite (NaNO₂) and sodium chloride (NaCl) are two chemical preservatives used in the meat industry that have exhibited antilisterial attributes. NaNO₂ was shown to be bacteriostatic at a pH of 6.0 and at a temperature of 5°C (Buchanan *et al.*, 1988). NaNO₂ (50 µg/ml) was also found to prevent the growth of *L. monocytogenes* at pH 5.3 when held at 20°C for 21 days (McClure *et al.*, 1991). By increasing the salt content from 3 to 3.5% NaCl, only a small affect on growth of the organism was observed (Junttila et al., 1989). NaCl (2%) alone has been shown to have a small inhibitory effect on *L. monocytogenes*. However, in combination with other antimicrobials such as 2-3% sodium lactate, growth can be completely inhibited (Chen and Shelef, 1991).

Organic acid dips incorporated into the production of ready-to-eat processed meats prior to packaging may also provide an additional measure that significantly reduces *L. monocytogenes*' growth. The use of lactic and acetic acid (0.5%) both prevent the growth of the organism on vacuum packaged frankfurters at 5°C for 90 days (Palumbo and Williams, 1993). In addition, the use of 0.1% acetic, citric, and lactic acids inhibits the organism, while the use of 0.3% acetic acid fully inactivated *L. monocytogenes* at 7, 13, 21, and 35°C (Ahamad and Marth, 1989). Although the use of organic acids is more successful at inhibiting *L. monocytogenes* than the salts of these acids, a higher concentration of these salts can be used without damaging the quality of

the food product (Buncic *et al.*, 1995). Sodium lactate, sodium acetate, and sodium diacetate have all shown potential for suppressing growth of *L. monocytogenes* in meats and poultry. These additives are “generally recognized as safe (GRAS)” for their use in foods, and are currently regulated by the USDA for their use in meat and poultry products.

B. *Listeria monocytogenes* and Ready-To-Eat Meat Products

1. Significance

The ability of *L. monocytogenes* to grow in many different processing environments causes it to be very difficult to eliminate from a food production facility. Although *L. monocytogenes* can be destroyed if heated to a high enough temperature, cross-contamination of the food product after the heat treatment followed by temperature abuse can allow the organism to multiply (Buncic *et al.*, 1990; Zaika *et al.*, 1989). Due to the risk of cross-contamination, post-processing treatments are needed to eliminate *L. monocytogenes* in ready-to-eat food products, such as frankfurters.

In a 1991 study, samples of vacuum packaged ready-to-eat meats were taken from retail stores to determine the prevalence of *L. monocytogenes*. The contamination level of this pathogen was observed to be 72% for corned beef and 33.8% for ham (Grau and Vanderlinde, 1991). The level of *L. monocytogenes* in ready-to-eat chicken and turkey products was reported by Rijpens *et al* (1996) to be 15.5%. Samples of retail frankfurters from 19 brands were also gathered to determine the presence of *L. monocytogenes* in supermarkets (Rijpens *et al.*, 1996). Of the samples taken, overall *L. monocytogenes* contamination was 7.5%. In another study, *L. monocytogenes* incidence in one sample brand was as high as 71% (Wang and Muriana, 1993). Frankfurters and deli meats are of particular interest for food safety due to the recent outbreaks of *L. monocytogenes*, in part because these meat products are sometimes eaten without thorough re-heating. Twenty-nine illnesses, including four deaths and three miscarriages/stillbirths were associated with *L. monocytogenes* contamination of deli turkey meat from Cargill Turkey Products, Inc. (CDC, 2000). In 1998, at least 50 illnesses caused by *L. monocytogenes* serotype 4b were reported to the CDC. The mode of transmission was identified as frankfurters and other meat products from Bil Mar Foods (CDC, 1998). Bil Mar Foods voluntarily

recalled these products due to post processing contamination of *L. monocytogenes*. In 1989, a case of listeriosis was documented, and isolates were taken from the patient and from opened and unopened packages of turkey frankfurters found in the patient's refrigerator. These isolates were confirmed as *L. monocytogenes*, where each isolate contained the same serotype and enzyme type of the organism (CDC, 1989). After the 1989 case of human listeriosis, USDA Food Safety and Inspection Service (FSIS) investigators and the Center for Disease Control (CDC) evaluated the facility to determine the points on the production line where contamination was occurring (CDC, 1989). The investigation led to the determination that minimal contamination occurred before the cooking step, while the majority of contamination occurred during the peeling step, immediately prior to packaging (Wenger et al., 1990). This investigation identifies that additional steps are needed before packaging to eliminate *L. monocytogenes* from ready-to-eat food products.

2. Governmental Regulation

The food industry in the United States considers *L. monocytogenes* as an adulterant and is currently under a "zero tolerance" policy for *L. monocytogenes* in ready-to-eat foods, because the infective dose is unknown. In May 1999, the Food Safety and Inspection Service (FSIS) announced strategies for controlling *L. monocytogenes* in ready-to-eat food products (FSIS, 1999). These steps include encouraging plants to re-evaluate their HACCP plans to prevent *L. monocytogenes* contamination and growth, recommending end-product testing for *L. monocytogenes* contamination, and educating consumers on the risk of listeriosis and prevention of *L. monocytogenes* growth (FSIS, 1999). In addition, the FSIS announced their long-term goals for controlling *L. monocytogenes* in ready-to-eat food products. These goals included a study of *L. monocytogenes* post-production growth and a risk assessment of *L. monocytogenes* in a variety of food products, especially ready-to-eat foods (FSIS, 1999).

The Food and Drug Administration (FDA) along with the FSIS released the *L. monocytogenes* risk assessment in January 2001. The risk assessment was completed to provide governmental agencies a way to evaluate policies and programs regarding the reduction of *L. monocytogenes* contamination in food products and the reduction of

listeriosis nationwide (USDA, 2001). The risk assessment provides information on foodborne listeriosis from 20 food categories, and predicts the potential risk of food products that have previously been associated with *L. monocytogenes* illnesses (CFSSAN, 2001). From the risk assessment, it was determined that the overall risk of acquiring foodborne listeriosis is very small, however the illness is very severe. The factors that affect exposure to *L. monocytogenes* were also stated. These include the amount and frequency of consumer consumption of a food product, the level of *L. monocytogenes* in the food product, the actual storage temperature of the product, the ability of the food to allow growth during refrigeration, and the length of storage time before consumption (CFSSAN, 2001). It was also found that new plans to reduce post-process contamination in food production facilities are needed, as well as new plans to educate consumers on proper at-home product storage. In addition, the risk assessment identified foods that are of higher risk for contamination and need additional steps to prevent illnesses. These include fresh soft cheese, deli meats, and deli salads, as well as those foods with a high incidence of illness due to post-process contamination, including frankfurters, pasteurized mild, and mold-ripened cheese (CFSSAN, 2001).

In May 2000, the President of the United States announced his plan to reduce the number of *L. monocytogenes* illnesses by 50% in five years. The plan involves the Secretary of Health and Human Services (HHS) and the Secretary of Agriculture (USDA) currently collaborating to identify steps to reduce the number of illnesses due to this organism (DHHS, 2000). *L. monocytogenes*' ubiquitous nature causes it to be an extremely difficult organism to eliminate from the processing environment. The *L. monocytogenes* risk assessment along with immediate research to develop treatments that inhibit *L. monocytogenes* growth in ready-to-eat foods, will be beneficial in reducing the number of illnesses due to this organism each year.

C. Chemical Preservatives

1. Sodium Lactate

Sodium lactate is approved, for use in fully cooked meat and poultry up to 4.8% by weight of the total formulation, as a flavoring agent and as a means of inhibiting certain pathogenic bacteria. (FDA, 2000). This antimicrobial has been shown to prolong

shelf life by lowering the water activity of foods (Chirife and Fontan, 1980). The ability of sodium lactate to lower water activity is not its only mechanism of inhibition. Other possible mechanisms include cytoplasmic acidification, specific anionic effect, and chelating action. DeWit and Rombouts (1989) demonstrated that adverse growing conditions, particularly sub-optimum temperatures, increases the antimicrobial effects of sodium lactate. This aspect of sodium lactate could be very useful in prohibiting the growth of *L. monocytogenes* at refrigeration temperatures. Chen and Shelef (1991) also showed that the inhibitory effect of sodium lactate was augmented, and the growth of *L. monocytogenes* was suppressed by decreased moisture content in the food product.

Sodium lactate's potential for inhibiting a number of pathogens has been demonstrated in a variety of food products. Sodium lactate (6.0%) was reported to delay the growth of the *Clostridium botulinum* toxin in vacuum packaged uncured turkey products for >18 days at 28°C (Miller *et al.*, 1992). In a study using smoked salmon, Pelroy discovered that 3% sodium lactate prevented any increase in *L. monocytogenes* during 40-50 days of storage at 5°C and 10°C (Pelroy *et al.*, 1993). Wederquist *et al* (1994) reported that *L. monocytogenes* growth was inhibited in turkey bologna using 2% sodium lactate at 4°C. Furthermore, sodium lactate (1.8%) was shown to reduce the numbers of *L. monocytogenes* when compared to the control in cooked ground beef stored at 4°C (Harmayani *et al.*, 1992). Although the same results did not occur in raw ground beef as in cooked ground beef, it was shown that sodium lactate inhibits *L. monocytogenes* more than the treatments of other additives such as kappa-carrageenan, sodium erythorbate, and a combination of sodium alginate, lactic acid, and calcium carbonate. Additionally, *L. monocytogenes* is greatly reduced by using 2.4% and 4.8% sodium lactate treatments and decreasing the temperature in minced beef products (McMahon *et al.*, 1999). Sodium lactate (4%) also suppresses *L. monocytogenes* growth in sterile comminuted chicken and beef when incubated at 5, 20, and 35°C (Shelef and Yang, 1990). Inhibition of *L. monocytogenes* by sodium lactate increases with increasing fat content and decreasing temperature (Hu and Shelef, 1996). This could be very useful in preventing the growth of *L. monocytogenes* in ready-to-eat food products that are held at refrigeration temperature.

A combination treatment involving sodium lactate and an additional antimicrobial is another possibility for increasing the safety of foods. The addition of 2.5% sodium lactate and 0.25% sodium acetate to sliced and spreadable vacuum-packaged serelat sausage and cooked ham could be used to increase the safety of the product for 4-6 weeks when stored at 4°C (Blom *et al.*, 1997). Also, the antilisterial potential for sodium lactate (2.5%) in combination with sodium diacetate (0.3% and 0.1%) was exhibited in turkey slurries at 4 and 25°C (Schlyter *et al.*, 1993(b)). These treatments provide a greater antilisterial effect than when either of them used alone. Additional research is needed to investigate other combinations of food additives, and their possible effect on the inhibition of *L. monocytogenes*.

Although there has been little research done involving the inhibition of *L. monocytogenes* in turkey frankfurters, there is definite potential for the use of sodium lactate as a secondary lethal treatment. Furthermore, even less research has been done involving combination treatments of sodium lactate and carbon dioxide. Additional studies needed immediately to identify any potential use for such combinations in ready-to-eat foods.

2. Sodium Diacetate

This antimicrobial is approved as a flavoring agent in meat and poultry products at a level up to .25% by weight of the total formulation (FDA, 2000). The use of sodium diacetate to inhibit the growth of *L. monocytogenes* is also approved up to a level of .25% by weight of the total formulation (FDA, 2000). Sodium diacetate, which contains acetic acid (40%) and sodium acetate, was first proven useful in the food industry as a mold inhibitor in baked products, then later in mixed poultry feed, ensiled whole kernel corn, and corn silage (Glabe and Maryanski, 1981). It was shown that there was 90% growth inhibition on potato dextrose agar of six species of molds by 0.05-0.2% sodium diacetate at pH 3.5, and by 0.15-0.5% sodium diacetate at pH 4.5 (Glabe and Maryanski, 1981). This antimicrobial, at a concentration of 32mM, was also shown to completely inhibit the growth of *L. monocytogenes* in BHI broth at temperatures of 20 and 5°C (Shelef and Addala, 1993). Inhibition was observed to increase with decreasing temperature.

More recent studies have shown the potential usefulness of sodium diacetate in meat and poultry products, and the effects of sodium diacetate combined with other antimicrobials to control the growth of *L. monocytogenes*. The addition of 0.5% sodium diacetate alone to turkey slurries exhibited a listericidal effect. However, a combination of 0.5% sodium diacetate and pediocin or sodium lactate showed even greater listericidal ability (Schlyter *et al.*, 1993(b)). It was also reported that 0.5% sodium diacetate causes only slight inhibition of *L. monocytogenes* in turkey slurries, yet the combination of 0.5% sodium diacetate and 0.75% ALTA 2341 (a commercial shelf-life extender) shows greater inhibition (Schlyter *et al.*, 1993(a)). Additional research using sodium diacetate in combination with other antimicrobial agents to control the growth of *L. monocytogenes* in ready-to-eat meat products is needed. The addition of sodium diacetate alone delays the growth of *L. monocytogenes*, however multiple barriers may provide additional protection from food-related listeriosis.

3. Sodium Acetate

Sodium acetate has also been known to exhibit antilisterial effects. Sodium acetate is currently approved as a flavoring agent in meat and poultry products at a level up to .25% by weight of the total formulation (FDA, 2000). This ingredient is not, however, approved for use as an antimicrobial agent in meat and poultry, because sufficient data has not been submitted to support this approval. Additional research is needed to verify the usefulness of sodium acetate in controlling the growth of pathogens.

Sodium acetate has proven useful for controlling pathogens in a variety of meat and poultry products. An uncured turkey product was able to remain free of the *Clostridium botulinum* neurotoxin for over 18 days at 28°C when treated with 6% sodium acetate (Miller *et al.*, 1992). Other studies have likewise shown the importance of sodium acetate in inhibiting the growth of *L. monocytogenes*. In vacuum packaged turkey bologna, sodium acetate was determined to significantly reduce the growth of *L. monocytogenes* at 4°C by only allowing a maximum growth of 1.33 log CFU/g after 70 days of storage (Wederquist *et al.*, 1994).

Recent studies have shown the effects of sodium acetate combined with other antimicrobial agents at inhibiting *L. monocytogenes*. A combination of sodium acetate,

EDTA, and ascorbic acid at 4°C and pH 4.0-4.5 was shown to increase the inactivation of *L. monocytogenes* in BHI broth compared to the use of either antimicrobial alone, and at higher incubation temperatures (Golden *et al.*, 1994). This combination of sodium acetate with EDTA and ascorbic acid demonstrates the usefulness of antimicrobial agent combinations at suppressing the growth of *L. monocytogenes*. The use of 2.5% sodium lactate and 0.25% sodium acetate at inhibiting *L. monocytogenes* in vacuum packaged serelat sausage and sliced, cooked ham is another example of inhibition by combination treatments. Individually, 2.5% sodium lactate and 0.25% sodium acetate both strongly inhibit the growth of *L. monocytogenes* (Blom *et al.*, 1997). However, in the same study, a combination of 2.5% sodium lactate, 0.25% sodium acetate, and 2.75% salt completely inhibited the organism (Blom *et al.*, 1997). Limited research is available using sodium acetate in combination with other treatments including CO₂ atmospheres on ready-to-eat meat products. Additional research is needed to determine combinations that are capable of preventing the growth of *L. monocytogenes*, which will prevent outbreaks in ready-to-eat foods.

D. Modified Atmosphere Packaging (MAP)

1. Definition of MAP

MAP techniques have been used for many years to increase the shelf life of many products. This type of packaging has recently become more common due to consumer demands for fresh foods with a prolonged shelf life. A modified atmosphere is a change in the gaseous atmosphere surrounding the product, still allowing respiration to occur and naturally alter the package environment (Brody, 1989).

The most common gases used in MAP are oxygen, nitrogen, and carbon dioxide. Foods packaged in oxygen will have stimulated aerobic bacterial growth and inhibited anaerobic bacterial growth. Oxygen is commonly used to maintain myoglobin in its oxygenated form, which creates the typical red color of fresh red meat (Brody, 1989). Nitrogen in MAP is primarily used to replace oxygen which will delay oxidative rancidity and provide inhibition of some aerobic microorganisms (Farber, 1990). Carbon dioxide is primarily used for its ability to increase the shelf life of refrigerated food products, and inhibit pathogenic microorganisms.

2. Inhibitory Nature of Carbon Dioxide

Carbon dioxide (CO₂) increases the shelf life of foods by inhibiting bacterial growth. The specific mechanism CO₂ uses to inhibit bacteria is unknown, but the general effect on bacteria is an increase in both the lag phase and the generation time of microorganisms (Daniels *et al.*, 1984). Some theories for the mechanism of inhibition include, changing nutrient uptake and absorption of the cell membrane, decreasing enzyme activity, causing an intracellular pH change by invading the cell membrane, and changing properties of the proteins in foods (Farber, 1990). Although the specific mechanism is unknown, it is known that at lower temperatures the solubility of CO₂ is higher, which increases the effectiveness of bacterial inhibition (Brody, 1989).

3. Factors Affecting Bacterial Inhibition by MAP

The inhibitory effect of CO₂ is affected by concentration, temperature, food composition, pH, water activity, volume of headspace gas, and type of organism (Daniels *et al.*, 1984). CO₂ is usually shown to have a greater affect on gram-negative organisms than on gram-positive organisms. In general, CO₂ also decreases the growth of microorganisms with increasing concentration, decreasing temperature, and decreasing pH. *L. monocytogenes* and *Pseudomonas fragi* exhibited higher growth at 20°C than at 4°C on cooked pork packaged in a 100% CO₂/nisin combination system (Fang and Lin, 1994). Although growth of *Yersinia enterocolitica*, *Aeromonas hydrophila* and *L. monocytogenes* was observed at 10°C, none of these organisms grew at 2°C in CO₂ atmospheres (Gill and Reichel, 1989). Both the lag and generation time of *L. monocytogenes* has been shown to increase as the CO₂ level is increased and as temperature and pH are decreased (Farber *et al.*, 1996).

4. Bacterial inhibition by MAP

Meat spoilage organisms, such as *Pseudomonas*, *Micrococcus*, and *Acinetobacter* are inhibited with as little as 25% CO₂ (Brody, 1989). In the presence of this concentration of CO₂, lactic acid bacteria, which are less affected by a CO₂ atmosphere, dominate and eventually spoil the food product. *Listeria monocytogenes* is capable of

growing in completely aerobic or anaerobic environments, as well as in the presence of small amounts of carbon dioxide. Growth of *L. monocytogenes* in vacuum-packaged products, such as frankfurters has also been documented at temperatures as low as 4°C (Buncic *et al.*, 1990). The effectiveness of carbon dioxide at inhibiting this microorganism generally increases with increasing concentration of CO₂ (Daniels *et al.*, 1984). Atmospheres containing large amounts of CO₂ (> 50% CO₂) have been shown to decrease the growth rate of *L. monocytogenes* as compared to growth of this pathogen in aerobic or anaerobic atmospheres. A 100% carbon dioxide atmosphere for chicken breast meat when stored at 6°C inhibited growth of *L. monocytogenes* (Hart *et al.*, 1990). This experiment also showed that a 100% carbon dioxide atmosphere was more effective than an atmosphere containing 30% CO₂ plus air, or a 30% CO₂ and 70% N₂ environment. Additionally, there seemed to be no difference between 30% CO₂ / 70% air and the 30% CO₂ / 70% N₂ atmosphere at any temperature (Hart *et al.*, 1990). CO₂ (at levels greater than 70%) was also determined to inhibit *L. monocytogenes* in turkey roll slices at 4° and 10°C (Farber and Daly, 1994).

Little data is available involving the combined effects of CO₂ with other processing treatments on the inhibition of *L. monocytogenes*, but the research that has been done in this area shows promising results. A synergistic effect on the inhibition of *L. monocytogenes* was observed on poultry with a 90% CO₂ / 10% O₂ atmosphere and a treatment of 10% lactate acid/sodium lactate buffer (Zeitoun and Debevere, 1991). This combination treatment was shown to be more effective than either treatment alone. An additional reduction in *L. monocytogenes* due to a combination of nisin and carbon dioxide was seen in cold-smoked salmon (Nilsson *et al.*, 1997). The combination of a high carbon dioxide atmosphere with nisin at 5°C provided a delayed growth of *L. monocytogenes* in the salmon as compared to either treatment individually (Nilsson *et al.*, 1997). A treatment of 2% lactic acid combined with a 74.8% CO₂/10.4% O₂/14.8% N₂ atmosphere resulted in an 8-day extended lag phase of *L. monocytogenes* compared to a treatment of 2% lactic acid with vacuum or air packaging (Pothuri, *et al.*, 1995). In addition, an environment of 44% CO₂/56% N₂ combined with sodium lactate extended the lag phase of *L. monocytogenes* by 10 days at 3.5°C (Barakat and Harris, 1999). These experiments show the possible inhibition of *L. monocytogenes* by the combination

of CO₂ and other chemical antimicrobials, and the importance of innovative research involving different treatment combinations. Combining the usage of CO₂ with sodium lactate, sodium acetate, or sodium diacetate are a just a few examples of treatments that could possibly be useful in eliminating *L. monocytogenes* in ready-to-eat meat products.

5. Advantages and Disadvantages of MAP

The use of MAP to store food products can increase shelf life by 50-400%, which reduces economic loss, allows for longer distribution distances, and supplies a better quality product (Farber, 1990). There are many advantages of using MAP to prolong the shelf life of a food product, but there are also a few disadvantages that should be noted. MAP can be more expensive than vacuum packaging or packaging with air. Also, different formulations of gases are needed for different products, strict temperature control are mandatory, and additional equipment is necessary for its use (Farber, 1990). An increased risk of food-borne illness by anaerobic or facultative anaerobic organisms may also occur.

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