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Inhibition of Listeria monocytogenes on frankfurters treated with organic acid salts

Zheng Lu
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Inhibition of *Listeria monocytogenes* on frankfurters treated with organic acid salts

by

Zheng Lu

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Meat Science

Program of Study Committee:
Joseph Sebranek, Major Professor
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Ames, Iowa
2004

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has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program
DEDICATION

This dissertation is dedicated to my husband, Chunwang, my grandma Xinzhu Cao, my mom Xueqin Zhang, my dad Zhiguang Lu and my little brother, Yanyi.
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Several listeriosis outbreaks linked to the consumption of food products have occurred in the last two decades. Post-processing contamination of frankfurters with *L. monocytogenes* after cooking or before packaging is one such major concern for the food industry and has become an important food safety issue for meat processors.

The hypothesis for this study was that surface dipping of frankfurters with chemical preservatives or their combinations will inhibit *L. monocytogenes* growth on frankfurters with the degree of inhibition dependent upon temperature.

Phase I of this study was an evaluation of different anti-microbials for effectiveness. Surface dipping solutions of organic acids, e.g. sodium diacetate (SD), sodium lactate (SL), potassium sorbate (PS) and potassium benzoate (PB), were used singly or in combinations at 6% total concentration to control *L. monocytogenes* growth on inoculated (10⁵ CFU/g) vacuum-packaged frankfurters stored at 4.4 °C for 14 days. The maximum population density of *L. monocytogenes* was decreased in SD, SD/PB and SD/SL/PB treatment groups compared with the others in Phase I. In Phase II these three treatments were investigated in more detail at different concentrations (3.0% and 6%) and storage temperatures (-2.2 °C, 1.1 °C, 4.4 °C, 10.0 °C and 12.8 °C) for up to 90 days. The growth of *L. monocytogenes* was significantly decreased when stored at lower temperature (-2.2 and 1.1 °C) compared with the other temperatures. Surface treatments with 6% SD, 6% SL/SD/PB, 3% SD/PB and 6% SD/PB effectively inhibited *L. monocytogenes* growth at 1.1 °C compared with the control group, and the treatment 6% SD was the most effective. The 6% SD/PB treatment increased meaty flavor score, and the 3% SL/SD/PB decreased smoke flavor score of the frankfurters (*P < 0.05*) as assessed by sensory panels, however, no sensory difference was observed in saltiness, sourness and pepper flavors among surface-treated non-inoculated frankfurters. The SL/SD/PB treatment at either 3 or 6% significantly increased the lightness (L*) and decreased the redness (a*) color values for frankfurters compared with the control group. Storage time longer than two months also significantly increased L* value and decreased a* value.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

*Listeria monocytogenes* is a gram-positive, psychrotrophic facultative anaerobe that can tolerate acids (Sorrells et al., 1989), sanitizers and antibiotics (Jay, J. M., 2000). Several listeriosis outbreaks linked to the consumption of food products have occurred in the last two decades. Post-processing contamination by *L. monocytogenes* after cooking or before packaging of frankfurters is a major concern for the meat industry and has become an important food safety issue. The sources of *L. monocytogenes* are widespread, including raw meat, ready-to-eat (RTE) meat, poultry, raw milk, pasteurized milk, dairy products and vegetables (Jay, J. M., 2000).

Ingestion of food-borne *L. monocytogenes* has been demonstrated to result in listeriosis in susceptible populations. The presence of *L. monocytogenes* in meat products has received increased attention worldwide, and researchers in both the United States and United Kingdom have suggested that all *L. monocytogenes* should be considered as potential pathogens. Furthermore, US federal regulatory agencies have established a “zero tolerance” policy for *L. monocytogenes* in RTE foods, due to the outbreaks of listeriosis (Jay, J. M., 2000) from these food products.

Grau and Vanderlinde (1992) found *L. monocytogenes* on 53% of vacuum-packaged, processed, deli- and luncheon-style meats. Differences in the composition in regard to pH, nitrite level and salt concentration were identified as important parameters for the survival and growth of listeriae (Jay, J. M., 2000). It has been suggested that chemical preservatives, such as diacetate (Islam et al., 2002), lactate (Maca et al., 1999), sorbate (Islam, et al., 2002) and benzoate (Islam et al., 2002), could affect the growth of microorganisms by acting on the cell wall and the cell membrane, destroying their structure and subsequently the transport mechanism of nutrients to the cell (Jay, J. M., 2000). The efficiency of most preservatives is governed by a dose-effect relationship. Also, use of chemical preservatives in combination often increases the degree of anti-microbial action and lowers the necessary concentration of individual substances (Jay, J. M., 2000). Because *L. monocytogenes* contamination on RTE meats is typically on the surface due to post-heating contamination, surface treatments of
these products with anti-microbial compounds seems to be a logical approach. However, more information is needed to determine the most effective inhibitors to use for surface treatment of frankfurters in order to inhibit *L. monocytogenes*.

Therefore, the purpose of this study was to evaluate four potential inhibitors (sodium lactate, sodium diacetate, potassium sorbate and potassium benzoate), alone and in combination, to assess their effectiveness against *L. monocytogenes* on frankfurters.

**Dissertation Organization**

This dissertation contains a general introduction, a literature review, four chapters on experimental work, and general conclusions. A general introduction is included at the beginning as Chapter 1. Chapter 2 includes a literature review in two sections. The first section is a review of *L. monocytogenes*; and the second section is a review of organic acid salts and their effects on *L. monocytogenes*. Four individual manuscripts, which are to be published, are individually designed as Chapter 3, 4, 5 and 6, a general conclusion including recommendations for future research comprises Chapter 7. These chapters are followed by an appendix, a list of references cited and acknowledgements at the end of this dissertation. The dissertation uses the standard format as prescribed by the thesis office at Iowa State University.
CHAPTER 2. LITERATURE REVIEW

A food safety perspective of *Listeria monocytogenes* in meat

A paper to be submitted to Food Microbiology

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Keywords: *Listeria monocytogenes*, foodborne listeriosis, food safety, meat

Abstract

*Listeria monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, and *L. ivanovii* are six currently recognized species of *Listeria spp.*, with serovars 1/2a and 4b of *L. monocytogenes* the most common pathogens among the 17 serovars of *Listeria spp.* *L. monocytogenes* is a gram-positive, non-spore-forming, non-acid-fast rod that is facultative anaerobic, psychrotrophic and tolerates acids, sanitizers and antibiotics. Listeriosis-susceptible human populations include organ transplant patients, patients with AIDS, HIV-infected individuals, pregnant women, patients with cancer and the elderly. Many meat products, including ready-to-eat (RTE) products such as frankfurters and deli meats, have been reported to be associated with foodborne listeriosis, therefore, handling, storage, processing and associated food supply systems must be carefully managed by food producers and processors to control the growth of *L. monocytogenes*. “Zero tolerance” of *L. monocytogenes* is required to assure the safety of food products with respect to this foodborne bacterial pathogen. To achieve control of this organism, it is necessary to have up-to-date understanding of the conditions that affect *L. monocytogenes* growth and survival, including factors such as nutrients, pH, temperature and a$_w$. Food processing technologies such as chemical preservatives, irradiation, bacteriocins, low temperature, pasteurization and MAP systems have been shown to impact growth of *L. monocytogenes*. Appropriate use of these technologies, particularly in combination, offers potential for improved control of *L. monocytogenes* on meat products.

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Introduction

The incidence and concern for *L. monocytogenes* in meat products has received increased attention worldwide, not only because of increased foodborne listeriosis over the last two decades (Bernard and Scott, 1999; Tompkin et al., 1999), but also because the unique characteristics of *L. monocytogenes* make this organism one of the most dangerous food pathogens. Meat products, including ready-to-eat (RTE) meat products, such as frankfurters and deli meats (CDC 2003; Farber and Peterkin, 1999; Samelis et al., 2001), have been reported to be the sources of listeriosis outbreaks; thus, determining the unique characteristics of *L. monocytogenes*, understanding the factors affecting *L. monocytogenes* growth and achieving improved control of *L. monocytogenes* in meat products have become very important objectives of food safety research.

**Taxonomy of Listeria spp.**

*Listeria monocytogenes* is a gram-positive, non-spore-forming, non-acid-fast rod. It was named after the British surgeon Lord Joseph Lister who pioneered the concept of antiseptic surgery in the 1860s to prevent surgical sepsis. For many years, *L. monocytogenes* was the only species recognized in the genus *Listeria*, which was originally named as *Bacterium monocytogenes* (Murray et al., 1926). The original name was derived from the observation that the rabbits became infected by large mononuclear leucocytosis.

The six currently recognized species are *L. monocytogenes*, *L. innocua*, *L. welshimexi*, *L. seeligeri*, *L. grayi*, and *L. ivanovii*; *L. ivanovii* has two subspecies, *ivanovii* and *londoniensis*. The six species of *Listeria* have been characterized by serotyping antigens and have given rise to 17 serovars. The primary pathogenic species, *L. monocytogenes*, is represented by 13 serovars, some of which are shared by *L. innocua* and *L. seeligeri*.

Surprisingly, different serovars of *Listeria* have shown some geographical differences, e.g. serovar 4b has accounted for 65-80% of all strains observed in the United States and Canada (Seeliger, H. P. R., 1961), but serovar 1/2a is the most frequently reported serovar in Eastern Europe, West Africa, Central Germany, Finland, and Sweden, while serovars 1/2a and 4b have been more often reported in equal proportions in France and the Netherlands (Seeliger and Höhne, 1979). Between January 1, 1966 and June 30, 1996, 60% of the 2,232
isolates from human cases in the United Kingdom were 4b with 17%, 11%, and 4% caused by 1/2a, 1/2b, and 1/2c, respectively (McLauchlin, J. 1997).

In addition to serotyping, a variety of other methods have been applied to species and subspecies characterization of listeriae, such as bacteriophage typing, multilocus enzyme electrophoresis (MEE) typing, restriction enzyme analysis (REA), pulsed field gel electrophoresis (PFGE), restriction fragment length polymorphisms (RFLP), and ribotyping (Jay, J. M., 2000).

**Characteristics of *L. monocytogenes***

*L. monocytogenes* is a facultative anaerobic, psychrotrophic bacteria that can tolerate acids (Conner et al., 1986; Conner et al., 1990; Doyle, M. P., 1988; Sorrells et al., 1989), sanitizers (Zottola, E. A., 1994) and antibiotics (Jay, J. M., 2000). The organism is commonly found in soil, water and on plant material, particularly that undergoing decay, with these environments being regarded as the natural habitat of the organism (Rocourt and Seeliger, 1985). Table 1 shows the persistence of *L. monocytogenes* in various natural and agricultural environments (Bell and Kyriakides, 1998; Fenlon, D. R., 1999). The ability to survive for long periods in a variety of environments may explain why the natural environment is a common reservoir of contamination for animal and plant products used as human food. Although *L. monocytogenes* is widely distributed, the total number of the organism present in most environmental habitats is very low.

The six recognized species of listeriae with differentiating characteristics, are listed in Table 2 (Bell and Kyriakides, 1998; Jay, J. M., 2000). The CAMP (Christie-Atkins-Munch-Petersen) test listed in table 2 is a synergistic haemolytic reaction in which a characteristic enhancement of the haemolytic reaction is obtained on sheep blood agar when a specific weakly beta haemolytic strain of *Staphylococcus aureus* is grown in close proximity to *L. monocytogenes* but not when grown in close proximity to *Rhodococcus equi* (Harrigan, W. F., 1998). It is considered as the definitive test for *L. monocytogenes*. Usually, an isolate that is CAMP positive with either *S. aureus* or *R. equi* will be considered as a presumptive *L. monocytogenes* isolate, but not necessarily a virulent one. However, the actual mechanisms of the *S. aureus-* and *R. equi-* *L. monocytogenes* reactions are unknown (MeKellar, R.C. 1994).
Foodborne Listeriosis

Ingestion of food-borne *L. monocytogenes* has been demonstrated to result in listeriosis in susceptible populations (Miller et al., 1997). With the increased foodborne listeriosis outbreaks worldwide (Bernard and Scott, 1999; Tompkin et al., 1999), the incidence and behavior of *L. monocytogenes* in meat products has received more and more attention, and researchers in both the United States and United Kingdom have suggested that all *L. monocytogenes* should be considered as potential pathogens (Brackett, R. E, 1988; Slutsker and Schuchat, 1999). Also, US federal regulatory agencies have established a "zero tolerance" policy for *L. monocytogenes* in RTE foods, due to the increased outbreaks of listeriosis from these food products.

**Virulence properties of *Listeria* spp.**

Among the *Listeria* species, *L. monocytogenes* is the pathogen of most concern for human health (Datta and Wentz, 1989) with the infection dose for sensitive groups being about 100 to 1000 cells (Jay, J. M., 2000). Although *L. ivanovii* has been reported to multiply in the mouse model, this organism does not cause infection until the population reaches $10^6$ CFU/ml. The other four species of *Listeria*, *L. innocua*, *L. seeligeri*, *L. grayi*, and *L. welshimeri*, are non-pathogens.

*L. monocytogenes* can cause a variety of infections, at locations including the uterus, bloodstream or central nervous system (Table 3). The most significant virulence factor associated with *L. monocytogenes* is listeriolysin O, which is produced during the exponential growth phase and causes haemolysis in the victims. The concern over listeriosis is not only due to the increased recent outbreaks, but also the relatively high mortality rate. It has been reported that listeriosis can result in approximately 30% fatalities (Newton et al., 1992), which is much higher than the mortality caused by pathogens such as *E. coli* or *Salmonella*.

The primary high-risk groups for listeriosis, in order of descending risk, are organ transplant patients, patients with AIDS, HIV-infected individuals, pregnant women, patients with cancer and the elderly. Individuals with AIDS, for example, suffer listeriosis 65-145 times more frequently than the general population (Jurado et al., 1993). Pregnant women are about 20 times more likely than other healthy adults to contact listeriosis (Tappero et al.,
Further, listeriosis in pregnant women can result in spontaneous abortion, stillbirth or birth of severely ill babies. However, the mother is rarely affected by listeriosis as the disease appears to focus on the fetus. New-born babies may also acquire listeriosis by postnatal infection from the mother or from other infected babies. Healthy non-pregnant adults may also suffer listeriosis even though the most vulnerable groups, e.g. the immunocompromised and the elderly, are those with reduced immune system function. In such groups, listeriosis usually results in meningitis (infection of the tissues surrounding the brain) and/or septicaemia (infection in the bloodstream).

Incidence of listeriosis

The first reported case of human listeriosis was in 1929, and since then the disease has been observed sporadically throughout the world (McLauchlin, J. 1987). Prior to 1974, 15 documented cases were reported in western France, but in 1975 and 1976 there were 115 and 54, respectively, and in 1987, there were 687 cases in France. In the United States, the estimated number of listeriosis cases in 1993 was 1,092 with 248 deaths (Jay, J. M., 2000), and in 2002 it was 2,500 with 500 deaths (Schuchat et al., 1992).

In recent years, a decrease in pregnancy-related cases, and increase in the vulnerable non-pregnant adults cases within the total population, has been recorded. This change may have occurred because pregnant women began to follow recommendations made in the late 1980s to avoid high-risk foods during pregnancy. Of 2005 listeriosis cases in England and Wales between 1983 and 1996, a total of 659 (33%) were associated with pregnancy (Bell and Kyriakides, 1998), and the remaining 1346 cases of listeriosis primarily related to immunocompromised and elderly groups.

Nature of L. monocytogenes infections and the listeriosis syndrome

Among the Listeria genus, the species L. monocytogenes is widely recognized as the principal human pathogen. The Public Health Laboratory Service (PHLS) of England and Wales identified the strains of Listeria isolated from 2237 human listeriosis cases in the UK, and all but two strains were L. monocytogenes. However, L. seeligeri and L. ivanovii, on rare occasions, have also been implicated in human infections.

L. monocytogenes is relatively easily killed by direct thermal pasteurization. Recent experiments using sealed bags (Beckers et al., 1987), sealed glass reaction vials (Donnelly et
al., 1987), or borosilicate glass tubes (Bradshaw et al., 1985; Bradshaw et al., 1987), immersed in water, demonstrated the inability of \textit{L. monocytogenes} to survive a heat treatment at 63 °C for 30 min or 72 °C for 15 s. However, as an intracellular parasite, \textit{L. monocytogenes} can grow inside leukocytes of humans or animals. This means that \textit{L. monocytogenes} may be afforded extra heat protection when it resides within these cells (Farber, J. M., 1989), and may still cause listeriosis outbreaks from pasteurized milk in such conditions. In 1983, an outbreak of listeriosis epidemiologically linked to whole and/or 2% pasteurized milk occurred in the Massachusetts area (Fleming et al., 1985). There were 49 patients involved with 29% mortality rate, but there was no evidence of improper pasteurization or of post-pasteurization contamination. This incident suggested the possibility of \textit{L. monocytogenes} survival during pasteurization (Farber, J. M., 1989), and showed that experiments done in an open test-tube system can give misleading results (Beckers et al., 1987; Donnelly et al., 1987).

\textit{L. monocytogenes} infections and listeriosis involve a variety of factors including host immunity, level of inoculum and virulence of the specific \textit{L. monocytogenes} strain. However, the mechanisms of pathogenicity of the organism are not clearly understood. It has been suggested that serotype may be an important indication for assessing the risk to an individual who has consumed food containing \textit{L. monocytogenes}. In the vast majority of large foodborne outbreaks, serotype 1/2a and 4b have been the epidemic strains (Table 4, Rocourt, J. 1994).

Immunological control of \textit{L. monocytogenes} in the body is achieved by T-lymphocytes and activated macrophages, and thus any condition that adversely affects these cells will exacerbate the course of listeriosis. The most effective drugs for treatment are coumermycin, rifampicin, and ampicillin. Ampicillin plus an aminoglycoside antibiotic appears to be the best combination (Espaze and Reynaud, 1988). Even with that regimen, antibiotic therapy for listeriosis may not be entirely satisfactory because ill patients and compromised hosts are more difficult to treat than competent hosts (Jay, J. M., 2000).

\textbf{Outbreaks of \textit{L. monocytogenes} Associated with Meats}

A large number of listeriosis outbreaks linked to the consumption of food products have occurred in the last two decades (Bernard and Scott, 1999; Tompkin et al., 1999). Those
listeriosis outbreaks were associated with variety of food products, including raw meat, RTE meat (Farber and Peterkin, 1999), poultry (Pinner et al., 1992; Schuchat et al., 1992), raw milk (Ryser, E. T., 1999), pasteurized milk (Fleming et al., 1985), dairy products and vegetables (Heisick et al., 1989). Post-processing contamination by *L. monocytogenes* after cooking or before packaging of RTE meat, such as deli-meats or frankfurters, is a major concern for the meat industry and has become an important food safety issue (Samelis et al., 2001). Grau and Vanderlinde (1992) reported that *L. monocytogenes* was found on 53% of vacuum-packaged, processed, deli- and luncheon-style meats. These are a significant concern because many of these products are consumed without heating. Table 4 summarizes information concerning a few of these outbreaks, indicating both the worldwide nature of outbreaks and the wide range of foods involved (Bell and Kyriakides, 1998, CDC, 2003; Jay, J. M., 2000).

**Raw meats**

The muscles of live animals are considered to be essentially sterile before slaughter, but can be easily contaminated by microorganisms, including *L. monocytogenes*, during slaughtering or processing. The incidence of *Listeria* on carcasses is about 0 - 9%, and in comminuted raw meats can be 80 - 100%. In the United States, the incidence of *Listeria spp.* on intact muscle cuts such as raw roasts and steaks ranges from 0 to 6%, with the incidence of *L. monocytogenes* in the same range. In contrast, comminuted raw meats showed a much higher incidence of *Listeria spp.*, ranging from 24 to 100%, while *L. monocytogenes* ranged from 0 to 25%. However, the incidence of *Listeria spp.* in raw meats, e.g. beef, pork and lamb, in the United Kingdom ranged from 59 to 88%, where *L. monocytogenes* was reported at an incidence of 28 to 40%, which is much higher than the United States. Other Europe countries, such as Ireland, Germany, Italy, Switzerland, and Spain, all have been found to have a high percentage of positive samples in raw meat products, particularly in ground meats. Japan had the highest incidence of *Listeria* in raw meats among Asian countries, with about 80 to 100% in the ground meats, with 40 to 61% being *L. monocytogenes*. Trinidad had the lowest incidence of *Listeria spp.* in raw meats and ground meats, with less than 11% positive samples found in either of these products (Farber and Peterkin, 1999).

**Cooked and RTE meats**
L. monocytogenes is obviously a greater concern in processed, RTE meats, which may be consumed without further heating, than in raw meat products that are cooked before consumption. Numerous worldwide surveys have been conducted since 1990 to determine the incidence of L. monocytogenes on RTE meat.

Several surveys on incidence of L. monocytogenes in retail wiener and frankfurters in the U.S. have been conducted, due to the listeriosis outbreaks associated with consumption of these products (Farber and Peterkin, 1999). A study of 19 brands of retail wiener, with a total of 93 samples, showed a 10% incidence of Listeria, including 8% incidence of L. monocytogenes. One brand among those 19 brands contained Listeria in 83% of the 24 packages studied, including 71% with L. monocytogenes (Wang and Muriana, 1994). Another survey found 20% contamination of Listeria in 30 packages of retail wiener, with 17% being L. monocytogenes. In France, 22% of 18 dry sausages were found positive for L. monocytogenes, whereas in Germany, 9% of mettwurst samples contained L. monocytogenes (Farber and Peterkin, 1999). Surveys of RTE meat products in Spain showed a 13% incidence of Listeria spp. in 32 cured sausage samples (Farber and Peterkin, 1999), with 12% being L. monocytogenes.

Factors Affecting Survival and Growth of L. monocytogenes

L. monocytogenes is present and will continue to be found in a wide variety of raw food materials. In order to assure the safety of food products with respect to this potential foodborne bacterial pathogen, handling, storage, processing and associated food supply systems must be managed by food producers and processors in a reliable way to control the growth of L. monocytogenes, particularly in RTE meat products. L. monocytogenes must be reduced from potentially harmful levels to 0 CFU/ml in RTE products. To achieve the zero tolerance objective, it is necessary to understand the conditions and factors that affect growth and survival of this organism.

Nutrients

Like most other bacteria, such as E. coli and Salmonella, nutrients are necessary for L. monocytogenes growth. According to research thus far, at least four B vitamins are required for proper growth of L. monocytogenes; biotin, riboflavin, thiamine, and thiocetic acid. Thiocetic acid is an α-lipolic acid and a growth factor for some bacteria and protozoa. The
amino acids, cysteine, glutamine, isoleucine, leucine, and valine are also required for growth of *L. monocytogenes* (Jay, J. M., 2000).

**Temperature**

A major food safety concern for *L. monocytogenes* derives from the ability of the organism to survive and sometimes grow slowly at temperatures used for refrigeration (0 °C to 8 °C). Therefore, refrigerated storage alone is no guarantee for prevention of growth (McClure et al., 1991). The relationships between growth parameters, i.e. lag time (days) and generation time (h), and temperatures are shown in Table 5. Obviously, lag time and generation time increase linearly as temperature decreases from 13 to 0 °C.

*L. monocytogenes* is a psychrotrophic bacteria, not very heat-resistant, and properly controlled cooking processes will achieve significant reductions in numbers of the organism. Cooking at 60 °C for 45 min or 75 °C for 30 s will kill $10^6$ CFU/ml *L. monocytogenes*. If the internal temperature of the product reaches 85 °C, $10^6$ CFU/ml *L. monocytogenes* will be killed within 1 s (Bell and Kyriakides, 1998).

**pH**

*L. monocytogenes* grows best in the pH range of 6 to 8, when all other growth conditions are optimal. Generally this pathogen grows within a temperature range of 1 °C to 45 °C, and some strains can grow at the pH range of 4.1 to 9.6 (Jay, J. M., 2000). In general, factors such as temperature of incubation, general nutrient composition of growth substrate, water activity ($a_w$), and the presence and quantity of NaCl and other salts or inhibitors influence the minimum pH at which bacteria may grow. Growth of *L. monocytogenes* in culture media was observed at a pH of 4.4 in less than 7 days at 30 °C (George et al., 1988), at a pH of 4.5 in tryptose broth at 19 °C, and at pH value of 4.66 in 60 days at 30 °C (Colburn et al., 1990).

When the pH of the environment becomes non-optimal (usually acidic), the pH will contribute to control of bacterial growth, including any *Listeria spp.* that may be present. Non-optimal pH may be the result of either the manufacturing process, e.g. cheese or fermented meats in which lactic acid is produced by the starter cultures used, or by the direct addition of an acidic component, e.g. oil and vinegar (acetate) dressing. Organic acid
solutions may also be utilized for surface applications of potentially contaminated food products.

When organic acids, e.g. acetic acid, lactic acid, citric acid, etc., are used as preservatives in foods, it is important to ensure that the correct concentration of undissociated acid, which is responsible for the antimicrobial activity, is available for bacterial growth inhibition. When determining the amount of total acid required to reach a particular concentration of undissociated acid, the pH value must be taken into account, since the proportion of undissociated acid present varies with pH values (Table 6). For example, at a neutral pH, most organic acids will have a limited effect on the growth of *L. monocytogenes* (Bell and Kyriakides, 1998).

**Water activity**

*L. monocytogenes* can not grow if the $a_w$ is less than 0.93, thus, reducing $a_w$ of the product by adding salt, evaporation, or freezing, can effectively prevent *L. monocytogenes* growth. It has been reported that *L. monocytogenes* can grow in about 10% NaCl, 0.025% thallous acetate, and 0.04% potassium tellurite, but it will not grow in the presence of 0.02% sodium azide (Jay, J. M., 2000). However, in the meat industry only a few meat products such as beef jerky have a suitably low $a_w$, and most meat products e.g. frankfurters have $a_w$ values over 0.98. Thus, it is not likely that $a_w$ in most RTE meat products is low enough to provide control of *L. monocytogenes*.

**Interactions**

Heat treatments applied to products with a sub-optimal pH for *L. monocytogenes*, and/or with a preservative containing an organic acid, can be expected to be more effective than the same heat treatment applied at the optimum pH for the organism. Combining sub-optimal physico-chemical conditions such as pH, temperature and $a_w$ usually has a greater effect than any of the individual factors used alone at the same level (Mossel et al., 1995). At pH value of 4.66, for example, time to visible growth of *L. monocytogenes* was 5 days at 30 °C with no NaCl added, 8 days at 30 °C with 4.0% NaCl, and 13 days at 30 °C with 6% NaCl (Cole et al., 1990).

**Practical Techniques to Inhibit *L. monocytogenes* Growth**
Accumulated evidence to date clearly demonstrates the need for the food industry to employ measures that minimize *L. monocytogenes* in foods. Many meat products, particularly RTE meats, present a significant risk for outbreaks of listeriosis. Most of the past outbreaks have been the result of failure in the control systems for the products. In most of these cases, hazard analysis and implementation of controls at the critical points identified could have prevented the outbreaks, providing the control systems were operated correctly. Bell and Kyriakides (Bell and Kyriakides, 1998) suggested that there is no more potentially dangerous product, than that manufactured by a complacent management who believe that their product is safe because of historical precedence. It is important to note that listeriosis outbreaks may be caused at any time that the controls inherent in the normal manufacture of these products are not applied correctly. This is true even for processes and products considered to be a very low concern in relation to *L. monocytogenes*.

**Chemical preservatives**

Control of *L. monocytogenes* growth might be achieved by a variety of chemical preservatives, since the pH value (Ravishankar and Harrison, 1999), nitrite level and salt concentration (Nerbrink et al., 1999) of food products have been shown to be three important parameters for the survival and growth of *Listeria*.

No *L. monocytogenes* will grow in or on food products with very high sodium chloride, e.g. 10% or more, not only because the significantly decreased $a_w$, but also because of the large difference in ion potential between bacteria cell walls (Jay, J. M., 2000). Nitrite is commonly used in cured meat products, such as frankfurters, for both cured meat color development and as a food preservative. The antimicrobial activity of nitrite has been recognized since the late 1920s (Robert et al., 1991), specifically for spore-forming bacteria because nitrite inhibits out-growth of germinated spores (Duncan and Foster, 1968). In combination with 5% NaCl, sodium nitrite at 200 mg/kg level has been shown to inhibit *L. monocytogenes* growth for 40 days at 5 °C in vacuum-packaged and film-wrapped smoked salmon (Pelroy et al., 1994), and significantly increased product shelf life. It has been suggested that chemical preservatives, such as diacetate (Islam et al., 2002; Lou and Yousef, 1999), lactate (Blom et al., 1997; Maca et al., 1999; Nerbrink et al., 1999), sorbate (El-Shenawy and Marth, 1988; Islam et al., 2002) and benzoate (Islam et al., 2002; Lou and...
Yousef, 1999), could affect the growth of *L. monocytogenes* by acting on microbial cell wall and cell membrane, destroying their structure and subsequently limiting the transport mechanism of nutrients to the cells (Jay, J. M., 2000). The efficiency of most preservatives is governed by a dose-effect relationship. Furthermore, use of chemical preservatives in combination often increases the degree of anti-microbial action and lowers the necessary concentration of individual substances (Jay, J. M., 2000).

Because *L. monocytogenes* contamination on RTE meats is typically on the surface due to post-heating contamination, surface treatments of these products with anti-microbial compounds seems to be a logical approach. However, more research is needed to determine the most effective *L. monocytogenes* inhibitors to use for surface treatment of RTE meats.

**Irradiation**

For food preservation, the primary interest in radiation applications is for electron-magnetic ionizing radiation. The different forms of ionizing radiation, with wavelengths of 2000 Å or less, such as ultraviolet (UV) light, beta ray, gamma ray, and X-ray, all have potential application in food preservation, because the shorter wavelengths result in greater damage to microorganisms.

UV light may result in bacterial cell death on food surfaces, due to the lethal mutation products that result from UV light action on cell nucleic acids. However, the poor penetrative capacities of UV light make it functional only as a surface treatment of certain foods. Beta rays also have relatively poor penetration power and are considered inadequate for food preservation (Mendonca, A. F., 2002).

Gamma radiation from $^{60}$Co or $^{137}$Cs, and electron beams from linear accelerators are the two most widely used techniques for irradiation of foods. Gamma is the cheapest form of radiation for food preservation, because the source elements are either by-products of atomic fission or atomic waste products. Gamma rays have excellent penetration power, as opposed to beta rays, but the $^{60}$Co and $^{137}$Cs have a half-life of about 5 and 30 years, respectively. These sources have to be changed periodically to maintain desired levels of radioactivity. Also, gamma radiation is not focused in one direction, but emits to all directions from the source, and can not be turned on or off, making it harder to control. Linear accelerators produce accelerated electron beams or can convert electrical power to X-ray power for
increased penetration capacity. Compared with gamma rays, the accelerated electron beams are much easier to use and safer during operation, because the electron beam current is adjustable, and easy to turn on or off.

Regardless of irradiation type, many factors including the irradiation dose, numbers and types of microorganisms, food composition, other preservation treatments, temperature, and atmospheric gas composition, are very important for microbial inactivation as a result of irradiation (Mendonca, A. F., 2002). It was reported that a dose of 0.8 kGy was sufficient to increase the heat sensitivity of *L. monocytogenes* in roast beef and gravy (Grant and Patterson, 1995).

Increased doses of ionizing radiation generally result in greater destruction of microorganisms. However, microbial destruction at a given irradiation dose will decrease under anaerobic or dry conditions because of the lower rate of oxidizing reactions that produce free radicals and toxic oxygen derivatives. Meat products packaged in air have been shown to be significantly more lethal to *L. monocytogenes* as a result of gamma radiation treatments than if packaged in either vacuum or modified atmospheres. There is a small but significant increase in the radiation sensitivity of *L. monocytogenes* on meat packaged in 100% CO₂ compared to 100% N₂ (Thayer and Boyd, 1999).

The composition of food, including liquid or solids content, protein content, and product thickness, also affect the inactivation of microorganisms by irradiation. Solid foods offer greater protection to microorganisms against irradiation than phosphate buffers or other liquid media. Similarly, increasing amounts of protein in foods tends to provide a protective effect. Interestingly, the sensitivity of *L. monocytogenes* to gamma radiation was reported to be unaffected by increasing levels of fat (0.39-32.5%) in various varieties of fish (Kamat and Thomas, 1998).

**Microwave heating**

Farber et al. (1998) examined the survival of *L. monocytogenes* on chicken skin heated by microwave energy. Broilers (<1.8 kg) and roasters (>1.8 kg) were microwave-heated according to manufacturers’ directions with a standing time of 10-15 minutes. One of 81 broilers and 9 of 93 roasters tested positive for *L. monocytogenes* after microwave heating. In an experiment comparing microwave heating with conventional cooking, one of the
broilers and none of the roasters were positive for Listeria. Ovens equipped with turntables, ovens of different cavity-sizes, or ovens of various wattage levels did not appear to affect destruction of L. monocytogenes. Farber et al. (1998) recommended that chickens be heated in a microwave oven to an internal temperature of > 74 °C, and that frozen foods be thawed before cooking. Stuffing should be cooked separately from the bird, foods should be rotated manually during cooking, and thicker portions of the food should be placed closer to the exterior of the microwave dish (Doores, S., 2002) to assure adequate heating in microwave ovens.

**Bacteriocins**

Bacteriocins are secondary metabolites produced by microorganisms and have been demonstrated to inhibit or kill a wide spectrum of other microorganisms. Bacteriocins used as food preservatives, must be nontoxic and heat stable without off-flavors or off-odors. It has been reported that Nisin A effectively inhibited L. monocytogenes growth on ricotta-type cheeses for 8 weeks (Davies et al., 1997). Pediocin AcH as a surface spray prevented growth of L. monocytogenes on a smear-surface soft cheese (Ennahar et al., 1998), and Pediocin PA-1 effectively inhibited growth of L. monocytogenes on dry fermented sausage (Foegeding et al., 1992). Linocin M-18 caused a 2-log reduction of L. monocytogenes and L. ivanovi on soft cheese (Eppert et al., 1997) while Lactocin 705 has been reported to inhibit growth of L. monocytogenes in ground beef (Vignolo et al., 1996).

**Low temperature**

* L. monocytogenes is a major food safety concern, at least in part because of its ability to grow at refrigeration temperatures (Juneja and Sofos, 2002). The minimum growth temperature for L. monocytogenes was reported to be 0 °C in chicken broth (Walker et al., 1990). According to Table 5, the lag phase is at least 3 days, and might be extended to 33 days, when products were stored at 0 °C. Lower storage temperature for meat product will decrease the L. monocytogenes protein synthesis rate (Jay, J. M., 2000). Therefore, storage of frankfurters and similar products below freezing is probably necessary to prevent L. monocytogenes growth by temperature alone.

**High temperature**
Pasteurization and sterilization are two commonly used heating methods for food preservation. Based on most research, *L. monocytogenes* is unable to survive at 65 °C for 30 min or 72 °C for 15 s, except when at intracellular locations such as in the leukocytes of animals (Farber, J. M., 1989).

Potential targets for heat damage have been implicated to explain various pathogen effects. These include proteins, enzymes and cellular membranes, as well as nucleic acids (Marquis et al., 1994). Heat is believed to be uniformly distributed in a cell, resulting in damage to only the most sensitive molecules, such as 30S ribosomal subunits, within that cell. Therefore, protection of the 30S subunit of *L. monocytogenes* ribosome is a critical mechanism for increased *L. monocytogenes* thermo-tolerance (Stephens and Jones, 1993). Differential scanning calorimetry has been used to show that osmotic and heat shock-induced thermo-tolerance of *L. monocytogenes* was increased by increased thermal stability of the 30S ribosomal subunit. The subunit was stabilized through cellular dehydration, which resulted in an increase in the internal solute concentration, including Mg\(^{+}\) ions, which may have contributed to tighter coupled particles on the 30S subunits.

The heat resistance of *L. monocytogenes* strain Scott A was reported to be influenced by growth temperature history and pH, including the type of acidulant used to adjust the pH (Juneja et al., 1998). Regardless of acid identity, increased temperature during growth resulted in significantly decreased D-values (*P < 0.05*) when the pH of the growth medium was 5.4; and significantly increased D-values (*P < 0.05*) when the pH of the growth medium was 7. At pH 5.4, adjusted with lactic acid, D-values ranged from 1.30 min at 10 °C to 1.14 min at 37 °C. At pH 5.4 adjusted with acetic acid, *L. monocytogenes* failed to grow at 10 °C; the D-values were 1.32 min and 1.22 min when the organism was grown at 19 °C and 37 °C, respectively. At pH 7, adjusted with lactic acid, D-values were 0.95, 1.12, and 1.28 min for cells grown at 10, 19, and 37 °C, respectively. The values ranged from 0.83 min at 10 °C to 1.11 min at 37 °C when the pH was adjusted with acetic acid. Thus, if conditions can be found that increase cell susceptibility, these conditions can be exploited to enhance inactivation (Juneja et al., 1998).
A log-linear model has been applied successfully to quantify the effects of the temperature, pH and salt concentration on the thermal inactivation of *L. monocytogenes*, where the natural logarithm D-value of *L. monocytogenes* is predicted as follows:

\[
\ln (\text{D-value of } L. \text{ monocytogenes}) = -61.4964 + 2.3019 (\text{temp}) + 1.2236 (\text{pH}) + 0.7728 (\text{salt}) + 1.0477 (\text{phos}) - 0.0102 (\text{temp}) \times (\text{pH}) - 0.0085 (\text{temp}) \times (\text{salt}) - 0.0566 (\text{temp}) \times (\text{phos}) - 0.0210 (\text{pH}) \times (\text{salt}) - 0.4160 (\text{pH}) \times (\text{phos}) + 0.1861 (\text{salt}) \times (\text{phos}) - 0.0217 (\text{temp})^2 - 0.0273 (\text{pH})^2 -0.0213 (\text{salt})^2 -13.1605 (\text{phos})^2
\]

(Juneja and Eblen, 1999).

Table 7 shows the results of observed and predicted D-values at 70-90 °C for *L. monocytogenes* in beef gravy according to the above model (Juneja and Eblen, 1999). The observed D-values showed that pH was the most important factor affecting *L. monocytogenes* growth when the temperature varied within a very narrow range (55 – 57.5 °C). Obviously, there were interaction effects between salt and pH, and phosphate and pH. However, the predicted D-value did not fit the observed D-value very well. Therefore, further research is necessary to discover a more reliable mathematical model for predicting the antilisterial effects of factors that may contribute to the control of *L. monocytogene*.

Packaging

Modified atmosphere packaging (MAP) is a packaging technique that utilizes an atmosphere modified so that its composition is different from that of air. Various MAP techniques are available, and their advantages and disadvantages should be considered before selection for centralized meat packaging (Tewari et al., 1999). In MAP, the initial atmosphere surrounding a food is typically altered by removal of O\(_2\) and addition of either CO\(_2\) or a combination of gases (O\(_2\), N\(_2\), and CO\(_2\)). The function of CO\(_2\) is to decrease the growth rate of microorganisms by increasing their lag phase and reducing product respiration, whereas N\(_2\) is used to displace O\(_2\) and act as inert filler to prevent package collapse when some of the CO\(_2\) is absorbed by moisture in the product. The package should have proper water vapor and O\(_2\) barrier properties to prevent subsequent spoilage through microbial growth (Juneja and Sofos, 2002). It was reported that *L. monocytogenes* growth was inhibited in both 50 and 80% CO\(_2\) environments on frankfurters for 3 weeks at 4 °C, and it grew in the present of 50% CO\(_2\) but not 80% CO\(_2\) during the additional 3 weeks of storage. However,
CO₂ at level of 80% may cause undesirable organoleptic changes in the product, CO₂ levels between 50 and 80% may be more practical (Farber and Peterkin, 1999).

Vacuum-packaging was the earliest form of MAP developed commercially and is still used for many products including cuts of fresh red meat, cured meats, hard cheeses, and ground coffee. The product is packed in film of low O₂ permeability, the air is evacuated, and entry of O₂ from outside is restricted. In the case of vacuum-packaged fresh meat, CO₂ increases to 10-20% within the package as the respiration of the meat quickly consumes the residual O₂ (Tewari et al., 1999). L. monocytogenes was reported to grow faster in vacuum pack compared with 20% and 30% CO₂ packs at 4 °C (Farber and Peterkin, 1999), therefore, vacuum packaging alone will not control L. monocytogenes growth.

Other Preservation Techniques

High pressure, high-intensity pulsed electric fields and magnetic fields are examples of additional non-thermal food preservation technologies that have potential to affect L. monocytogenes and need to be investigated for practical applications in the meat industry to provide more options for inhibiting L. monocytogenes growth (Juneja and Sofos, 2002).

Conclusions

Listeriosis is a foodborne illnesses primarily caused by L. monocytogenes, a gram-positive, non-spore-forming, non-acid-fast, facultative anaerobic, psychrotrophic rod that can tolerate acids, sanitizers and antibiotics, and exists ubiquitously. The reported average mortality of foodborne listeriosis is approximately 30% (Newton et al., 1992), which is much higher than the mortality caused by E. coli O157:H7 or Salmonella spp. Such high mortality and the increased frequency of outbreaks of listeriosis involving meat products has made L. monocytogenes a major food safety concern in the meat industry over the last two decades.

RTE meat products have been reported to be the source of listeriosis in susceptible populations (Jurado et al., 1993; Tappero et al., 1995), e.g., organ transplant patients, patients with AIDS, HIV-infected individuals, pregnant women, patients with cancer and the elderly. Handling, storage, processing and associated food supply systems must be carefully managed by food producers and processors to control the growth of L. monocytogenes in food products, particularly in RTE meats. To assume safety of food products, L. monocytogenes must be reduced from potentially harmful levels to 0 CFU/ml (Jay, J. M., 2000).
Research has contributed greatly to understanding conditions and factors that affect *L. monocytogenes* growth and survival, including factors such as nutrients (Jay, J. M., 2000), pH (Bell and Kyriakides, 1998, Colburn et al., 1990; George et al., 1988), temperature (Bell and Kyriakides, 1998, McClure et al., 1991), and $a_w$ (Jay, J. M., 2000). Chemical preservatives (Blom et al., 1997; El-Shenawy and Marth, 1988; Islam et al., 2002; Jay, J. M., 2000; Lou and Yousef; 1999; Maca et al., 1999), irradiation (Juneja and Sofos, 2002; Kamat and Thomas, 1998; Thayer and Boyd, 1999), bacteriocins, low temperature, pasteurization (Juneja and Eblen, 1999; Marquis et al., 1994; Stephens and Jones, 1993), and MAP (Tewari et al., 1999) packaging systems have also each been shown to impact growth of *L. monocytogenes*. However, further research to determine how those inhibitory factors interact with each other is necessary to achieve further improvements in control of *L. monocytogenes* in meat products.
TABLE 1. Survival of *L. monocytogenes* in Various Environmental Samples (Bell and Kyriakides, 1998; Fenlon, D. R., 1999)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage temperature (°C)</th>
<th>Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sterile soil (I)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Outside-winter/spring</td>
<td>154</td>
</tr>
<tr>
<td>clay soil (I)</td>
<td>24-26</td>
<td>225</td>
</tr>
<tr>
<td>sealed tubes</td>
<td>24-26</td>
<td>67</td>
</tr>
<tr>
<td>fertile soil (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sealed tubes</td>
<td>24-26</td>
<td>295</td>
</tr>
<tr>
<td>cotton-plugged tubes</td>
<td>24-26</td>
<td>67</td>
</tr>
<tr>
<td>top soil (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>exposed to sunlight</td>
<td>NG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>not exposed to sunlight</td>
<td>NG</td>
<td>182</td>
</tr>
<tr>
<td>moist soil</td>
<td>NG</td>
<td>~497</td>
</tr>
<tr>
<td>dry soil</td>
<td>NG</td>
<td>&gt;730</td>
</tr>
<tr>
<td>soil</td>
<td>4-12</td>
<td>240-311</td>
</tr>
<tr>
<td>soil</td>
<td>18-20</td>
<td>201-271</td>
</tr>
<tr>
<td>Fecal material</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cattle feces (NC)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>182-2190</td>
</tr>
<tr>
<td>moist horse/sheep feces (I)</td>
<td>Outside</td>
<td>347</td>
</tr>
<tr>
<td>dry horse/sheep feces (I)</td>
<td>Outside</td>
<td>730</td>
</tr>
<tr>
<td>sheep feces</td>
<td>Outside</td>
<td>242</td>
</tr>
<tr>
<td>liquid manure</td>
<td>Summer</td>
<td>36</td>
</tr>
<tr>
<td>liquid manure</td>
<td>Winter</td>
<td>106</td>
</tr>
<tr>
<td>sewage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sewage sludge cake (NC)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28-32</td>
<td>35</td>
</tr>
<tr>
<td>surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td>interior</td>
<td>48-56</td>
<td>49</td>
</tr>
<tr>
<td>sprayed on field</td>
<td>Outside</td>
<td>&gt;56</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sterilized pond water (I)</td>
<td>Outside</td>
<td>7</td>
</tr>
<tr>
<td>unsterilized pond water (I)</td>
<td>Outside</td>
<td>&lt;7-63</td>
</tr>
<tr>
<td>pond water</td>
<td>35-37</td>
<td>346</td>
</tr>
<tr>
<td>pond water</td>
<td>15-20</td>
<td>299</td>
</tr>
<tr>
<td>pond water/ice</td>
<td>2-8</td>
<td>790-928</td>
</tr>
<tr>
<td>pond/river water</td>
<td>37</td>
<td>325</td>
</tr>
<tr>
<td>pond/river water</td>
<td>2-5</td>
<td>750</td>
</tr>
<tr>
<td>water</td>
<td>Outside</td>
<td>140-300</td>
</tr>
<tr>
<td>distilled water (I)</td>
<td>4</td>
<td>&lt;9</td>
</tr>
<tr>
<td>Animal feed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>silage (NC)</td>
<td>4</td>
<td>450</td>
</tr>
<tr>
<td>silage (NC)</td>
<td>5</td>
<td>180-2190</td>
</tr>
<tr>
<td>mixed feed (I)</td>
<td>Outside</td>
<td>188-275</td>
</tr>
<tr>
<td>oats (I)</td>
<td>Outside</td>
<td>150-300</td>
</tr>
<tr>
<td>hay (I)</td>
<td>Outside</td>
<td>145-189</td>
</tr>
<tr>
<td>straw (NC/I)</td>
<td>ca.22</td>
<td>365</td>
</tr>
<tr>
<td>straw (I)</td>
<td>Outside</td>
<td>47-207</td>
</tr>
<tr>
<td>straw</td>
<td>Outside-summer</td>
<td>23</td>
</tr>
<tr>
<td>straw</td>
<td>Outside-winter</td>
<td>135</td>
</tr>
</tbody>
</table>

<sup>a</sup> Inoculated.<br>
<sup>b</sup> Not given.<br>
<sup>c</sup> Naturally contaminated.
### TABLE 2. Some differentiating characteristics of the recognized species of *Listeria* (2, 34)

<table>
<thead>
<tr>
<th>Species</th>
<th>Xylose</th>
<th>Lactose</th>
<th>Galactose</th>
<th>Rhamnose</th>
<th>Mannitol</th>
<th>Hippurate</th>
<th>CAMP test</th>
<th>Beta</th>
<th>Mol%</th>
<th>Serovars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydrolysis</td>
<td>S. aureus</td>
<td>R. equi</td>
<td>Hemolysis</td>
<td>G+C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>+1</td>
<td>v²</td>
<td>v</td>
<td>+³</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>37-39</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>36-38</td>
</tr>
<tr>
<td><em>L. seeligeri</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td><em>L. welshimeri</em></td>
<td>+</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td><em>L. ivanovii</em></td>
<td>+</td>
<td>+</td>
<td>v</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>37-38</td>
</tr>
<tr>
<td><em>L. grayi</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>41-42</td>
</tr>
</tbody>
</table>

1 - means that most strains are positive

2 v - variable

3 + means most strains positive

4 w - weak

* 1/2a, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, "7"

** same as for *L. monocytogenes* and *L. Innocua*, but no "5" or "7"
<table>
<thead>
<tr>
<th>Type of listeriosis</th>
<th>Nature of infection</th>
<th>Severity</th>
<th>Time to onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoonotic infection</td>
<td>Local infection of skin lesions</td>
<td>Mild and self-resolving</td>
<td>1-2 days</td>
</tr>
<tr>
<td>Neonatal infection</td>
<td>Infection of new-born babies from infected mother during birth or due to cross-infection from one neonate in the hospital to other babies</td>
<td>Can be extremely severe, resulting in meningitis and death</td>
<td>1-2 days (early onset) usually from congenital infection prior to birth 5-12 days (late onset) following cross-infection from another infant</td>
</tr>
<tr>
<td>Infection during pregnancy</td>
<td>Acquired following the consumption of contaminated food</td>
<td>Mild flu-like illness or asymptomatic in the mother but serious implications for unborn infant including spontaneous abortion, foetal death, stillbirth and meningitis. Infection is more common in third trimester</td>
<td>Varies from 1 day to several months</td>
</tr>
<tr>
<td>Infection of non-pregnant adults</td>
<td>Acquired following the consumption of contaminated food</td>
<td>Asymptomatic or mild illness, which may progress to central nervous system infections such as meningitis. Most common in immunocompromised or elderly</td>
<td>Illness may occur within 1 day or up to several months</td>
</tr>
<tr>
<td>Listeria food poisoning</td>
<td>Consumption of food with exceptionally high levels of L. monocytogenes, &gt; 107 per ml</td>
<td>Vomiting and diarrhoea, sometimes progressing to bacteraemia but usually self-resolving</td>
<td>&lt; 24 h after consumption</td>
</tr>
<tr>
<td>Year</td>
<td>Country</td>
<td>Cases (deaths)</td>
<td>Food</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>----------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>1980-1981</td>
<td>Canada</td>
<td>41 (18)</td>
<td>Coleslaw</td>
</tr>
<tr>
<td>1983</td>
<td>USA</td>
<td>49 (14)</td>
<td>Pasteurized milk</td>
</tr>
<tr>
<td>1985</td>
<td>USA</td>
<td>142 (48)</td>
<td>Mexican-style soft cheese</td>
</tr>
<tr>
<td>1983-1987</td>
<td>Switzerland</td>
<td>122 (34)</td>
<td>Vacherin cheese</td>
</tr>
<tr>
<td>1987-1989</td>
<td>UK</td>
<td>&gt;350 (&gt;90)</td>
<td>Belgian pâté</td>
</tr>
<tr>
<td>1992</td>
<td>New Zealand</td>
<td>4 (2)</td>
<td>Smoked mussels</td>
</tr>
<tr>
<td>1992</td>
<td>France</td>
<td>270 (63)</td>
<td>Pork tongue in asoic</td>
</tr>
<tr>
<td>1994</td>
<td>USA</td>
<td>45 (0)</td>
<td>Chocolate milk</td>
</tr>
<tr>
<td>1995</td>
<td>France</td>
<td>20 (4)</td>
<td>Raw-milk soft cheese</td>
</tr>
<tr>
<td>1998</td>
<td>USA</td>
<td>105</td>
<td>Deli meats and hot dogs</td>
</tr>
<tr>
<td>2000</td>
<td>USA</td>
<td>5</td>
<td>Turkey deli meats</td>
</tr>
<tr>
<td>2002</td>
<td>USA</td>
<td>43</td>
<td>Turkey deli meats</td>
</tr>
</tbody>
</table>
TABLE 5. Growth rate guide for *L. monocytogenes* at different temperatures (Mossel et al., 1995)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Lag time (days)</th>
<th>Generation time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>3-33</td>
<td>62-131</td>
</tr>
<tr>
<td>2 - 3</td>
<td>2-8</td>
<td>NA</td>
</tr>
<tr>
<td>4 - 5</td>
<td>NA</td>
<td>13-25</td>
</tr>
<tr>
<td>5 - 6</td>
<td>1-3</td>
<td>NA</td>
</tr>
<tr>
<td>7 - 8</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>9 - 10</td>
<td>&lt; 1.5</td>
<td>NA</td>
</tr>
<tr>
<td>10 - 13</td>
<td>NA</td>
<td>5-9</td>
</tr>
</tbody>
</table>

NA - not available.
TABLE 6. Percentage of total undissociated organic acid present at different pH values

(Bell and Kyriakides, 1998)

<table>
<thead>
<tr>
<th>pH value</th>
<th>Acetic acid</th>
<th>Citric acid</th>
<th>Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>84.5</td>
<td>18.9</td>
<td>39.2</td>
</tr>
<tr>
<td>5</td>
<td>34.9</td>
<td>0.41</td>
<td>6.05</td>
</tr>
<tr>
<td>6</td>
<td>5.1</td>
<td>0.006</td>
<td>0.64</td>
</tr>
<tr>
<td>7</td>
<td>0.54</td>
<td>&lt;0.001</td>
<td>0.064</td>
</tr>
</tbody>
</table>
TABLE 7. Observed and predicted D-values at 70-90 °C of *L. monocytogenes* in beef gravy (Juneja and Eblen, 1999)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>pH</th>
<th>%NaCl</th>
<th>%Phosphate</th>
<th>D-value observed (min)</th>
<th>D-value predicted (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>4</td>
<td>0.0</td>
<td>0.0</td>
<td>5.35</td>
<td>1.68</td>
</tr>
<tr>
<td>55</td>
<td>4</td>
<td>6.0</td>
<td>0.0</td>
<td>12.49</td>
<td>2.24</td>
</tr>
<tr>
<td>57.5</td>
<td>5</td>
<td>4.5</td>
<td>0.10</td>
<td>6.92</td>
<td>1.52</td>
</tr>
<tr>
<td>57.5</td>
<td>5</td>
<td>4.5</td>
<td>0.20</td>
<td>10.61</td>
<td>0.78</td>
</tr>
</tbody>
</table>
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Use of Organic Acid Salts for Control of *Listeria monocytogenes* on Ready-to-Eat (RTE) Meat Products

A paper submitted to the Journal of Food Protection

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Keywords: food safety, *Listeria monocytogenes*, organic acids, organic acid salts, frankfurters

Abstract

Organic acids and their salts have received considerable attention as potential inhibitors for prevention of *Listeria monocytogenes* growth in food products. While these compounds are recognized as inhibitors, less is known about the mechanisms of inhibition of *L. monocytogenes* growth, the inhibitory characteristics of different organic acids, such as lactate, sorbate, benzoate, and diacetate, and the factors affecting the antilisterial activity of these compounds. Organic acids or their salts, such as diacetate, lactate, sorbate, and benzoate, may inhibit the growth of *L. monocytogenes* by acidifying cell cytoplasm, diffusing the proton motive force, destroying ATP synthesis and subsequently disrupting the transport of nutrients to the cell. The efficiency of most organic acids is governed by a dose-effect relationship, and use of organic acids in combination often increases the degree of antimicrobial action, lowering the necessary concentration of individual substances. Using surface treatments with organic acids or their salts for *L. monocytogenes* control on ready-to-eat meat products is a logical approach, because *L. monocytogenes* contamination on these products typically results from post-heating contamination and is restricted to product surfaces. Recent research has reported synergistic effects between organic acids, such as benzoate, acetate, lactic acid and citric acid. The storage temperature, pH, water activity (\(a_w\)), NaCl and nitrite concentration of RTE meats, and the type and level of organic acids used for surface treatments are major factors affecting the antilisterial activities of organic acids and their salts on these products.

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Introduction

It is estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Schlundt, J., 2002). While one tenth of the deaths are due to listeriosis, there are only 2,500 cases of listeriosis annually (CDC, 2003). Such high mortality rate has made *L. monocytogenes* one of the most dangerous pathogens in food products. Further, the number of cases of foodborne listeriosis has increased in the past 15-25 years (Sofos, J. N., 2002).

*L. monocytogenes* is widely spread in nature and can be found ubiquitously. It has been reported that 53% of vacuum-packaged, processed, ready-to-eat (RTE) meats, such as frankfurters, deli- and luncheon-style meats, were found to be *L. monocytogenes* positive (Grau and Vanderlinde, 1992). Since the increased L. monocytogenes outbreaks, there have been more and more studies conducted to identify the factors potentially affecting the survival and growth of *L. monocytogenes*, e.g., nutrients (Jay, J. M., 2000), temperature (Bell and Kyriakides, 1998; McClure et al., 1991; Walker et al., 1990), pH (George et al., 1988; Colburn et al., 1990; Ravishankar and Harrison, 1999), water activity (Jay, J. M., 2000), nitrite level (Lou and Yousef, 1999) and salt concentration (Cole et al., 1990; Nerbrink et al., 1999).

Recent research has focused on the antilisterial effects of organic acids and their salts including diacetate (Bedie et al., 2001; Islam et al., 2002; Lou and Yousef, 1999; Palumbo and Williams, 1994), lactate (Bedie et al., 2001; Blom et al., 1997; Maca et al., 1999; Nerbrink et al., 1999; Palumbo and Williams, 1994; Samelis et al., 2002), sorbate (El-Shenawy and Marth, 1988; Islam et al., 2002) and benzoate (Islam et al., 2002; Lou and Yousef, 1999). Further research has begun to investigate how these organic acids affect *L. monocytogenes* growth (Jay, J. M., 2000). It has been reported that the efficiency of most organic acids is governed by a dose-effect relationship, and using organic acids in combination may increase the degree of anti-microbial action and lower the necessary concentration of individual substances (Jay, J. M., 2000).

Application of the organic acids or their salts for RTE meats logically seem best as surface treatments because *L. monocytogenes* contamination on the products is typically on the surface following peeling and packaging.
Mechanisms of Organic Acids Inhibition on *L. monocytogenes*

The antilisterial mechanism of organic acids is linked to damage of cellular sites or inhibition of metabolic functions that are most sensitive to effects of the organic acid. Usually, the major damage or inhibition associated with the antimicrobial mechanism of a food preservative is termed the primary lesion, with the lesions occurring as a result of the primary lesion considered as secondary lesions. The primary lesion for *L. monocytogenes* caused by organic acids or their salts is acidification of the cytoplasm and inactivation of the ATP synthesis enzyme (ATPase) (Jay, J. M., 2000). Because of the diffusion of proton motive forces, the primary lesion will lead to secondary lesions, such as ATP synthesis depletion, protein denaturation, and inhibition of nutrient transport (Jay, J. M., 2000).

It is well known that ATP is the necessary energy supply for living cells, and ATP synthesis is accomplished through an elegant proton-pumping system that occurs inside mitochondria, which are special double-membrane-bound organelles. There are three individual reactions of interest that occur in mitochondria for ATP generation through oxidative phosphorylation. These include oxidation, reduction and phosphorylation as shown in the following:

Oxidation: NADH → NAD\(^+\) + H\(^+\) + 2e\(^-\) \hspace{1cm} \Delta G^o = -158.2 \text{ kJ}

Reduction: \(\frac{1}{2} \text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}\) \hspace{1cm} \Delta G^o = -61.9 \text{ kJ}

Phosphorylation: ADP\(^3\) + HPO\(_4\)\(^2-\) + H\(^+\) \rightarrow ATP\(^4\) + H\(_2\)O \hspace{1cm} \Delta G^o = +30.5 \text{ kJ}

A reaction with \(\Delta G^o < 0\) means it can occur spontaneously. The synthesis of ATP (phosphorylation), is a non-spontaneous reaction, but it is coupled with two spontaneous reactions, the oxidation of NADH and the reduction of O\(_2\).

The three key steps in ATP synthesis are shown in Figure 1 and 2.

Electrons are transferred from NADH, through a series of electron carriers, to O\(_2\) which serves as the terminal electron acceptor in aerobes and facultative anaerobes growing aerobically. The electron carriers are proteins embedded in the inner mitochondrial membrane. Transfer of electrons by these carriers generates a proton (H\(^+\)) gradient across the inner mitochondria membrane, with more hydrogen ions (H\(^+\)) outside of the cell membrane, making it acidic, with more hydroxyl ions (OH\(^-\)) inside of the cell membrane. Thus, H\(^+\) and...
OH⁻ ions are separated by the cell membrane, and form a potential gradient that is positive outside and negative inside (Fig. 1). ATP is synthesized when ATPase, a special enzyme which is necessary for ATP generation (ADP + Pi → ATP), comes into living cell with H⁺ spontaneously diffusing back across the inner mitochondria membrane. Thus, the large positive free energy of ATP synthesis is overcome by the even larger negative free energy associated with proton flow across the concentration gradient (Casiday et al., 2003) (Fig. 2).

Undissociated short chain lipophilic acids can enter the cell through cell membranes very easily in undissociated form, and will dissociate to H⁺ and A⁻ immediately after passing through the cell membrane. This will decrease the cell membrane potential, diffuse the proton motive force, stop ATP generation and destroy cell functions. Without ATP generation, nutrients can not be transported, enzymes can not function, proteins can not be synthesized as usual, DNA is denatured, cell lysis may occur and bacteria may die quickly (Gould et al., 1983).

As a Listeria inhibitor, lipophilic acids and their salts are most effective in their undissociated or protonated form because they are able to penetrate the cytoplasmic membrane of L. monocytogenes more effectively.

Organic Acids and Their Salts

Growth and inactivation rates for L. monocytogenes vary markedly in the presence of different acids. Lactic acid, acetic acid, benzoic acid and sorbic acid are most often used and are generally-recongnized-as-safe (GRAS) substances approved by U.S. Food and Drug Administration Center (FDA) (Table 1). Most organic acids permitted in meat products are applied as acidulants, e.g., acetic and lactic acids, whereas others, particularly their salt forms, are used as preservatives, e.g., potassium sorbate and sodium benzoate. Effectiveness of these weak organic acids as antimicrobial agents is related to the amount of the undissociated acid that is present. Concentration of the undissociated form of a weak organic acid, which is related to pH of the medium and the pK of the acid, can be calculated using the Henderson-Hasselbalch equation (Chaplin, M. 2002). For example, at pH 5, 35.5% of acetic (pKa 4.74) and 5.8% of L-lactic acid (pKa 3.79) will be undissociated. Undissociated organic acids can pass through the cell membrane, dissociate inside the cytoplasm, and interfere with
metabolic processes of the microbial cell. The antimicrobial action of these acids is attributed to cytoplasm acidification, as well as the specific antimicrobial effect of the particular anionic species. Several of the organic acids and their salts have been studied for *L. monocytogenes* inhibition in RTE meat products (Doores, S. 1993).

**Sodium lactate**

Lactic acid (CH\(_3\) - CHO\(_2\) - COOH; \(pK_a = 3.79\); MW 90.08 Da) is an end product of natural fermentations, produced by lactic acid bacteria including *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Carnobacterium*. As one of the oldest known antimicrobial agents, lactic acid at 2.5-5.0% was reported to inhibit *C. botulinum*, *Clostridium perfringens*, *C. sporogenes*, *L. monocytogenes*, *Salmonella*, *S. aureus*, and *Yersinia enterocolitica* (Brackett, R. E. 1987; Doores, S. 1993; Juneja and Sofos, 2002) in turkey (Maas et al., 1989; Meng and Genigeorgis, 1993), chicken (Meng and Genigeorgis, 1993; Shelef and Yang, 1991) and beef (Chen and Shelef, 1992; Harmayani et al., 1993; Papadopoulos, et al., 1991; Shelef and Yang, 1991; Unda et al., 1991).

Recent studies showed that sodium lactate in frankfurter formulations could control *L. monocytogenes* on the product at 4 °C. At 4 °C, *L. monocytogenes* growth was effectively suppressed for 35 days by 1.8% sodium lactate (Samelis et al., 2002), for 70 days by 3% sodium lactate, and for 120 days by 6% sodium lactate (Bedie et al., 2001). At 5 °C, a 5% sodium lactate solution used for dipping frankfurters was reported to effectively inhibit *L. monocytogenes* growth for 90 days (Palumbo and Williams, 1994). However, it was reported that the *L. monocytogenes* growth in the presence of 0.1 % lactic acid was observed, because the *L. monocytogenes* inhibition effect also depended on the concentration of the organic acid salts (Lou and Yousef, 1999).

**Sodium diacetate**

Acetic acid (CH\(_3\)COOH; \(pK_a = 4.75\); MW 60.05 Da) is the primary component of vinegar. Its sodium, potassium and calcium salts are used as food preservatives and to inhibit *L. monocytogenes* growth on frankfurters (Juneja and Sofos, 2002). Some derivatives of acetic acid including diacetate salts and dehydroacetic acid have also been used (Juneja and Sofos, 2002). Sodium diacetate (CH\(_3\)COOH - CH\(_3\)COONa, \(pK_a = 4.75\)), which contains
about 40% acetic acid, is used as an acidulant, flavoring agent, and antimicrobial agent in RTE meat products (Shelef and Addala, 1994).

Either sodium acetate or sodium diacetate used at 0.25% on frankfurter surfaces can inhibit *L. monocytogenes* growth for 20 to 35 days (Bedie et al., 2001). However, with higher concentration, such as 5% or 15%, it can inhibit *L. monocytogenes* growth on frankfurters up to 90 days at 5 °C (Palumbo and Williams, 1994), and up to 14 days at 13 °C (Islam et al., 2002). At 5 °C, using 2.5% acetate and 2.5% citric acid in combination on frankfurters, not only effectively inhibited *L. monocytogenes* growth, but also had a listericidal effect (Palumbo and Williams, 1994).

A comparison of pH values permitting growth at 16, 20 and 35 °C showed that acetic acid was most inhibitory (pH 5.04) followed by lactic (pH 4.73), citric (pH 4.53), malic (pH 4.46), and hydrochloric acid (pH 4.46) (Ahamad and Marth, 1989; El-Shenawy and Marth, 1989; Farber et al., 1989; Sorrells et al., 1989). Therefore, differences in antilisterial activity of acidulants depend on type of acid rather than on pH alone.

**Potassium benzoate**

Benzoic acid (C₆H₅COOH, pKₐ = 4.19, MW 122.12 Da) and sodium benzoate (C₆H₅COONa) were the first antimicrobial compounds permitted in foods by FDA (Jay, J. M., 2000). Benzoic acid is obtained naturally from cranberries, plums, prunes, apples, strawberries, cinnamon, cloves, and most berries. At pH range of 2.5–4.5, it is largely undissociated and has the most effective antilisterial activity (Juneja and Sofos, 2002) in that pH range. At pH 4.00, 60% of the compound is undissociated, whereas at a pH of 6.0, only 1.5% is undissociated. This means that benzoic acid and its sodium salts are restricted in effectiveness to high-acid products. Also, usage of benzoates in RTE meat products is limited to a level of 0.1%, because of its “peppery” or burning taste.

The concentration of sodium benzoate required to inhibit growth of *L. monocytogenes* at 4 or 13 °C in tryptose broth is 0.05-0.1% (500-1000 µg/ml) at pH 5.0 (El-Shenawy and Marth, 1988). At pH 5.6, the microorganism is able to grow at 4 °C with 0.05% sodium benzoate, while 0.2% sodium benzoate is required to inhibit the microorganism for 9 days at 13 °C. At pH 5.6 and 21 °C, 0.25% sodium benzoate restricted *L. monocytogenes* growth to less than 1 log for 5 days (El-Shenawy and Marth, 1988).
Potassium sorbate

Sorbic acid (CH3-CH=CH-CH=CH-COOH; pKa 4.75, MW 112.3 Da) was first isolated from the oil of unripened rowanberries of the mountain ash tree (Sofos and Busta, 1993). Either sorbic acid or its calcium, sodium, or potassium salts have been used to inhibit *L. monocytogenes* in meat products including frankfurters (Jay, J. M., 2000). Among the GRAS organic acids, the antimicrobial activity of sorbic acid is the greatest when the compound is in the undissociated state, thus at pH 6.0 or higher, it has considerably less antilisterial activity (Juneja and Sofos, 2002).

Potassium sorbate and sorbic acid have been widely used to extend the shelf life of many foods, including butter, cheese, and meats. The ability of potassium sorbate and sorbic acid to inhibit *L. monocytogenes* has been assessed in laboratory media and several foods, and it was reported to inhibit the hemolytic activity of listeriolysis O of *L. monocytogenes* by reacting with cysteine (Kouassi and Shelef, 1995). Moir and Eyles (Moir and Eyles, 1992) measured the minimum inhibitory concentrations (MICs) of sorbate against *L. monocytogenes* Scott A in buffered BHI broth. These authors reported MICs of 400 to 600 and > 5000 mg/L at pH 5 and 6, respectively, when the culture was incubated at 35 °C, and 1500 mg/L at pH 6 and 5 °C. In another study, Trypticase Soy Broth (TSB) was prepared to contain 500, 1500, and 3000 ppm potassium sorbate and pH of the medium was adjusted to 5.0 or 5.6 using HCl or organic acids (acetic, tartaric, lactic, or citric), followed by inoculation with *L. monocytogenes*, and incubation at 13 or 35 °C (El-Shenawy and Marth, 1991). When compared with HCL as an acidulant, the antilisterial activity of sorbate was enhanced more by organic acids, with acetic and tartaric acids being more effective than lactic and citric acids.

The antilisterial ability of potassium sorbate is also related to temperature and pH (El-Shenawy and Marth, 1988). The lower the storage temperature and pH of the medium, the greater was the effectiveness of sorbates against *L. monocytogenes*. In the absence of potassium sorbate, generation times for *L. inmonocytogenes* in TSB at pH 5.6 decreased from 1.13 days to 49 min as the incubation temperature increased from 4 to 35 °C. Addition of 2500 ppm potassium sorbate prevented *Listeria* growth at 4 °C and led to complete demise of...
the organism after 66 days, whereas listeriae grew with a generation time of 9 h in the same sorbate-containing medium incubated at 35 °C.

**Benzoic Acid Derivatives**

Methyl, propyl, and heptyl parabens are esters of p-hydroxybenzole acids, approved as food additives in several countries (Jay, J. M., 2000; Lou and Yousef, 1999). These benzoic acid derivatives usually remain undissociated at pH values up to 8.5, because of the higher pKa values than benzoic acid. Therefore, parabens retain antilisterial activity over a wider pH range (Davidson, P. M. 1993; Davidson, P. M. 1997).

**Fatty Acids and Related Compounds**

Antilisterial activity of free fatty acids, particularly those of medium chain length, has been demonstrated. In addition to their primary function as food emulsifiers, some fatty acid esters, particularly monoacylglycerols (monoglycerides) and esters of sucrose, inhibit a wide spectrum of microorganisms, including *L. monocytogenes* (Lou and Yousef, 1999).

**Synergistic Effects of Combinations of GRAS Preservatives**

It was reported that combinations of lactate with NaCl, nitrite, or low temperature enhanced the overall antibacterial effect against *L. monocytogenes* (Chen and Shelef, 1992; Pelroy et al., 1994; Weaver and Shelef, 1993). The antilisterial activity of sodium lactate was improved by combination with 400 IU/mL nisin in a buffered BHI broth at pH 5.5 and 4 °C (Buncic et al., 1995), and it was further enhanced by addition of 0.5% polyphosphate. *Listeria* populations decreased by 2.2-2.4 and 4.2 logs after 28 and 20 days following treatment with lactate/nisin and lactate/nisin/polyphosphate combinations, respectively (Lou and Yousef, 1999). Using 1.8% sodium lactate alone in frankfurters inhibited *L. monocytogenes* growth for 70 days, however, when combined with sodium acetate, sodium diacetate, or glucono-δ-lactone (GDL), the inhibition effect could be extended to 120 days (Samelis et al., 2002). A recent study showed that, at 5 °C, a combination of 0.5% acetic acid with 0.5% citric acid had stronger antilisterial effect than acetic, citric or tartaric acid alone at 1% (Palumbo and Williams, 1994).

The antilisterial activity of sodium benzoate was greatly enhanced after pH adjustment with other acids (El-Shenawy and Marth, 1989). Recent research has shown that the acetic and tartaric acids were most effective in enhancing the antilisterial effects of
sodium benzoate followed by lactic and citric acid (Lou and Yousef, 1999). At 35 °C, adding acetic or tartaric acid to adjust the pH of the medium to 5.0, 1500 ppm sodium benzoate resulted in complete inactivation of *L. monocytogenes* after 96 h. However, the organism remained viable at least 78 h longer if hydrochloric acid was used to adjust the medium pH to 5.0.

Potassium sorbate at 0.3% was reported to prevent growth of *L. monocytogenes* at an initial inoculation of $10^7$ CFU/mL during 6 weeks of incubation, but no bactericidal effect was observed. However, strong listericidal effects were observed when sorbate was used in combination with 125 ppm sodium nitrite, 0.5%polyphosphate, or 400 IU/mL nisin. Neither nitrite nor nisin alone had listericidal effects on *L. monocytogenes*. Adding nitrite to the three-agent combination reduced populations by 3.7 logs after 37 days, with the same reduction being achieved in 12 days by incorporation of 0.5% polyphosphate. However, there was no antilisterial interaction observed between sorbate and 4% sodium lactate (Lou and Yousef, 1999).

Factors Affecting the Antilisterial Activity of Organic Acid Salts

To achieve the maximum antilisterial activity of organic acid salts on RTE meat products, it is essential to obtain a thorough knowledge and understanding of the processes used to produce these products and the growth control parameters existing in both the process and the finished products (Bell and Kyriakides, 1998). For example, it was reported that the antilisterial activity of organic acids in frankfurters depended upon the *Listeria* characteristics (Davidson, P. M., 2002), pH value, water activity and the storage conditions, including storage time, temperature, and atmosphere. Recent research has suggested that the factors that influence the antilisterial activity of organic acids on frankfurters, usually acted in an interactive manner (Davidson, P. M., 2002). Heat treatments applied to frankfurters formulated with or dipped in an organic acid are expected to be more effective for *L. monocytogenes* inactivation than the same heat treatment applied to a product at the optimum pH for the organism (Bell and Kyriakides, 1998). Combining sub-optimal physico-chemical conditions such as pH, temperature and water activity usually has a greater effect than any of the individual factors used at the same level (Bell and Kyriakides, 1998; Mossel et al., 1995).
pH

*L. monocytogenes* grows best in the pH range of 6 to 8, when all other growth conditions are optimal. It is not surprising that the pH value of food products is one of the most important factors influencing the antilisterial effectiveness of organic acids and their salts (Jay, J. M., 2000). With a lower pH, a greater proportion of the acid is in the protonated form and a greater level of the antilisterial activity is usually observed. At 30 °C, growth of *L. monocytogenes* in culture media has been reported to occur in less than 7 days at pH 4.4 (George et al., 1988), and 60 days at pH 4.66 (Colburn et al., 1990). However, no *L. monocytogenes* growth occurs at pH below 4.1 or above 9.6 (Jay, J. M., 2000). Usually, the minimum growth pH of a bacterium is highly correlated to the temperature of incubation, general nutrient composition of growth substrate, water activity ($a_w$), and the presence and quantity of NaCl and other salts or inhibitors (Colburn et al., 1990).

If the pH value of RTE meats becomes non-optimal for *Listeria spp.* growth as a result of surface dipping or mixing with an acidic component, e.g. lactic acid or diacetate, the pH alone will contribute to the control of population growth for any *Listeria spp.* that may be present, including *L. monocytogenes*. When organic acids, such as acetic, lactic, citric, etc., are used as preservatives in foods, it is important to ensure that the correct concentration of undissociated acid, which is responsible for the antimicrobial activity, is available for *L. monocytogenes* growth inhibition. The proportion of undissociated acid present varies with pH (Table 2), thus, it must be taken into account when determining the amount of total acid required at a specific pH to give a particular concentration of undissociated acid. At neutral pH, most organic acids will have a limited effect on the growth of *L. monocytogenes* (Bell and Kyriakides, 1998).

The pKa of a weak acid indicates how much of the compound will be in the most active form in a given food application, and most organic acids have pKa's of pH at 3.0-5.0 as described earlier (Doores, S. 1993). Thus, the use of organic acids may be limited in some RTE meats because the sensory quality may not be acceptable with a pH less than 5.0.

**Temperature**

*L. monocytogenes* is not a very heat-resistant organism. It was reported that *L. monocytogenes* was easily killed by pasteurization at 65 °C for 30 min or 75 °C for 30
seconds (Beckers et al., 1987; Bradshaw et al., 1985; Bradshaw et al., 1987; Donnelly et al., 1987) unless it was located within leukocytes (Farber, J. M. 1989).

However, *L. monocytogenes* can survive and grow slowly at the temperatures used for refrigeration even as low as 0 to 1 °C (Junttila et al., 1988; Seeliger and Jones, 1986; Walker et al., 1990). Therefore, there is no guarantee for prevention of *L. monocytogenes* growth by refrigerated storage alone (McClure et al., 1991).

Behavior of *L. monocytogenes* on frankfurters treated with organic acids is affected by both pH and the incubation temperature (Lou and Yousef, 1999). In this study, polymyxin-Tryptose Phosphate Broth containing 0, 0.003, 0.03, and 0.3 M lactic acid was adjusted to pH 5, 6, and 7 using HCl or NaOH, inoculated to contain $10^3$ *L. monocytogenes* CFU/mL, and incubated at 35 or 4 °C. When incubated at 35 °C and pH 5, *Listeria* populations decreased in broth that contained 0.3 and 0.03 M lactic acid, whereas rapid growth occurred with 0 and 0.003 M lactic acid after a prolonged lag period. At pH 5 and 4 °C, *Listeria* was eliminated after 27-42 days from broth containing 0.3 M lactic acid, while populations of the pathogen remained unchanged in the same medium containing the three lower concentrations of lactic acid. At pH 6 and in the presence of 0.3 M lactic acid, the *Listeriae* population increased 2 logs at 35 °C and did not grow at 4 °C. However, at pH 6 and 4 °C, *Listeria* growth occurred at the three lower concentrations (0, 0.03, and 0.003 M) of lactic acid. At pH 7, *Listeriae* grew at 35 °C, regardless of lactic acid concentration; however, at 4 °C the pathogen only grew in media containing less than 0.03 M lactic acid with a 7-day lag time. During extended incubation at both 13 and 35 °C, the presence of 0.3 and 0.5% citric acid in TSB was most injurious to *L. monocytogenes* followed in order by similar concentrations of lactic and acetic acid. Acid-injured *Listeriae* survived approximately nine times longer at 13 °C than 35 °C (Ahamad and Marth, 1990).

The relationships between growth parameters, lag time (days), generation time (hours), and temperatures are shown in Table 3. Obviously, lag time and generation time is increased linearly as temperature is decreased in the temperature range of 0 to 13 °C.

**Water activity**

There are no reports that *L. monocytogenes* can grow at $a_w$ values less than 0.93 (Jay, J. M., 2000). However, because most RTE meat products have $a_w$ values of greater than 0.93,
it is not possible to control *L. monocytogenes* growth by lowering $a_w$ of these products. Exceptions might include dried products such as dried sausage, jerky and some snack sticks.

**NaCl**

At 30 °C and pH 4.66, the lag phase for *L. monocytogenes* has been reported to increase from 5 days to 8 days after adding 4% NaCl, and to 13 days after adding 6% NaCl. However, NaCl concentration in processed meats seldom exceeds 2.5% which is not high enough to inhibit *L. monocytogenes* (Cole et al., 1990).

**Nitrite**

Nitrite is a necessary component in cured meat products, with the maximum added amount of nitrite limited to 156 ppm. It is reported that the antilisterial effects of sorbate on frankfurters was enhanced with 125 ppm of nitrite, and the combined sorbate and nitrite showed listericidal effects (Lou and Yousef, 1999). However, there is generally less than 10% of the added nitrite remaining in cured meats after cooking, therefore, the added nitrite in frankfurters may not have a great effect on control of *L. monocytogenes* contamination that occurs after cooking.

**Food composition**

Food composition is another factor affecting the antilisterial activity of the organic acids. This relates to the hydrophobicity of the antimicrobial molecule. Antimicrobials must be lipophilic to attach and pass through the cell membrane, but also should be at least partially soluble in the aqueous phase (Davidson, P. M. 1997). A food that is high in fat will cause the antimicrobial to be solubilized in the lipid phase and may reduce its availability to inhibit microorganisms in the water phase (Rico-Munoz and Davidson, 1983).

**Conclusions**

Recent research has reported that organic acids, such as diacetate, lactate, sorbate and benzoate or their salts affect the growth of microorganisms such as *L. monocytogenes* by acting on the cell wall and the cell membrane, destroying their structure and subsequently the transport mechanism of nutrients to the cell (Jay, J. M., 2000). Recent research has also shown that efficiency of those organic acids is governed by a dose-effect relationship. Synergistic effects have been observed between benzoate and other organic acids, such as acetate, lactic acid and citric acid. Combined with nitrite or nisin, the antilisterial activity of
both lactate and sorbate may be enhanced. Also, addition of polyphosphate to lactate and nisin combinations has been reported to further increase the antilisterial activity of the combination. On the other hand, there was no synergistic antilisterial effect observed for sorbate with 4% sordium lactate. Therefore, use of organic acids or their salts in combinations may increase the degree of anti-microbial action and lower the necessary concentration of individual substances but each must be considered on a case-by-case basis. Moreover, surface treatment of RTE meats with organic acids or their salts is more efficient than using organic acids or their salts as a product ingredient because *L. monocytogenes* contamination on these products is typically on the surface as a result of post-heating contamination. The storage temperature, pH, water activity (a\textsubscript{w}), NaCl and nitrite concentration of RTE meats, and the type and level of organic acids used are major factors affecting the antilisterial activities of organic acids and their salts.

Because of the large number of variables that exist in processed meat products, more research is needed to determine the most effective inhibitory treatments for *L. monocytogenes* in specific RTE meat products.

From Casiday et al. (2003) with permission.


From Casiday et al. (2003) with permission.
TABLE 1. *Maximum use of some GRAS organic acids as chemical food preservatives (Jay, J. M., 2000)*

<table>
<thead>
<tr>
<th>Organic Acids</th>
<th>Maximum tolerance in food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid/Lactate</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sorbic acid/Sorbates</td>
<td>0.2%</td>
</tr>
<tr>
<td>Benzoic acid/Benzoates</td>
<td>0.1%</td>
</tr>
<tr>
<td>Sodium diacetate</td>
<td>0.32%</td>
</tr>
<tr>
<td>Propionic acid/propionates</td>
<td>0.32%</td>
</tr>
<tr>
<td>Parabens&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1%</td>
</tr>
<tr>
<td>Dehydroacetic acid</td>
<td>65 ppm</td>
</tr>
</tbody>
</table>

<sup>a</sup> NA means not available.

<sup>b</sup> Methyl-, propyl, and heptyl- esters of p-hydroxybenzoic acid.
TABLE 2. Percentage of total undissociated organic acid present at different pH values
(Bell and Kyriakides, 1998)

<table>
<thead>
<tr>
<th>Organic acid</th>
<th>pH value</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>84.5</td>
<td>34.9</td>
<td>5.1</td>
<td>0.54</td>
</tr>
<tr>
<td>Citric acid</td>
<td>18.9</td>
<td>0.41</td>
<td>0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>39.2</td>
<td>6.05</td>
<td>0.64</td>
<td>0.064</td>
</tr>
</tbody>
</table>
TABLE 3. Growth rate guide for *L. monocytogenes* at different temperatures (Mossel et al., 1995)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Lag time (days)</th>
<th>Generation time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>3-33</td>
<td>62-131</td>
</tr>
<tr>
<td>2 - 3</td>
<td>2-8</td>
<td>NA <em>a</em></td>
</tr>
<tr>
<td>4 - 5</td>
<td>NA</td>
<td>13-25</td>
</tr>
<tr>
<td>5 - 6</td>
<td>1-3</td>
<td>NA</td>
</tr>
<tr>
<td>7 - 8</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>9 - 10</td>
<td>&lt; 1.5</td>
<td>NA</td>
</tr>
<tr>
<td>10 - 13</td>
<td>NA</td>
<td>5-9</td>
</tr>
</tbody>
</table>

*a* NA means not available
References


CHAPTER 3. INHIBITION OF *Listeria monocytogenes* ON FRANKFURTERS TREATED WITH ORGANIC ACID SALTS

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Keywords: food safety, organic acid salts, *Listeria monocytogenes*, frankfurters

Abstract

Inhibition of *Listeria monocytogenes* by four chemical compounds, sodium lactate (SL), sodium diacetate (SD), potassium sorbate (PS), and potassium benzoate (PB), used either singly or in all possible combinations, on frankfurters was evaluated. The compounds were applied as a 6% dipping solution for surface treatment of the frankfurters. The treated frankfurters were inoculated with a five-strain cocktail of *L. monocytogenes* (Scott A, H7764 1/2 a, H7962 4b, H7762 4b, and H7969 4b) using 1 ml with $10^5$ cells per 90.8 gram package containing 2 frankfurters. Packages were stored at 10 °C for 14 days, and *L. monocytogenes* was counted at two-day intervals. The results indicated that surface dipping of frankfurters with SD, SD/PB, or SD/SL/PB were the three most effective treatments for inhibiting *L. monocytogenes* growth compared with the other treatments in this study. The maximum population density of *L. monocytogenes* was lowered, and generation time and lag phase were increased after surface treatments with 6% SD, SD/PB, or SD/SL/PB solutions. There was no adverse effect on sensory scores for meatiness, sourness, pepper flavor, or saltiness of the frankfurters after surface application of these organic acids.

Introduction

*Listeria monocytogenes* is a gram-positive, facultative anaerobe, psychrotrophic bacteria that can tolerate acids (Sorrells et al., 1989), sanitizers and antibiotics (Jay, J. M., 2000). Several listeriosis outbreaks linked to the consumption of food products have occurred

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in the last two decades. Post-processing contamination by *L. monocytogenes* after cooking or before packaging of ready-to-eat (RTE) processed meats is a major concern for the meat industry and has become an important food safety issue. The sources of *L. monocytogenes* are widespread, including raw meat, poultry, raw milk, pasteurized milk, dairy products and vegetables (Jay, J. M., 2000).

Ingestion of food-borne *L. monocytogenes* has caused listeriosis in susceptible populations. The presence of *L. monocytogenes* in meat products has received increased attention worldwide, and researchers in both the United States and United Kingdom have suggested that all *L. monocytogenes* should be considered as potential pathogens. Furthermore, US regulatory agencies have established a “zero tolerance” policy for *L. monocytogenes* in RTE foods, due to the outbreaks of listeriosis (Jay, J. M., 2000) from these food products.

Grau and Vanderlinde (1992) found *L. monocytogenes* on 53% of vacuum-packaged, processed, deli- and luncheon-style meats. Differences in product composition with regard to pH, nitrite level and salt concentration were identified as important parameters for the survival and growth of *Listeriae* (Jay, J. M., 2000). It has been suggested that chemical preservatives, such as lactate (Maca et al., 1999), diacetate, sorbate and benzoate (Islam et al., 2002), could affect the growth of microorganisms by acting on the cell wall and the cell membrane, destroying their structure and subsequently the transport mechanism of nutrients to the cell (Jay, J. M., 2000). The efficiency of most preservatives is governed by a dose-effect relationship. Also, use of chemical preservatives in combination often increases the degree of anti-microbial action and lowers the necessary concentration of individual substances (Jay, J. M., 2000). Because *L. monocytogenes* contamination on RTE meats is typically on the surface due to post-heating contamination, surface treatments of these products with anti-microbial compounds seems to be a logical approach. However, more information is needed to determine the most effective antimicrobials to use for surface treatment of frankfurters in order to inhibit *L. monocytogenes*.

Therefore, the purpose of this study was to evaluate four potential inhibitors (sodium lactate, sodium diacetate, potassium sorbate and potassium benzoate), alone and in combination, to assess their effectiveness against *L. monocytogenes* on frankfurters.
Materials and Methods

Manufacturing process

Meat trimmings, consisting of boneless beef (80/20 lean/fat ratio), lean pork (50/50 lean/fat ratio) and pork fat (20/80 lean/fat ratio), were formulated into frankfurters. The three sources of meat were singly ground using a 0.79 cm grinder plate and mixed prior to sampling for measurement of actual fat content. Frankfurters were formulated on a 25% fat basis for the meat block. Beef trim, salt, sodium erythorbate, sodium nitrite, seasonings and one-half of the total water (ice) were chopped for approximately 3 minutes in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA), until temperature reached 40 °F. The pork trim, pork fat and remaining one-half of the ice were then added and this composite was chopped for an additional 3 minutes, until temperature reached 55 °F. The meat batter was placed into a vacuum stuffer (Risco® Model RS 4003-165; Stoughton, MA) and stuffed into 22 mm diameter, peelable cellulose casings (Devro Teepak™Wienie-Pak® Coastal Corrugated Inc., N. Charleston, SC) and linked (Poly-clip® Gmbh & Co. KG, Germany) at 8.5 cm in length.

A conventional cooking-smoking cycle of 2.5 hours for frankfurters was used in a humidity-controlled smoke house (Alkar, DEC International Inc., Lodi, WI) to achieve a cooked product internal temperature of 71.1 °C. The franks were showered and then chilled for approximately 16-18 hours in a 4 °C cooler.

Experimental design

Four organic acid salts, sodium lactate (SL), sodium diacetate (SD), potassium benzoate (PB), and potassium sorbate (PS) were used either singly or in all possible combinations as a 6% dip solution (wt/vol) for the frankfurters. Preliminary experiments showed that dipping for 3 min resulted in an approximately 0.08% pickup of the compounds or combinations by the frankfurters. Surface-treated products were placed in bags (2 frankfurters per package) and inoculated with a 5-strain cocktail of *L. monocytogenes*, to reach a final level of 10⁵ cells per package (90.8 g). Each of the treatments included a treated but uninoculated control. The 16 treatments utilized for both inoculated and un-inoculated frankfurters are shown in Table 1.
Frankfurters were stored at 10 °C for 14 days. Packages were analyzed for *L. monocytogenes* counts immediately after inoculation and then every 48 hours during storage. Background bacteria, i.e. mesophilic aerobic plate counts (APC), lactic acid bacteria and yeast/mold counts were also enumerated.

**Microorganisms**

The inoculum included 5 *L. monocytogenes* strains: Scott A (human isolate), H7764 (serotype 1/2a, deli meat isolate), H7762 (serotype 4b, hotdog isolate), H7962 (serotype 4b, hotdog isolate), and H7969 (serotype 4b, hotdog isolate). The scott A strain was obtained from the National Animal Disease Center (NADC), and the other four strains were obtained from the Centers for Disease Control and Prevention, Atlanta, GA (CDC), as clinical isolates from the Bil Mar Foods outbreak of 1998-1999.

The *L. monocytogenes* cultures were individually grown in Trypticase Soy Broth containing 0.6% Yeast Extract (TSB-YE broth, Difco Laboratories, Becton Dickinson and Company, Sparks, MD) in a rotating platform incubator at 35 °C for 24 hours. Then, 1 ml of culture from each individual stain was combined to give a 5-ml mixed culture of *L. monocytogenes*, and the mixed culture was transferred to 500-ml TSB-YE broth and incubated at 35 °C for 24 hours to reach the stationary phase. The final concentration of the original 5-strain mixture of *L. monocytogenes* was 9.30 log CFU /ml, and the culture was serially diluted to achieve 10^5 cells / ml for the inoculum. The number of viable cells in the inoculum was verified by plate count methods. Typical colonies were gram-stained and analyzed using API *Listeria* kits (BioMerieux, Inc., Hazelwood, MO).

Frankfurters were surface inoculated with the 5-strain mixture of *L. monocytogenes* by adding 1 ml of inoculum (10^5 cells / ml) into each package containing two frankfurters. Average package weight was 90.8g. The packages were hand-massaged 5-10 s to evenly distribute the inoculum on the surface of each frankfurter immediately after inoculation. After vacuum-packaging and sealing, all frankfurters were placed in U.N.-approved biosafety shipping containers (AirSea-Atlanta, GA) with cold packs. A temperature recorder was placed in one of the shipping containers to monitor temperature, and the containers were shipped overnight to Silliker Laboratories (Silliker Laboratories, INC., South Holland, IL) for microbial analysis.
Packaging

Both uninoculated and inoculated products were vacuum-sealed in polyvinyl chloride (PVC) high-barrier bags (Cryovac® B-540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5-0.6 g at 100 °F, 100% RH/100 sq. in. /24h; oxygen transmission rate = 3-6 CC at 40 °F /m²/24 h/0% RH) after treatments. Two frankfurters were included in each package.

Chemical analyses

Proximate analyses for moisture, fat and protein were conducted on the frankfurters according to procedures outlined by the AOAC (1990).

Sodium lactate, SD, PS, and PB analyses were determined on the surface-treated frankfurters as outlined in the AOAC (1990), in order to verify that the formulations did not exceed the allowable limit (9 CFR 318.7) of 0.1 percent for SD, PB and PS which was in effect at the time of this work.

Microbiological analyses

Detection of *L. monocytogenes* was done according to the U.S. Department of Agriculture/Food Safety Inspection Service method (USDA, 1998), except that quad plates were used during the confirmation (Evanson et al., 1991).

Samples of the control and inoculated products were analyzed immediately upon receipt (days 0) and at 2, 4, 6, 8, 10, 12 and 14 days of storage at 10 °C. A single package was randomly selected from each set at each interval. Packages were aseptically opened, the contents transferred to a sterile stomacher bag and homogenized for two minutes with Butterfield’s phosphate buffer added at a 1:1 weight ratio. Twenty-five gram aliquots of homogenate were added to 100 grams diluent in sterile bottles and homogenized by manual agitation to make a 1:10 dilution. Subsequent dilutions were performed with 9.9 ml tubes of Butterfield’s phosphate buffer. Control samples were analyzed for aerobic mesophilic bacteria (Plate Count Agar incubated at 35 °C for 48 hours), lactic acid bacteria (DeMan, Rogasa, Sharpe (MRS) agar with overlay agar incubated at 30 °C for 5 days), yeast and mold (Potato Dextrose Agar incubated at 25 °C for 5 days) and *L. monocytogenes*. *L. monocytogenes* was enumerated with a pour plate technique using an underlay of non-selective Trypticase Soy Agar with 0.6% yeast extract (TSAYE). After the TSAYE hardened,
an overlay of Modified Oxford agar (MOX) was added. Small white/gray colonies with black halos were considered typical for *L. monocytogenes*. Test samples were analyzed for *L. monocytogenes* by plating (TSAYE/MOX overlay) and 3-tube MPN techniques. The MPN sequence was UVM (University of Vermont) tubes (20-24h at 30 °C), Fraser Broth tubes (48 hours at 35 °C), and MOX plates (24-48 hours at 35 °C). Two samples per testing interval with typical colonies were confirmed as *Listeria* by HL (Horse Blood Overlay Agar) plates (24 hours at 35 °C) (USDA, 1998), QUAD plates (Evanson, et al., 1991) and *Listeria* Micro-ID test kits (Donnelly et al., 1992).

**Sensory evaluation**

Twelve panelists were recruited from the faculty, staff, and students of Iowa State University for the sensory panel evaluations. The University’s Human Subjects in Research Committee approved the project and the panelists were compensated for their participation. Panelists were trained to evaluate salt, sour, meaty, and pepper flavors in two one-hour training sessions. Testing was conducted in partitioned booths and under fluorescent lighting conditions. A line scale with a numerical value of 15 units, was used with descriptive anchors indented 0.5 unit from each end of the line. Samples were scored for meaty, salty, sour, or black pepper flavors, where “0” represented none and “15” represented a high flavor intensity. Only uninoculated, treated frankfurters were used for the sensory evaluation.

**Curve fitting and shelf life prediction**

A modified Gompertz sigmoid curve was used to fit the *L. monocytogenes* growth curve, and three growth curve parameters, lag phase duration time, generation time, and maximum population density, were calculated by applying estimates of the 4 parameters (a, b, X₀, and Y₀) from the Gompertz equation (Buchanan and Bagi, 1999; Franses, P. H., 2002). In this study, curve fitting was performed using Sigma Plot 2000 software (SPSS Science, Chicago, IL, USA).

The Gompertz equation is: $Y = Y_0 + a \times \exp \left(-\exp \left(-\frac{(X - X_0)}{b}\right)\right)$, where $X = \text{time at which } \log_{10} \text{count is } Y$; $X_0 = \text{time at which absolute growth rate is at a maximum}$; $Y = \log_{10} \text{count at a time } X$; $Y_0 = \text{asymptotic log count as } X \text{ decreases indefinitely}$; $a = \text{asymptotic amount of growth that occurs as } X \text{ increases indefinitely}$; $1/b = \text{relative growth rate at } X_0$. 

(Wei and Fang, 2001)
These parameters were used to derive maximum population density, generation time, and lag phase for \( L.\ monocytogenes \) as follows:

\[
\text{Maximum population density} = a + Y_0; \\
\text{Lag phase (days)} = X_0 - 0.9624 \times b; \\
\text{Generation time (days)} = \frac{0.81828 \times b}{a}.
\]

Statistical analyses

A complete block design was used for the microbiological analyses and a balanced incomplete block design was used for the sensory evaluation. The data on \( L.\ monocytogenes \) counts were analyzed by Sigma Plot 2000 software after translation to logarithms. Both growth curve parameters of \( L.\ monocytogenes \) and sensory evaluation data were analyzed by the MIXED procedure in SAS V8.1 (SAS Inc. 2000). The treatment effects were considered to be significantly different when \( P \)-values were less than or equal to 0.05, and means were separated using the Tukey test. The experiment was replicated twice.

Results and Discussion

Chemical analyses

The fat, moisture and protein content of the finished products were 24.90%, 56.54% and 15.86%, respectively. There were no significant differences for fat, moisture or protein content in the frankfurters as formulated \((P > 0.05)\).

Microbial analyses

Microorganism counts for both \( L.\ monocytogenes \) and indigenous bacteria, i.e. mesophilic aerobic plate counts (APC), lactic acid bacteria and yeast/mold, were less than 1 log (10 CFU/g) for all of the treated but uninoculated controls (data not shown).

Counts of \( L.\ monocytogenes \) for inoculated frankfurters are shown in table 2. \( L.\ monocytogenes \) numbers (log CFU/g) for each treatment were measured every other day for 14 days. The initial concentration of \( L.\ monocytogenes \) inoculated frankfurters was about 3-log (2.78-2.98), i.e. ~1000 CFU/g for all of the 16 treatments, and there was no difference between treatments at day 0 \((P > 0.05)\). At day 2, the control group (3.24-log CFU/g) showed markedly larger counts than PS (2.92-log CFU/g), SL/SD/PS (2.97-log CFU/g), and SL/PB/PS (2.96-log CFU/g) treatment groups \((P < 0.05)\), and numerically higher \( L.\ monocytogenes \) numbers \((P > 0.05)\) than all of the other treatments. Sodium diacetate and
PB/PS treatments showed significant effects \( P < 0.05 \) on day 4, while the other treatment groups were similar to the control group. It should be noted that the effects of organic acids are not always positive in terms of food safety (Lou and Yousef, 1997). Slightly higher \textit{L. monocytogenes} counts, for example, were observed for the SL treatment than for the control after day 4, though the difference was not significant \( P > 0.05 \). Sodium lactate is considered a significant inhibitor of \textit{L. monocytogenes} but the SL surface treatment based on a 0.1% pick-up in this study may be considerably lower than minimum lethal concentration (MLC) necessary to prevent the growth of \textit{L. monocytogenes}. Further, \textit{L. monocytogenes} that have been exposed to low SL concentration and survived, may repair themselves during storage and begin to multiply, becoming more tolerant of the acid. Lou and Yousef (1997) reported that exposure to acid induced stress responses in \textit{Listeriae} which made the bacteria more tolerant of acidity. In addition, Sorrells et al. (1989) reported that acetic acid had stronger antilisterial activity than lactic acid at 10 °C.

The SD (2.74-log CFU/g), PB (3.00-log CFU/g), SD/PB (2.75-log CFU/g), SL/SD/PB (2.94-log CFU/g), and SD/PB/PS (2.97-log CFU/g) treatments successfully inhibited \textit{L. monocytogenes} growth at 6 days after inoculation \( P < 0.05 \), and at 8 days the populations of \textit{L. monocytogenes} on frankfurters were significantly lowered by SD (3.09-log CFU/g), PB (3.41-log CFU/g), SL/SD (3.49-log CFU/g), SD/PB (3.44-log CFU/g), SD/PS (3.56-log CFU/g), PB/PS (3.39-log CFU/g), SL/SD/PB (3.35-log CFU/g), SL/PB/PS (3.68-log CFU/g), and SD/PB/PS (3.71-log CFU/g) treatments compared to the control group \( P < 0.05 \). The counts of \textit{L. monocytogenes} on treated frankfurters became higher than the initial inoculation density for all 16 treatments after 8 days. At day 10, the \textit{L. monocytogenes} populations of SD (3.08-log CFU/g), PB (3.27-log CFU/g), SL/SD (2.98-log CFU/g), SD/PB (3.20-log CFU/g), or SL/SD/PB (3.34-log CFU/g) treatments were less than 0.5 log CFU/g higher than the initial inoculation level, and were significantly lower than the control group which was 2-log units higher than the initial inoculation. Suppression of \textit{L. monocytogenes} growth on frankfurters with SD, PB, SL/SD, SD/PB or SL/SD/PB treatments was observed for as long as 10 days at 10 °C. At day 12, frankfurters with SD (3.33-log CFU/g), SL/SD (3.52-log CFU/g), or SD/PB (3.32-log CFU/g) treatments had lower \textit{L. monocytogenes} populations than the control group (4.72-log CFU/g, \( P < 0.05 \)), and at day 14, SD (3.39-log
CFU/g), PB (3.81-log CFU/g), SL/SD (3.79-log CFU/g), SD/PB (3.58-log CFU/g), SD/PS (4.00-log CFU/g), PB/PS (3.84-log CFU/g) and SL/SD/PB (3.24-log CFU/g) all had significantly lower \textit{L. monocytogenes} populations relative to the control group (5.26-log CFU/g, \(P < 0.05\)). The most common denominator of the ingredient combinations used that were effective seemed to be sodium diacetate. The antilisterial activity of SL was not improved by addition of PS which is consistent with a report from Buncic et al. (1995) showing that 0.3% sorbate did not have synergistic effects against \textit{L. monocytogenes}. On the contrary, Schlyter et al. (1993) showed that antilisterial activity of SL was synergistically enhanced by addition of SD. Since SL, PS, SL/PB, SL/SD/PS, or SL/SD/PB/PS surface treatments in our study did not effectively inhibit \textit{L. monocytogenes} growth on frankfurters, they do not appear to be effective as \textit{L. monocytogenes} inhibitors at the concentrations used.

On the other hand, the anti-listerial effects of SD, SD/PB or SL/SD/PB treatments were quite significant. When contaminated frankfurters were surface-dipped with SD, SD/PB or SL/SD/PB treatment, growth of the inoculated \textit{L. monocytogenes} was suppressed during refrigerated storage. Addition of PB and SL may enhance the anti-listerial effects of SD.

According to Jay, J. M. (2000), organic acids can interact with other preservatives to enhance their antibacterial effects.

All of the growth curves from the experimental treatments were fitted to the modified sigmoid Gompertz equation. Three growth parameters for \textit{L. monocytogenes} on frankfurters, maximum population density (log CFU/g), lag phase duration time (days), and generation time (days), were calculated according to the equations (1), (2), and (3) which were listed under the topic of “curve fitting and shelf life prediction” in “materials and methods” section, and the values are shown in Figure 1, 2, and 3 respectively.

Figure 1 compares the treatment effects on maximum population density of \textit{L. monocytogenes}. The overall treatment effects on maximum population density of \textit{L. monocytogenes} were not significant (\(P > 0.05\)). Also, no significant difference was observed between the control group and the other treatment groups (\(P > 0.05\)). However, the SL/SD treatment had a significantly higher (\(P < 0.05\)) maximum population density (8.72-log CFU/g) than all but five of the other treatments. While not significant, the maximum population densities of treatments with SD, SD/PB or SL/SD/PB were more than 1.4 units lower than
the control group (Fig. 1). Thus, those three treatments, SD (3.52-log CFU/g), SD/PB (3.55-log CFU/g), or SL/SD/PB (3.73-log CFU/g), appear to have the greatest potential, among those evaluated, to affect *L. monocytogenes*.

The overall treatment effects on the lag phase of *L. monocytogenes* were not significant (*P* > 0.05, Fig. 2). However, a significantly longer lag phase was observed for treatments with SD/PB (10.19 day), and SL/SD/PB (10.69 days) compared with the control group (3.10 days, *P* < 0.05). Also, the lag phases for treatments with SD (7.50 days), or SL/SD (10.00 days) were more than 2.3 times longer than the control group, and such increases could be quite meaningful for frankfurters during storage. The lag phase of *L. monocytogenes* for frankfurters with SL (1.88 days), PS (1.55 days) or SD/PS (2.65 days) treatments were decreased slightly compared with the control group, and such decreases resulted in a significant difference (*P* < 0.05) from SL/SD, SD/PB, or SL/SD/PB treatments, which prolonged the lag phase for 7 more days than control group. Therefore, the SD, SD/PB, and SL/SD/PB treatments again appear to be most likely, among the treatments studied, to affect *L. monocytogenes* growth on frankfurters. Both the lag phase data and the maximum population density data were consistent in this respect. Woolthuis and Smulders (1985) found a lengthened lag phase and ultimately, a decreased maximum growth rate of microorganisms as a result of SL treatment. However, there was no *L. monocytogenes* growth inhibition demonstrated by the SL treatment in our study. These results are consistent with the conclusions of Maca et al. (1999) that SL levels were inversely related to microbial growth. In our study, less than 0.1% pick-up by the frankfurters probably did not result in enough SL to inhibit *L. monocytogenes* growth.

Figure 3 shows the effects of inhibitors on the generation time of *L. monocytogenes* in days. The overall treatment effects on the generation time of *L. monocytogenes* were not significant, and no significant differences were observed between the control group and the other treatment groups (*P* > 0.05). The generation times for *L. monocytogenes* on the frankfurters treated with SD (4.10 days), SL/SD (8.26 days), SD/PB (7.12 days), or SL/SD/PB (7.12 days) were at least twice as long as all other treatments, and more than 5.5 times longer than the control group. However, a large degree of variation in the data made the differences insignificant for the SD, SL/SD, SD/PB, and SL/SD/PB treatments.
The lag phase duration time calculated by equation (2) in days for treatments SL/SD (117475 days), SD/PB (1037244 days), SD/PS (-0.17 days) in the first replication, and SL (-10.2 days), SL/SD (-6.0 days) in the second replication are not reasonable values. The generation time calculated by equation (3) in days for treatments SL/SD (-146040 days), SD/PB (-1240891 days), and SL/SD/PB (-8.0 days) in the first replication also showed unreasonable results. The lag phase duration time is the time period from stationary phase to exponential phase, and generation time is the time needed for the population of organisms to double, consequently, neither of these growth estimates could realistically be negative. The negative estimates may have occurred because some of the growth curves obtained from the growth data of \textit{L. monocytogenes} for treatments in this study, were not typical microbiology growth curves. A possible reason for non-typical growth is that the \textit{L. monocytogenes} population increased very slowly, due to inhibitory effects of the treatments. The collected data also showed a large variation such that clear lag duration time could not be easily observed, particularly for SD/PS or SL/SD treatments. Consequently, the Gompertz equation could not predict \textit{L. monocytogenes} growth properly from those non-typical growth curves. This was the case for treatments SL, SL/SD, SD/PB, SD/PS, and SL/SD/PB, therefore, those data had to be treated as missing data, and imputed data used for statistical analysis (imputation is a procedure that uses available information and some assumptions to derive substitute values for missing values (Johnson and Wichern, 2002). There was no such problem with the maximum population density data. Thus, selecting the most effective \textit{L. monocytogenes} treatments according to their maximum population density seemed to be more feasible and reliable in this study than using lag phase duration time or generation time.

Temperature is a very important external factor affecting \textit{L. monocytogenes} growth. This study was conducted at 10 °C for 14 days, and the lag phase duration times for all treatments were less than 11 days. The maximum population density was at least 0.5 log unit larger than the initial inoculation concentration after 10 days. Obviously, the shelf life of all 16 treatments was less than 2 weeks at 10 °C. Thus, frankfurters stored at 10 °C are not likely to achieve control of 3-logs of \textit{L. monocytogenes} contamination even with surface treatments evaluated in this study. However, most \textit{L. monocytogenes} contamination on commercial frankfurters is much lower than 3-log unit density, thus, to fully assess effectiveness of these
compounds, it is necessary to conduct a more complete study of *L. monocytogenes* inhibition by SD, SD/PB, and SL/SD/PB using a variety of storage temperatures and including an extended storage time.

**Sensory evaluation**

No statistically significant differences were observed between the control group and the other treatments for salty, sour, meaty, or black pepper flavors (Table 3). Therefore, the surface treatments did not affect the flavor of the frankfurters. This result is consistent with Islam et al. (2002) and Maca et al. (1999) who reported that low concentration (0.1%) of added chemical compounds such as lactate, did not have adverse effects on sensory scores for frankfurters and beef rounds, respectively. However, a high concentration of SD (25% dip solution, 1 min) (Islam et al., 2002) significantly decreased flavor and overall sensory scores for the uncooked turkey frankfurters.

The addition of SL/SD increased acidity of the frankfurters and the SL, PS, SD/PS, or PB/PS treatments decreased acidity of the frankfurters compared with the control group, as shown by sourness scores in sensory evaluation (Table 3). These changes were not statistically significant from the control group, but resulted in significantly higher sourness scores for frankfurters from the SL/SD (5.1) treatment compared with those from the SL (3.8), PS (3.9), SD/PS (3.7), or PB/PS (3.9) treatments (Table 3). Similarly, PB, PS, PB/PS, SL/PB/PS, SD/PB/PS or SL/SD/PB/PS treatments increased meaty flavor of the frankfurters, and SL, SL/PB, SL/PS, SD/PS treatments decreased meaty flavor of the frankfurters. These changes were not significantly different from the control group, but were significantly higher in meaty flavor for the PB (9.1), PS (8.6), PB/PS (8.5), SL/PB/PS (8.7), SD/PB/PS (8.7) and SL/SD/PB/PS (8.7) treatments, compared with SL (7.9), SL/PB (7.8), SL/PS (7.4), SD/PS (8.0) treatments (Table 3). There were no significant differences observed in saltiness and black pepper flavor among the 16 different treatments (Table 3).

**Added compounds**

Analyses for the concentrations of the added compounds, (sodium lactate, sodium diacetate, potassium benzoate, and potassium sorbate), in the frankfurters, showed that all were below 0.1%, ranging from 0.82% to 0.99%, as intended when the project was designed (data not shown).
Conclusions

Based on the results of this study, the maximum population density was the best criteria to use for selection of treatments with greatest potential impact on *L. monocytogenes*. Consequently, treatments with SD, SD/PB, or SL/SD/PB appear to have the most potential for surface application on frankfurters to achieve *L. monocytogenes* inhibition. Storage of inoculated frankfurters at 10 °C did not suppress *L. monocytogenes* in any treatments for longer than 2 weeks, thus, lower temperature is necessary for frankfurter storage even if these inhibitors are included. To determine long-term effectiveness of these compounds, it is necessary to conduct a more complete study of *L. monocytogenes* inhibition by SD, SD/PB, or SL/SD/PB with a range of storage temperatures and an extended storage time.

The Gompertz equation may be used to calculate growth curve parameters of *L. monocytogenes* on frankfurters, providing typical growth curves are obtained. Therefore, with more complete growth curve data, *L. monocytogenes* population on frankfurters with various inhibitory compounds may be predicted on the basis of storage temperature, storage period, and initial population.
TABLE 1. Treatments with sodium lactate (SL), sodium diacetate (SD), potassium benzoate (PB), and potassium sorbate (PS) and combinations utilized for frankfurters

<table>
<thead>
<tr>
<th>Treatment number</th>
<th>Uninoculated</th>
<th>Inoculated with $10^5 L. monocytogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>SL</td>
<td>SL</td>
</tr>
<tr>
<td>3</td>
<td>SD</td>
<td>SD</td>
</tr>
<tr>
<td>4</td>
<td>PB</td>
<td>PB</td>
</tr>
<tr>
<td>5</td>
<td>PS</td>
<td>PS</td>
</tr>
<tr>
<td>6</td>
<td>SD/SL (1:1)</td>
<td>SD/SL (1:1)</td>
</tr>
<tr>
<td>7</td>
<td>SD/PB (1:1)</td>
<td>SD/PB (1:1)</td>
</tr>
<tr>
<td>8</td>
<td>SD/PS (1:1)</td>
<td>SD/PS (1:1)</td>
</tr>
<tr>
<td>9</td>
<td>SL/PB (1:1)</td>
<td>SL/PB (1:1)</td>
</tr>
<tr>
<td>10</td>
<td>SL/PS (1:1)</td>
<td>SL/PS (1:1)</td>
</tr>
<tr>
<td>11</td>
<td>PB/PS (1:1)</td>
<td>PB/PS (1:1)</td>
</tr>
<tr>
<td>12</td>
<td>SD/SL/PB (1:1:1)</td>
<td>SD/SL/PB (1:1:1)</td>
</tr>
<tr>
<td>13</td>
<td>SD/SL/PS (1:1:1)</td>
<td>SD/SL/PS (1:1:1)</td>
</tr>
<tr>
<td>14</td>
<td>SD/PB/PS (1:1:1)</td>
<td>SD/PB/PS (1:1:1)</td>
</tr>
<tr>
<td>15</td>
<td>SL/PB/PS (1:1:1)</td>
<td>SL/PB/PS (1:1:1)</td>
</tr>
<tr>
<td>16</td>
<td>SD/SL/PB/PS (1:1:1:1)</td>
<td>SD/SL/PB/PS (1:1:1:1)</td>
</tr>
<tr>
<td>Treatment</td>
<td>Day 0</td>
<td>Day 2</td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Control</td>
<td>2.88 a</td>
<td>3.24 a</td>
</tr>
<tr>
<td>SL ²</td>
<td>2.87 a</td>
<td>3.21 ab</td>
</tr>
<tr>
<td>SD ³</td>
<td>2.83 a</td>
<td>3.15 abc</td>
</tr>
<tr>
<td>PB ⁴</td>
<td>2.78 a</td>
<td>3.06 abc</td>
</tr>
<tr>
<td>PS ⁵</td>
<td>2.95 a</td>
<td>2.92 c</td>
</tr>
<tr>
<td>SL/SD</td>
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<td>SL/PB</td>
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<tr>
<td>SL/PS</td>
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</tr>
<tr>
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<td>SL/SD/PS</td>
<td>2.88 a</td>
<td>2.97 bc</td>
</tr>
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<td>SL/PB/PS</td>
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<td>2.96 bc</td>
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<td>SD/PB/PS</td>
<td>2.97 a</td>
<td>3.13 abc</td>
</tr>
<tr>
<td>SL/SD/PB/PS</td>
<td>2.89 a</td>
<td>3.01 abc</td>
</tr>
</tbody>
</table>

a-f Values in the same column with different letters are significantly different ($P \leq 0.05$)

¹ Mean of the microbial number
² Sodium lactate
³ Sodium diacetate
⁴ Potassium benzoate
⁵ Potassium sorbate


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flavor characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Control</td>
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</tr>
<tr>
<td>SL</td>
<td>9.1 a</td>
</tr>
<tr>
<td>SD</td>
<td>8.7 a</td>
</tr>
<tr>
<td>PB</td>
<td>8.4 a</td>
</tr>
<tr>
<td>PS</td>
<td>8.5 a</td>
</tr>
<tr>
<td>SL/SD</td>
<td>8.7 a</td>
</tr>
<tr>
<td>SL/PB</td>
<td>8.1 a</td>
</tr>
<tr>
<td>SL/PS</td>
<td>8.9 a</td>
</tr>
<tr>
<td>SD/PB</td>
<td>8.7 a</td>
</tr>
<tr>
<td>SD/PS</td>
<td>8.6 a</td>
</tr>
<tr>
<td>PB/PS</td>
<td>8.0 a</td>
</tr>
<tr>
<td>SL/SD/PB</td>
<td>8.6 a</td>
</tr>
<tr>
<td>SL/SD/PB</td>
<td>8.9 a</td>
</tr>
<tr>
<td>SL/PB/PS</td>
<td>8.2 a</td>
</tr>
<tr>
<td>SD/PB/PS</td>
<td>8.6 a</td>
</tr>
<tr>
<td>SL/SD/PB/PS</td>
<td>9.0 a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.59</td>
</tr>
</tbody>
</table>

a-b Values in the same column with different letters are significantly different ($P \leq 0.05$)
FIGURE 1. Effects of inhibitors on the maximum population density of L. monocytogenes on frankfurters stored at 10°C

a-b Values represented by the bars with different letters are significantly different ($P \leq 0.05$)
FIGURE 2. Effects of inhibitors on the lag phase of L. monocytogenes on frankfurters stored at 10 °C

a-c Values represented by the bars with different letters are significantly different ($P \leq 0.05$)
FIGURE 3. Effects of inhibitors on the generation time of L. monocytogenes on frankfurters stored at 10 °C

a-b Values represented by the bars with different letters are significantly different (P ≤ 0.05)
References


CHAPTER 4. ORGANIC ACID SALTS FOR CONTROL OF \textit{Listeria monocytogenes} GROWTH ON FRANKFURTERS

A paper to be submitted to \textit{The Journal of Food Protection}

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Keywords: food safety, organic acid salts, \textit{Listeria monocytogenes}, frankfurters

Abstract

Effects of sodium diacetate (SD), sodium diacetate + potassium benzoate (SD/PB), and sodium lactate + sodium diacetate + potassium benzoate (SL/SD/PB) on control of \textit{Listeria monocytogenes} growth on frankfurters were evaluated at -2.2, 1.1, 4.4, 10.0 and 12.8 °C for up to 90 days. The compounds were added as a 3 or 6% dipping solution for surface treatment of the frankfurters. The treated frankfurters were inoculated with a five-strain cocktail of \textit{L. monocytogenes} (Scott A, H7764 1/2 a, H7962 4b, H7762 4b, and H7969 4b) using 1 ml with $10^4$ cells per 90.8 gram package containing 2 frankfurters. The maximum population density of \textit{L. monocytogenes} was lowered, and generation time and lag phase were increased after surface treatments with 6% SD, 6% SL/SD/PB, 3% SD/PB and 6% SD/PB solutions at 1.1°C. The results indicated that surface dipping of frankfurters with SD at 6% was the most effective treatment for inhibiting \textit{L. monocytogenes} growth compared with the other treatments in this study. Under the conditions of this study, \textit{L. monocytogenes} survived in refrigerated storage even in the presence of the additives tested.

Introduction

Ingestion of food-borne \textit{L. monocytogenes} has been demonstrated to result in listeriosis in susceptible populations (Miller et al., 1997). Meat products, including ready-to-eat (RTE) meat products, such as frankfurters and deli meat (CDC, 2003; Farber and Peterkin. 1999; Samelis et al., 2001), have been reported to be the sources of listeriosis outbreaks.

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Post-processing contamination by \textit{L. monocytogenes} after cooking or before packaging of RTE meat, such as deli-meats or frankfurters, is a major concern for the meat industry and has become an important food safety issue (Samelis et al., 2001), because many of these products are consumed without heating. US regulatory agencies have established a “zero tolerance” policy for \textit{L. monocytogenes} in RTE foods (Jay, J. M., 2000), due to the increased outbreaks of listeriosis from these food products. To achieve the zero tolerance objective, it is necessary to understand the conditions and factors that affect growth and survival of this organism.

Recent research has focused on the antilisterial effects of organic acids and their salts including lactate (Samelis et al., 2002), diacetate, sorbate and benzoate (Islam et al., 2002). Further research has begun to investigate how these organic acids affect \textit{L. monocytogenes} growth (Jay, J. M., 2000). It has been reported that the efficiency of most organic acids is governed by a dose-effect relationship, and using of organic acids in combination may increase the degree of anti-microbial action and lower the necessary concentration of individual substances (Jay, J. M., 2000). Application of the organic acids or their salts for RTE meats logically seem best as surface treatments because \textit{L. monocytogenes} contamination on the products is typically on the surface following peeling and packaging.

It was reported that the antilisterial activity of organic acids in frankfurters depended upon the \textit{Listeria} characteristics, pH value, water activity and the storage conditions, including storage time, temperature, and atmosphere (Davidson, P. M., 2002). Recent research has suggested that the factors that influence the antilisterial activity of organic acids on frankfurters, usually act in an interactive manner (Davidson, P. M., 2002). Combining sub-optimal physico-chemical conditions such as pH, temperature and water activity usually has a greater effect than any of the individual factors used at the same level (Mossel et al., 1995; Bell and Kyriakides, 1998). Heat or cold temperature treatments applied to frankfurters formulated with or dipped in an organic acid are expected to be more effective for \textit{L. monocytogenes} inactivation than the same organic acid treatment alone (Bell and Kyriakides, 1998).

The purpose of this study was to evaluate three potential organic acid salts (sodium diacetate, the combination of sodium diacetate and potassium benzoate, and the combination
of sodium lactate, sodium diacetate and potassium benzoate), at two concentrations (3 or 6%) and a range of storage temperatures (-2.2, 1.1, 4.4, 10.0 or 12.8 °C) to assess potential inhibition of *L. monocytogenes* on frankfurters.

**Materials and Methods**

**Manufacturing process**

Meat trimmings, consisting of boneless beef (80/20 lean/fat ratio), lean pork (50/50 lean/fat ratio) and pork fat (20/80 lean/fat ratio), were formulated into frankfurters. The three sources of meat were singly ground using a 0.79 cm grinder plate and mixed prior to sampling for measurement of actual fat content. Frankfurters were formulated on a 25% fat basis for the meat block. Beef trim, salt, sodium erythorbate, sodium nitrite, seasonings and one-half of the total water (ice) were chopped for approximately 3 minutes in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA), until temperature reached 40 °F. The pork trim, pork fat and remaining one-half of the ice were then added and this composite was chopped for an additional 3 minutes, until temperature reached 55 °F. The meat batter was placed into a vacuum stuffer (Risco® Model RS 4003-165; Stoughton, MA) and stuffed into 22 mm diameter, peelable cellulose casings (Devro Teepak™Wienie-Pak® Coastal Corrugated Inc., N. Charleston, SC) and linked (Poly-clip® Gmbh & Co. KG, Germany) at 8.5 cm in length.

A conventional cooking-smoking cycle of 2.5 hours for frankfurters was used in a humidity-controlled smoke house (Alkar, DEC International Inc., Lodi, WI) to achieve a cooked product internal temperature of 71.1 °C. The franks were showered and then chilled for approximately 16-18 hours in a 4 °C cooler.

**Experimental design**

This study included seven different surface treatments, including a control with no surface dip solution for the frankfurters, sodium diacetate alone at 3 or 6% (total wt/vol, SD), sodium diacetate + potassium benzoate at 3 or 6% (total wt/vol, SD/PB), and sodium lactate + sodium diacetate + potassium benzoate at 3 or 6% (total wt/vol, SL/SD/PB). Preliminary experiments showed that dipping for 3 min resulted in an approximately 0.08% pickup by the frankfurters. Surface-treated products were placed in bags (2 frankfurters per package) and inoculated with a 5-strain cocktail of *L. monocytogenes*, to reach a final level of $10^4$ cells per
package (90.8 g). Each of the treatments also included a treated but uninoculated control. The 7 treatments utilized for both inoculated and non-inoculated frankfurters are shown in Table 1.

Frankfurters were stored at −2.2, 1.1, 4.4, 10.0 or 12.8°C for up to 90 days. Packages were analyzed for *L. monocytogenes* counts immediately after inoculation and then every 48 hours during 90 days of storage. Background bacteria, i.e. mesophilic aerobic plate counts (APC), lactic acid bacteria and yeast/mold counts were also enumerated.

**Microorganisms**

The inoculum included 5 *L. monocytogenes* strains: Scott A (human isolate), H7764 (serotype 1/2a, deli meat isolate), H7762 (serotype 4b, hotdog isolate), H7962 (serotype 4b, hotdog isolate), and H7969 (serotype 4b, hotdog isolate). Scott A strain was obtained from National Animal Disease Center (NADC), and the other four strains were obtained from the Centers for Disease Control and Prevention, Atlanta, GA (CDC), as clinical isolates from the Bil Mar Foods outbreak of 1998-1999.

The *L. monocytogenes* cultures were individually grown in Trypticase Soy Broth containing 0.6% Yeast Extract (TSB-YE broth, Difco Laboratories, Becton Dickinson and Company, Sparks, Maryland) in a rotating platform incubator at 35 °C for 24 hours. Then, 1 ml of culture from each individual stain was combined to give a 5-ml mixed culture of *L. monocytogenes*, and the mixed culture was transferred to 500-ml TSB-YE broth and incubated at 35 °C for 24 hours to reach the stationary phase. The final concentration of the original 5-strain mixture of *L. monocytogenes* was 9.30 log CFU/ml, and the culture was serially diluted to achieve 10^5 cells/ml in the inoculum. The number of viable cells in the inoculum was verified by plate count methods. Typical colonies were gram-stained and then analyzed by using API *Listeria* kits (bioMerieux, Inc., Hazelwood, MO).

Frankfurters were surface inoculated with the 5-strain mixture of *L. monocytogenes* by adding 1 ml of the inoculum (10^4 cells/ml) into each package. Average package weight was 90.8 g. The packages were hand-massaged 5-10 s to evenly distribute the inoculum on the surface of each frankfurter immediately after inoculation. After vacuum-packaging, all frankfurters were placed in U.N. approved biosafety shippers (AirSea-Atlanta, GA) with cold packs. A temperature recorder was placed in one of the biosafety shippers look for assurance
of refrigeration, and the samples were shipped overnight to Silliker Laboratories for microbial analysis.

**Packaging**

Both uninoculated and inoculated products were packaged using polyvinyl chloride (PVC) high-barrier bags (Cryovac® B-540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5-0.6 g at 100 °F, 100% RH/100 sq. in. /24h; oxygen transmission rate = 3-6 CC at 40 °F /m²/24 h/0% RH) after the appropriate treatments.

**Measurements**

**Chemical analyses**

Proximate analyses for moisture, fat and protein were conducted on cooked product samples according to procedures outlined by the AOAC (1990).

Sodium lactate, SD, and PB analyses were determined on the surface-treated frankfurters as outlined in the AOAC (1990), in order to verify that the formulations did not exceed the allowable limit (9 CFR 318.7) of 0.1 percent for SD and PB which was in effect at the time of this work.

**Microbiological analyses**

Detection of *L. monocytogenes* was done according to the U.S. Department of Agriculture/Food Safety Inspection Service method (USDA, 1998), except that quad plates were used during the confirmation (Evanson, et al., 1991).

One hundred packages of franks of each of the seven treatments (Control, 3% SD, 6% SD, 3%SL/SD/PB, 6% SL/SD/PB, 3% SD/PB, and 6% SD/PB) were stored at one of 5 temperatures (-2.2, 1.1, 4.4, 10.0, and 12.8 °C). Half of the packaged franks were inoculated with *L. monocytogenes*, the other packages served as uninoculated control samples. Packages of the untreated frankfurters and all treatments stored at -2.2, 1.1, 4.4, 10 and 12.8 °C were analyzed initially (day 1) and every other day for 90 days or until counts of *Listeria* reached 10⁶ colony forming units/g for two consecutive intervals.

For microbial counting, packages were aseptically opened, the contents transferred to a sterile stomacher bag and homogenized for two minutes with Butterfields-phosphate buffer added at a 1:1 weight ratio. Twenty-five gram aliquots of homogenate were added to 100 grams diluent in sterile bottles and homogenized by manual agitation to make a 1:10 dilution.
Subsequent dilutions were performed with 9.9 ml tubes of Butterfield’s phosphate buffer. Control samples were analyzed for aerobic mesophilic bacteria (Plate Count Agar incubated at 35 °C for 48 hours), lactic acid bacteria (DeMan, Rogasa, Sharpe (MRS) agar with overlay agar incubated at 30 °C for 5 days), yeast and mold (Potato Dextrose Agar incubated at 25 °C for 5 days) and *L. monocytogenes*. *L. monocytogenes* was enumerated with a pour plate technique with an underlay of non-selective Tryptase Soy Agar with 0.6% yeast extract (TSAYE). After the TSAYE hardened, and overlay of modified Oxford agar was added. Small white/gray colonies with black haloes were considered typical for *L. monocytogenes*. Test samples were analyzed for *L. monocytogenes* by plating methods (TSAYE/MOX overlay).

**Curve fitting and shelf life prediction**

A modified Gompertz sigmoid curve was used to fit *L. monocytogenes* growth curves, and three growth curve parameters for *L. monocytogenes* on frankfurters, lag phase duration time, generation time, and maximum population density, were calculated by applying estimates of 4 parameters (a, b, X₀, and Y₀) from the Gompertz equation (Buchanan and Bagi, 1999; Franses, P. H., 2002). In this study, curve fitting was performed using Sigma Plot 2000 software (SPSS Science, Chicago, IL, USA).

The Gompertz equation is: $Y = Y₀ + a \times \exp (-\exp (-\frac{X - X₀}{b}))$, where $X =$ time at which log₁₀ count is $Y$; $X₀ =$ time at which absolute growth rate is at a maximum; $Y = \log_{10}$ count at a time $X$; $Y₀ =$ asymptotic log count as $X$ decreases indefinitely; $a =$ asymptotic amount of growth that occurs as $X$ increases indefinitely; $1/b =$ relative growth rate at $X₀$ (Wei and Fang, 2001).

These parameters were used to derive maximum population density, generation time, and lag phase from the data on *L. monocytogenes* as follows:

- Maximum population density = $a + Y₀$; \hspace{1cm} equation (1)
- Lag phase (days) = $X₀ - 0.9624 \times b$; \hspace{1cm} equation (2)
- Generation time (days) = $0.81828 \times \frac{b}{a}$. \hspace{1cm} equation (3)

**Statistical analyses**

A complete block design was used for the microbiological analyses. The data on *L. monocytogenes* counts were analyzed by Sigma Plot 2000 (SPSS Science, Chicago, IL, USA).
software after translation to logarithms. Growth curve parameters of *L. monocytogenes* were analyzed by the MIXED procedure in SAS V8.1 (SAS Inc. 2000). The treatment effects were considered to be significantly different when *P*-values were less than or equal to 0.05, and means were separated using Tukey test. The experiment was repeated twice.

**Results and Discussion**

**Chemical analyses**

The fat, moisture and protein content of the finished products were 25.62%, 55.76% and 17.12%, respectively. There were no significant differences for fat, moisture or protein content (data not shown) in the frankfurters as formulated (*P* > 0.05).

**Microbial analyses**

Microorganism counts for both *L. monocytogenes* and indigenous bacteria, i.e. mesophilic aerobic plate counts (APC), lactic acid bacteria and yeast/mold, were less than 1 log (10 CFU/g) for all 7 uninoculated controls (data not shown).

The growth curve of *L. monocytogenes* for each treatment at each temperature was fitted to the modified sigmoid Gompertz equation. The three growth parameters of *L. monocytogenes* on frankfurters, maximum population density in log CFU/g calculated by Equation (1), lag phase in days calculated by Equation (2), and generation time in days calculated by Equation (3), are shown in tables 2, 3 and 4 respectively.

Table 2 shows the effects of inhibitors on the maximum population density of *L. monocytogenes* of frankfurters. Significant temperature effects were observed (*P* < 0.05), but there were no significant treatment effects or interaction effects between temperature and treatments (*P* > 0.05). The maximum population density of *L. monocytogenes* on frankfurters was significantly reduced when stored at -2.2 °C compared with 1.1 °C or higher temperatures. Storage at 1.1 °C also significantly reduced the maximum population density of *L. monocytogenes* relative to storage at 4.4, 10.0 or 12.8 °C. However, no significant differences were observed in maximum population density of *L. monocytogenes* when stored at 4.4, 10.0, or 12.8 °C.

The overall treatment effects on the lag phase of *L. monocytogenes* were not significant (*P* > 0.05, Table 3). However, significantly longer lag phases were observed for treatments with lower storage temperatures (-2.2, 1.1 or 4.4 °C) compared with the higher
temperature groups (10.0 or 12.8 °C, \( P < 0.05 \)). No significant interaction between temperature and treatment was observed.

Table 4 shows the effects of inhibitors on the generation time of \( L. \) monocytogenes in days. Treatments, temperatures and the interaction between treatments and temperatures all had significant effects on the generation time of \( L. \) monocytogenes \( (P < 0.05) \). The generation time for \( L. \) monocytogenes on frankfurters treated with 6% SD (21.74 days) was greater than all of the other treatments in this study including the control group (1.79 days). Obviously, 6% SD was the most effective among the 7 treatments used in this study for extending the generation time of \( L. \) monocytogenes on frankfurters. Lower storage temperatures (-2.2, 1.1 or 4.4 °C) also significantly increased the generation time of \( L. \) monocytogenes relative to higher storage temperatures (10.0 or 12.8 °C).

Temperature is one of the most important external factors affecting \( L. \) monocytogenes growth. This study was conducted at -2.2, 1.1, 4.4, 10.0 and 12.8°C for up to 90 days. At -2.2 °C, no \( L. \) monocytogenes growth on frankfurters was observed for any of the 7 treatments for 90 days, therefore, no statistical difference was obtained among those 7 treatments for maximum population density, lag phase duration time, or generation time of \( L. \) monocytogenes. At 1.1 °C, the 6% SD treatment strongly inhibited \( L. \) monocytogenes growth for 90 days, with the maximum population density of \( L. \) monocytogenes remaining lower than the initial inoculation. Statistically, the treatments with 6% SD, 6% SL/SD/PB, 3% SD/PB and 6% SD/PB all significantly lowered the maximum population density of \( L. \) monocytogenes on frankfurters compared with control and the 3% SD group. The lag phase for \( L. \) monocytogenes resulting from treatments with 3% SD (44.51 days), 6% SD (> 90 days), 6% SL/SD/PB (45.00 days), 3% SD/PB (57.50 days) and 6% SD/PB (49.08 days) were significantly longer than the control group, therefore, all of these organic acid salts effectively increased shelf life of the frankfurters. At 4.4 °C, \( L. \) monocytogenes grew on surface of the frankfurters for all treatments. The 6% SD treatment appeared to have the lowest maximum population density (4.43 log CFU/g) among the 7 treatments, but this was not significantly lower than the control group (6.65 log CFU/g). The lag phase for \( L. \) monocytogenes resulting from 3% SD (50.06 days), 6% SD (23.57 days), 3% SL/SD/PB (30.00 days), 3% SD/PB (39.60 days) and 6% SD/PB (36.95 days) seemed longer than the
control group (18.04 days), but these were also not statistically significant ($P > 0.05$).

However, the generation time for *L. monocytogenes* following with treatment of 6% SD (5.68 days) was significantly greater than for the other 6 treatments, and it was about three times as long as the control group (1.80 days). Consequently, *L. monocytogenes* can survive during refrigerated storage of frankfurters even in the presence of these organic acid salts, a study by Harmayani et al. (1993) also concluded that *L. monocytogenes* was capable of surviving in refrigerated storage in the presence of the additives (1.8% SL, 0.1% sodium erythorbate, and 1% kappa-carrageenan) tested.

At 10.0 and 12.8 °C, none of the treatments reduced *L. monocytogenes* growth and the maximum population density reached levels above 5.54 log CFU/g. The lag phase varied from 6.50 to 15.00 days at 10.0 °C, and 4.09 to 12.00 days at 12.8 °C.

Across the temperatures of -2.2, 1.1, 4.4, 10.0 and 12.8 °C, the overall mean of generation time (0.67 – 1.11 days) by treatment with 6% SD (2.55 days) was significantly greater than the control, 3% SD, 6% SL/SD/PB, 3% SD/PB and 6% SD/PB treatments in this study ($P<0.05$). The generation time for *L. monocytogenes* treated with 3% SL/SD/PB was 0.91 day, lower than 6% SD/PB (1.05 days) and 6% SL/SD/PB (1.11 days) treatments. However, this was not significantly lower than the 6% SD treatment, because missing values for the 3% SL/SD/PB treatment at 10.0 and 12.8 °C in the first replication made the standard error for the 3% SL/SD/PB treatment higher than for the other 6 treatments. However, the Tukey test $P$-value between the 3% SL/SD/PB and 6% SD treatments is 0.056, very close to the critical cut-off point of 0.05.

**Added compounds**

The concentrations of the added compounds, (sodium lactate, sodium diacetate, and potassium benzoate), in the frankfurters, were all below 0.1%, ranging from 0.82 to 0.99, as was intended when the project was designed (data not shown).

**Conclusions**

Surface treatments with 3% SD, 6% SD, 3% SL/SD/PB, 3% SD/PB and 6% SD/PB effectively inhibited *L. monocytogenes* growth at -2.2, 1.1 and 4.4 °C compared to the control group, and the treatment of 6% SD was the most effective.
The results of this study, however, also suggest that under the conditions used for this study, *L. monocytogenes* is capable of surviving refrigerated storage even in the presence of the additives tested, and storage temperatures lower than 4.4 °C are necessary for frankfurters even with surface treatments utilizing organic acid salts.
Table 1. Treatments for frankfurters at −2.2, 1.1, 4.4, 10.0 or 12.8 °C

<table>
<thead>
<tr>
<th>Treatment number</th>
<th>Uninoculated</th>
<th>Inoculated with $10^4$ <em>L. monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>3% SD$^1$</td>
<td>3% SD</td>
</tr>
<tr>
<td>3</td>
<td>6% SD$^2$</td>
<td>6% SD</td>
</tr>
<tr>
<td>4</td>
<td>3% SL/SD/PB$^3$ (1:1:1)</td>
<td>3% SL/SD/PB (1:1:1)</td>
</tr>
<tr>
<td>5</td>
<td>6% SL/SD/PB$^4$ (1:1:1)</td>
<td>6% SL/SD/PB (1:1:1)</td>
</tr>
<tr>
<td>6</td>
<td>3% SD/PB $^5$ (1:1)</td>
<td>3% SD/PB (1:1)</td>
</tr>
<tr>
<td>7</td>
<td>6% SD/PB $^6$ (1:1)</td>
<td>6% SD/PB (1:1)</td>
</tr>
</tbody>
</table>

$^1$ 3% solution of sodium diacetate  
$^2$ 6% solution of sodium diacetate  
$^3$ 3% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination  
$^4$ 6% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination  
$^5$ 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination  
$^6$ 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Treatment</th>
<th>Mean*</th>
<th>Temperatures (°C)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>-2.2</td>
<td>1.1</td>
<td>4.4</td>
<td>10.0</td>
<td>12.8</td>
<td></td>
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<tr>
<td>Control</td>
<td></td>
<td>5.30</td>
<td>ab</td>
<td>0.95 a A</td>
<td>4.95 b B</td>
<td>6.65 ab B</td>
<td>6.96 a B</td>
<td>7.00 a B</td>
</tr>
<tr>
<td>3%SD</td>
<td></td>
<td>5.59</td>
<td>a</td>
<td>1.38 a A</td>
<td>5.75 b B</td>
<td>6.39 ab B</td>
<td>7.16 a B</td>
<td>7.30 a B</td>
</tr>
<tr>
<td>6%SD</td>
<td></td>
<td>4.23</td>
<td>b</td>
<td>0.74 a A</td>
<td>0.82 a A</td>
<td>4.43 a B</td>
<td>7.75 a C</td>
<td>7.44 a C</td>
</tr>
<tr>
<td>3%SL/SD/PB</td>
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<td>4.69</td>
<td>ab</td>
<td>2.14 a A</td>
<td>4.19 ab AB</td>
<td>5.18 ab B</td>
<td>5.54 a B</td>
<td>6.41 a B</td>
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<tr>
<td>6%SL/SD/PB</td>
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<td>a</td>
<td>1.84 a A</td>
<td>3.10 ab A</td>
<td>7.42 b B</td>
<td>7.55 a B</td>
<td>7.82 a B</td>
</tr>
<tr>
<td>3%SD/PB</td>
<td></td>
<td>5.13</td>
<td>ab</td>
<td>1.63 a A</td>
<td>3.17 ab A</td>
<td>6.30 ab B</td>
<td>7.62 a B</td>
<td>6.95 a B</td>
</tr>
<tr>
<td>6%SD/PB</td>
<td></td>
<td>5.01</td>
<td>ab</td>
<td>1.22 a A</td>
<td>3.51 ab A</td>
<td>6.44 ab B</td>
<td>7.06 a B</td>
<td>6.83 a B</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td>1.41</td>
<td>A</td>
<td>3.64 B</td>
<td>6.12 C</td>
<td>7.09 C</td>
<td>7.11 C</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Overall mean across all temperatures (-2.2, 1.1, 4.4, 10.0 and 12.8 °C)

Different letters A-C within each row indicate significant differences (P < 0.05)

Different letters a-b within each column indicate significant differences (P < 0.05)
Table 3. Effects of inhibitors on the lag phase (Days) of *L. monocytogenes* on frankfurters

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Overall mean*</th>
<th>Temperatures (°C)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-2.2</td>
<td>1.1</td>
<td>4.4</td>
<td>10.0</td>
<td>12.8</td>
</tr>
<tr>
<td>Control</td>
<td>9.54 a NG</td>
<td>21.96 a A</td>
<td>18.04 a A</td>
<td>6.50 a A</td>
<td>4.09 a A</td>
<td></td>
</tr>
<tr>
<td>3% SD</td>
<td>22.68 a NG</td>
<td>44.51 a A</td>
<td>50.06 a A</td>
<td>11.38 a A</td>
<td>7.34 a A</td>
<td></td>
</tr>
<tr>
<td>6% SD</td>
<td>16.05 a NG</td>
<td>NG</td>
<td>23.57 a A</td>
<td>12.63 a A</td>
<td>11.96 a A</td>
<td></td>
</tr>
<tr>
<td>3% SL/SD/PB</td>
<td>18.28 a NG</td>
<td>NA</td>
<td>30.00 a A</td>
<td>15.00 a A</td>
<td>12.00 a A</td>
<td></td>
</tr>
<tr>
<td>6% SL/SD/PB</td>
<td>9.69 a NG</td>
<td>45.00 a A</td>
<td>15.26 a A</td>
<td>7.23 a A</td>
<td>6.58 a A</td>
<td></td>
</tr>
<tr>
<td>3% SD/PB</td>
<td>18.75 a NG</td>
<td>57.50 a B</td>
<td>39.60 a AB</td>
<td>9.58 a A</td>
<td>7.08 a A</td>
<td></td>
</tr>
<tr>
<td>6% SD/PB</td>
<td>16.03 a NG</td>
<td>49.08 a B</td>
<td>36.95 a AB</td>
<td>9.32 a AB</td>
<td>1.83 a A</td>
<td></td>
</tr>
<tr>
<td>Overall mean</td>
<td></td>
<td>29.96 B</td>
<td>9.70 A</td>
<td>6.20 A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Overall mean cross the temperatures of 4.4, 10.0 and 12.8 °C

NG means no growth

NA means not available due to the large variation of the growth data

Different letters A-B within each row indicate significant differences (*P* ≤ 0.05)

Different letters a-b within each column indicate significant differences (*P* < 0.05)
Table 4. Effects of inhibitors on the generation time (days) of *L. monocytogenes* on frankfurters

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Overall Mean*</th>
<th>-2.2</th>
<th>1.1</th>
<th>4.4</th>
<th>10.0</th>
<th>12.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.88 a</td>
<td>NG</td>
<td>4.52 a B</td>
<td>1.80 a A</td>
<td>0.44 a A</td>
<td>0.41 a A</td>
</tr>
<tr>
<td>3% SD</td>
<td>0.67 a</td>
<td>NG</td>
<td>1.90 a A</td>
<td>1.07 a A</td>
<td>0.68 a A</td>
<td>0.25 a A</td>
</tr>
<tr>
<td>6% SD</td>
<td>2.55 b</td>
<td>NG</td>
<td>NG</td>
<td>5.68 b B</td>
<td>0.90 a A</td>
<td>1.07 a A</td>
</tr>
<tr>
<td>3% SL/SD/PB</td>
<td>0.91 ab</td>
<td>NG</td>
<td>NA</td>
<td>2.13 a A</td>
<td>0.37 a A</td>
<td>0.06 a A</td>
</tr>
<tr>
<td>6% SL/SD/PB</td>
<td>1.11 a</td>
<td>NG</td>
<td>2.11 a A</td>
<td>2.47 a A</td>
<td>0.77 a A</td>
<td>0.10 a A</td>
</tr>
<tr>
<td>3% SD/PB</td>
<td>0.72 a</td>
<td>NG</td>
<td>3.38 a A</td>
<td>1.56 a A</td>
<td>0.51 a A</td>
<td>0.10 a A</td>
</tr>
<tr>
<td>6% SD/PB</td>
<td>1.05 a</td>
<td>NG</td>
<td>3.17 a A</td>
<td>1.95 a A</td>
<td>0.42 a A</td>
<td>0.78 a A</td>
</tr>
<tr>
<td>Overall Mean</td>
<td></td>
<td></td>
<td></td>
<td>2.38 B</td>
<td>0.58 A</td>
<td>0.40 A</td>
</tr>
</tbody>
</table>

* Overall mean across temperatures (4.4, 10.0 and 12.8 °C)

NG = no growth

NA = not available due to large variation of the growth data

Different letters A-B within each row indicate significant differences (*P* < 0.05)

Different letters a-b within each column indicate significant differences (*P* < 0.05)
References


CHAPTER 5. THE EFFECTS OF ORGANIC ACID SALT SOLUTIONS ON SENSORY AND OTHER QUALITY CHARACTERISTICS OF FRANKFURTERS

A paper to be submitted to *The Journal of Food Science*

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Keywords: food safety, organic acid salts, *Listeria monocytogenes*, frankfurters

Abstract

The objective of this study was to investigate the technical feasibility of using organic acid salts for surface treatment of frankfurters to inhibit bacterial growth. To be practically effective, such treatments for frankfurters should have no adverse effects on meat quality attributes, including sensory quality, color, or texture. The 6% SD/PB treatment significantly increased meat flavor and the 3% SL/SD/PB treatment significantly decreased smoke flavor compared with the control group, but there was no significant difference between controls and the surface-treated frankfurters when comparing salty, sour, or pepper flavors. On the other hand, the SL/SD/PB at either 3 or 6% significantly increased the lightness (L*) and decreased the redness (a*) value for frankfurters compared with the control group. For the storage time that was longer than two months, the L* value significantly increased and the a* value decreased. Evaluation of quality characteristics showed that after surface treatment with organic acid salts, no differences were observed between controls and treated frankfurters for pH, nitrite concentration or sodium content.

Introduction

With the increased concern for food safety by the meat industry, more and more of the industry has begun using organic acid salts to inhibit *L. monocytogenes* growth, particularly after USDA issued the “zero” tolerance policy for ready-to eat (RTE) meat.

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products. It has been reported that meat products, surface treated with organic acids, e.g. lactate (Blom et al., 1997; Maca et al., 1999; Nerbrink et al., 1999), diacetate (Islam et al., 2002; Lou and Yousef, 1999), benzoate (Islam et al., 2002; Lou and Yousef, 1999) and sorbate (El-Shenawy and Marth, 1988; Islam et al., 2002), or their salts effectively inhibited *L. monocytogenes* growth on frankfurters. Recent research has suggested that combinations of the organic acids or their salts may be more effective for inhibition of *L. monocytogenes* growth in meat products than when these compounds are used alone (Jay, J. M., 2000). However, a major concern for use of the organic acids and their salts is the potential decrease in sensory and other quality characteristics of the products, because some organic acid salts reduce product pH (Blom et al., 1997; Samelis et al., 2001; Islam et al., 2002). Moreover, Islam et al. (2002) reported that frankfurters treated with SD had slightly lower scores for flavor and overall acceptance because of a strong initial acetic acid odor, though some reports said that the combination of the lactate and acetate did not adversely affect the sensory properties of the bologna (Blom et al., 1997), therefore, it is required to test sensory panel to evaluate their influence on product quality. Previous studies in this laboratory (Lu et al., 2004a; Lu et al., 2004b) demonstrated that the sodium diacetate (SD), sodium diacetate + potassium benzoate (SD/PB) and sodium lactate + sodium diacetate + potassium benzoate (SL/SD/PB) treatments effectively inhibited *L. monocytogenes* growth, and the treatments are potential useful chemical preservatives for *L. monocytogenes* growth inhibitors, but there is no published data describing the sensory evaluation results of the organic acid salts treatments. Therefore, it was important to conduct a study to evaluate the effects of surface dipping with SD, the SD/PB, or SL/SD/PB treatment at either 3% or 6% on sensory and some other quality characteristics of frankfurters.

The purpose of this study was to evaluate frankfurters for potential quality changes following surface treatment with organic acid salt solutions and subsequently determine the feasibility of using organic acid salts to inhibit bacteria growth on frankfurters.

**Materials and Methods**

**Manufacturing process**

Meat trimmings, consisting of boneless beef (80/20 lean/fat ratio), lean pork (50/50 lean/fat ratio) and pork fat (20/80 lean/fat ratio), were formulated into frankfurters. The three
meat sources were each ground using a 0.79 cm grinder plate and mixed prior to sampling for measurement of actual fat content. Frankfurters were formulated on a 25% fat basis. Beef trim, salt, sodium erythorbate, sodium nitrite, seasonings and one-half of the total added water (ice) were chopped for approximately 3 minutes in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA), until the temperature reached 40 °F. The pork trim, pork fat and remaining one-half of the ice were then added and this composite was chopped for an additional 3 minutes, until temperature reached 55 °F. The meat batter was placed into a vacuum stuffer (Risco® Model RS 4003-165; Stoughton, MA) and stuffed into 22 mm diameter, peelable cellulose casings (Devro Teepak®Wienie-Pak® Coastal corrugated Inc., N. Charleston, SC) and linked (Poly-clip® Gmbh & Co. KG, Germany) at 8.5 cm in length. Two separate batches were produced on different days to provide two complete experimental replications.

A conventional cooking-smoking cycle of 2.5 hours for frankfurters was used in a humidity-controlled smoke house (Alkar, DEC International Inc., Lodi, WI) to achieve a cooked product internal temperature of 71.1 °C. The franks were showered and then chilled for approximately 16-18 hours in a 4 °C cooler.

Experimental design

This study included seven treatment groups for the frankfurters including a control that received no antimicrobial treatment. The six treated groups of frankfurters were dipped into the following antimicrobial solutions: sodium diacetate alone at 3 or 6% (total wt/vol, SD), sodium diacetate + potassium benzoate at 3 or 6% (total wt/vol, SD/PB), and sodium lactate + sodium diacetate + potassium benzoate at 3 or 6% (total wt/vol, SL/SD/PB). Preliminary experiments showed that dipping for 3 min resulted in approximately 0.08% total pickup of the compounds by the frankfurters. Frankfurters were stored at 4.4°C for up to 90 days. Sensory evaluation, proximate analysis, concentration of the chemical preservatives, nitrite content, color, texture, pH value, and sodium chloride concentration of the frankfurters were determined during storage.

Packaging

Products were packaged under vacuum in polyvinyl chloride (PVC) high-barrier bags (Cryovac® B-540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5-
0.6 g at 100 °F, 100% RH/100 sq. in. /24h; oxygen transmission rate = 3-6 CC at 40 °F /m²/24 h/0% RH) following the surface-dipping treatments.

Measurements

Sensory evaluation

Frankfurters from each production batch were evaluated for sensory properties immediately after processing, and after four weeks of storage. Sample preparation and testing procedures for evaluating sensory attributes of frankfurters were according to the American Society for Testing and Materials (ASTM, 1973), Civille and Szczesniak (1973) and Meilgaard et al. (1991). Frankfurters were cooked in a microwave oven on a ribbed Litton Ware bacon and meat tray for two minutes and fifteen seconds. The tray was turned 90° after 75 seconds of cooking. Seven frankfurters were cooked on a tray and the frankfurters were placed between the grooves, each frank separated by a groove to prevent contact between them. After cooking, 60 mm pieces were cut from the center of each of the frankfurters, placed in covered 4 oz. Styrofoam containers, and served to the panelists while warm. The containers were labeled with three-digit random codes numbers.

Twelve panelists were recruited from the faculty, staff, and students of Iowa State University. The University’s Human Subjects in Research Committee approved the project and the panelists were compensated for their participation. Panelists were trained to evaluate salt, sour, cured-meat, pepper and smoke-like flavors in two one-hour training sessions. During training, panelists were familiarized with the tasting techniques to be used during the evaluation procedure and with the computer software scoring system. To familiarize panelists with the flavor attributes of smoke-like and cured-meat flavors, commercial turkey-and-pork frankfurters were used as well as control frankfurters from this experiment. To create a stronger salt note, commercial frankfurters were boiled in 5% salt water for five minutes and air-dried before serving. Intense pepper flavor was demonstrated by boiling commercial frankfurters for five minutes in water that had been seasoned with pepper (one-fourth teaspoon of course ground pepper in one cup of water) and boiled for 5 minutes before filtering. To create a sour note, frankfurters were immersed in vinegar for 4 minutes and air dried before cooking in the microwave oven.
Three sessions were conducted for each evaluation period and twelve panelists evaluated seven samples in each session. Water and unsalted crackers were available to panelists. Panelists were instructed to rinse with water between samples. Testing was conducted in partitioned booths and under fluorescent lighting conditions. A line scale (numerical value of 15 units) was used with descriptive anchors (none and high) indented 0.5 unit from each end of the line.

**Color**

The Hunter L*, a*, b*, values of frankfurters were measured using a Minolta colorimeter (CR-300, Ramsey, NJ). Two measurements were conducted on the surface of frankfurters through the polyvinyl chloride (PVC) package film.

**Texture**

Texture profile analysis (TPA) as described by Bourne (1978) was performed using an Instron Universal Testing Machine, Texture Analyzer (Model TA.XT 2 I, Godalming, UK), to assess skin toughness and interior firmness by measuring puncture resistance and interior texture with a 3-mm puncture probe. Five frankfurters from each treatment were measured in the center and the end of each link. The probe was programmed to penetrate 12 mm into the samples following measurement of the surface skin resistance. Penetration speed was 1.5 mm / sec, all samples were measured at room temperature 3 h after removal from the refrigerator.

**pH**

Duplicate 5 gram portions of meat samples were placed in a 250 mL beakers of distilled water and homogenized for 30 sec using a biohomogenizer. The pH was measured with Accumet ® 925 pH/ion meter (Fisher Scientific company, 711 Forbes Avenue Pittsburgh, PA 15219).

**Sodium chloride (NaCl) concentration**

Sodium chloride concentration was measured by the Quantab method (Quantab Chloride Titrator, Ames, Co. Miles Laboratories, Inc.). Measured chloride ion concentration in the meat extract was used to calculate the percentage of sodium chloride.
Nitrite

Nitrite (NaNO₂) analysis was determined on raw and cooked frankfurters according to procedures outlined by AOAC International (1992).

Statistical analyses

A complete random block design was used for the analyses. The data on sensory evaluation, color values, pH, sodium chloride concentration and texture profiles were analyzed by the MIXED procedure of the Statistical Analysis System (SAS, 2000). The treatment effects were considered to be significantly different when P-values were less than or equal to 0.05, and means were separated using the Tukey test. The experiment was replicated twice.

Results and Discussion

Added compounds

The measured concentration of the added compounds was between 0.082 and 0.098% for the surface treated frankfurters, just below the target level of 0.10% (data not shown).

Sensory evaluation

The 6% SD/PB treatment significantly increased meaty flavor and 3% SL/SD/PB treatment markedly decreased smoke flavors compared with control group, however, there was no significant treatment effect observed on salty, sour or pepper flavors among the 7 treatments (Table 1). Blom et al. reported that there was no adverse effect on sensory properties after combination of 2.5% lactate (w/v) and 0.25% acetate (w/v) treatment, but the combination of SL, SD, and PB decreased smoke flavor of the frankfurters. In contrast, no lower scores were observed in sour and meaty flavor for frankfurters with SD treatment as was reported by Islam et al. (2002).

The 6% SD treatment made the frankfurters much more salty than the 3% SD and 3% SL/SD/PB groups (P < 0.05). Sodium diacetate at 6% showed the highest sourness among the 7 treatments, and was significantly more sour than the 3% SL/SD/PB, 6% SL/SD/PB, 3% SD/PB, and the 6% SD/PB treatment. The 3% SD treatment was significantly more sour than the 6% SL/SD/PB treatment. The 6% SD/PB treatment significantly increased the meaty flavor of the frankfurters compared with the control, 3% SD and 6% SD treatments (P < 0.05), and the 6% SL/SD/PB treatment has significantly higher in meaty flavor than the 6%
SD treatment. The results suggest that, SD increased sourness but decreased the meaty flavor of the frankfurters. In contrast, SL/SD/PB and SD/PB treatments decreased sourness but increased the meaty flavor of the frankfurter, and the higher level of the organic acid salts tended to have a greater effect.

The difference in pepper flavor between SD and SL/SD/PB treatments was significant \( (P < 0.05) \). The SL/SD/PB treatment increased pepper taste, but SD declined pepper flavor of the frankfurters. Lower levels of the organic acid salts showed stronger effects on pepper flavors. Frankfurters in all of the six organic acid salts treatment showed a lower smoke flavor than controls, but only the 3% SL/SD/PB treatment was significantly different \( (P < 0.05) \).

No time effect or interaction between treatment and time were observed for any of the sensory properties evaluated in this study. Therefore, vacuum packaged frankfurters stored at 4.4 °C for up to 90 days did not change in terms of sensory characteristics during storage.

**Color**

The lightness value \( (L^* ) \) for frankfurters was significantly affected by the different treatments and the storage time \( (P < 0.05) \), but not the treatment-time interactions (Table 2). The 3% SD/SL/PB (53.58) and 6% SD/SL/PB (53.10) treatments had significantly higher \( L^* \) values than the control (51.42, \( P < 0.05 \)). However, there was no significant difference in \( L^* \) value between control and the other treatments, thus, only the combination of SD/SL/PB affected the lightness of frankfurters. The storage period was also a factor affecting the lightness of frankfurters. When the storage period was extended to as long as 4 months, the frankfurters became lighter when compared to the first two months \( (P < 0.05) \). The \( L^* \) values however, were 52.76 vs. 52.18 and 52.14 respectively and the difference is not likely to have a practical importance.

The “a*” value of the frankfurters was also affected by both the treatment and the storage time, but not the treatment-time interaction. The control (16.37) and 6% SD (16.43) treatments were redder than 3% SD/SL/PB and 6 % SD/SL/PB treatments (14.89 and 15.16 respectively), but there was no difference in redness between the control and the other treatments.
Storage time was a factor affecting the redness of frankfurters. The “a*” values during the first two months showed no difference, but was significantly increased in the 3rd and 4th month (16.83 and 17.35 vs. 14.74 and 14.25 respectively). These results suggest a decline in redness of frankfurters with storage time, which after 60 days became significant. Overall, the interaction between treatments and storage time showed a trend for decreased redness with storage time, but the interaction was not significant. This trend for decreasing “a*” values was consistent with the results of the “L*” value measurement that were increased.

The same as “L*” and “a*” values, the “b*” value of the frankfurters was affected by both the treatments and the storage time, but not their interactions. The 3% SD (17.97), 3% SL/SD/PB (18.06), 6% SL/SD/PB (18.36), and 3% SD/PB (18.28) treatments showed significantly lower “b*” values than the control group (19.61, \( P < 0.05 \)). The “b*” value for the first two months did not change, but it decreased significantly at the 3rd month (\( P < 0.05 \)), and continued to decrease significantly at the 4th month (\( P < 0.05 \)).

Therefore, the color of the frankfurters did not fade for at least 1 month, but the redness of the frankfurters declined after 60 days of storage, which in conjunction with slightly greater “L*” values suggest that some color fading occurred.

**Texture**

The texture measurements of the frankfurters showed that the peak force was significantly affected by storage time (\( P < 0.05 \)), but no significant effects observed by the treatments or the treatment-time interaction, and the mean-force was most affected by either treatment nor storage time (Table 3). Therefore, the different organic acid salt treatments did not affect surface and inner texture.

**Nitrite**

The nitrite concentration of the raw frankfurter batter (44 ppm) after formulation was considerably higher than frankfurters (22 – 24 ppm) at day 1 after cooking, but there was no significant difference for the nitrite concentration of the cooked frankfurters among the seven treatments. However, the nitrite concentration dropped quickly to about 1 ppm or less for both raw and cooked samples after 10 days (Table 4).
NaCl

No effects of treatment, storage time, or interaction for NaCl and Cl⁻ concentration in the frankfurters were observed (Table 5). These results were consistent with the results of the sensory evaluation in the salty group shown in Table 1.

pH

Organic acid salts treatments, storage time, and their interaction did not have statistical significant effects on pH value for frankfurters (Table 5). Samelis et al. (2001), however, reported that the combinations of sodium lactate (1.75%) with sodium diacetate (0.25%) decreased the pH of frankfurters, and Islam et al. (2002) reported that the frankfurters absorbed 0.24-0.26% SD solutions significantly decrease the pH values. Therefore, it is necessary to decrease the concentration of the organic acid salts in frankfurters to prevent the significant pH value dropping and the adversely effects on frankfurter flavors.

Conclusions

The 6% SD/PB treatment increased meaty flavor, but the 3% SL/SD/PB decreased smoke flavor of the frankfurters. There was no significant difference, however, between the control group and the organic acid salt surface-treated frankfurters in salty, sour, and pepper flavors as determined by sensory evaluation. The SL/SD/PB at either 3 or 6% significantly increased the lightness (L*) and decreased the redness (a*) value for frankfurters compared with the control group, and the storage time longer than two month also significantly increased the L* value and decreased the a* value. The peak force of the texture was significantly decreased after one-month storage but became tougher after 3 month storage. No difference was observed between the control group and the other treatments for pH value, nitrite or sodium content.
Table 1. Effects of surface organic acid treatments on salty, sour, meaty, and black pepper flavors of frankfurters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flavor Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salty</td>
</tr>
<tr>
<td>Control</td>
<td>10.05 ab</td>
</tr>
<tr>
<td>3% SD^1</td>
<td>9.63 a</td>
</tr>
<tr>
<td>6% SD^2</td>
<td>10.29 b</td>
</tr>
<tr>
<td>3% SL/SD/PB^3</td>
<td>9.50 a</td>
</tr>
<tr>
<td>6% SL/SD/PB^4</td>
<td>9.93 ab</td>
</tr>
<tr>
<td>3% SD/PB^5</td>
<td>9.74 ab</td>
</tr>
<tr>
<td>6% SD/PB^6</td>
<td>10.06 ab</td>
</tr>
<tr>
<td>SEM</td>
<td>0.24</td>
</tr>
</tbody>
</table>

^1 3% solution of sodium diacetate
^2 6% solution of sodium diacetate
^3 3% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination
^4 6% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination
^5 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination
^6 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination

a-c Values in the same column with different letters are significantly different (P ≤ 0.05)
Table 2. Color measurements of frankfurters following different organic acid treatments during storage

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.42 ab</td>
<td>16.37 c</td>
<td>19.61 bc</td>
</tr>
<tr>
<td>3% SD</td>
<td>52.77 bcd</td>
<td>15.69 abc</td>
<td>17.97 a</td>
</tr>
<tr>
<td>6% SD</td>
<td>51.90 abc</td>
<td>16.43 c</td>
<td>18.56 ab</td>
</tr>
<tr>
<td>3% SL/SD/PB</td>
<td>53.58 d</td>
<td>14.89 a</td>
<td>18.06 a</td>
</tr>
<tr>
<td>6% SL/SD/PB</td>
<td>53.10 cd</td>
<td>15.16 ab</td>
<td>18.36 a</td>
</tr>
<tr>
<td>3% SD/PB</td>
<td>52.58 abcd</td>
<td>15.81 abc</td>
<td>18.28 a</td>
</tr>
<tr>
<td>6% SD/PB</td>
<td>51.36 a</td>
<td>16.19 bc</td>
<td>19.78 c</td>
</tr>
<tr>
<td>SEM</td>
<td>0.57</td>
<td>0.88</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Month 1          | 52.18 a | 16.83 b | 19.71 c |
Month 2          | 52.14 a | 17.35 b | 20.03 c |
Month 3          | 52.47 ab| 14.74 a | 18.12 b |
Month 4          | 52.76 b | 14.25 a | 16.77 a |
SEM              | 0.46    | 0.83    | 0.90    |

1 3% solution of sodium diacetate
2 6% solution of sodium diacetate
3 3% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination
4 6% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination
5 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination
6 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination

a-d Values in the same column with different letters are significantly different \((P \leq 0.05)\)
Table 3. Texture measurements of frankfurters during storage following different organic acid treatments

<table>
<thead>
<tr>
<th></th>
<th>Force (kg)</th>
<th>Mean-Force (1:2, kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.57 a</td>
<td>0.20 a</td>
</tr>
<tr>
<td>3% SD</td>
<td>0.55 a</td>
<td>0.20 a</td>
</tr>
<tr>
<td>6% SD</td>
<td>0.54 a</td>
<td>0.19 a</td>
</tr>
<tr>
<td>3% SL/SD/PB</td>
<td>0.54 a</td>
<td>0.21 a</td>
</tr>
<tr>
<td>6% SL/SD/PB</td>
<td>0.52 a</td>
<td>0.20 a</td>
</tr>
<tr>
<td>3% SD/PB</td>
<td>0.55 a</td>
<td>0.20 a</td>
</tr>
<tr>
<td>6% SD/PB</td>
<td>0.52 a</td>
<td>0.18 a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Month 1</td>
<td>0.55 b</td>
<td>0.20 a</td>
</tr>
<tr>
<td>Month 2</td>
<td>0.51 a</td>
<td>0.18 a</td>
</tr>
<tr>
<td>Month 3</td>
<td>0.52 a</td>
<td>0.19 a</td>
</tr>
<tr>
<td>Month 4</td>
<td>0.56 b</td>
<td>0.21 a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.03</td>
<td>0.01</td>
</tr>
</tbody>
</table>

1 3% solution of sodium diacetate
2 6% solution of sodium diacetate
3 3% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination
4 6% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination
5 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination
6 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination

*a-b* Values in the same column with different letters are significantly different (*P* ≤ 0.05)
Table 4. Nitrite concentration of frankfurters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrite concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Day</td>
</tr>
<tr>
<td>Raw sample</td>
<td>44</td>
</tr>
<tr>
<td>Control</td>
<td>22</td>
</tr>
<tr>
<td>3% SD</td>
<td>24</td>
</tr>
<tr>
<td>6% SD</td>
<td>23</td>
</tr>
<tr>
<td>3% SL/SD/PB</td>
<td>23</td>
</tr>
<tr>
<td>6% SL/SD/PB</td>
<td>24</td>
</tr>
<tr>
<td>3% SD/PB</td>
<td>23</td>
</tr>
<tr>
<td>6% SD/PB</td>
<td>22</td>
</tr>
</tbody>
</table>

1 3% solution of sodium diacetate
2 6% solution of sodium diacetate
3 3% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination
4 6% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination
5 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination
6 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination
Table 5. Sodium chloride, Cl⁻ ion concentration and pH values of frankfurters for 90 days

<table>
<thead>
<tr>
<th></th>
<th>NaCl %</th>
<th>Cl⁻ (ppm)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.97 a</td>
<td>11929 a</td>
<td>6.20 a</td>
</tr>
<tr>
<td>3% SD¹</td>
<td>1.97 a</td>
<td>11925 a</td>
<td>6.19 a</td>
</tr>
<tr>
<td>6% SD²</td>
<td>1.97 a</td>
<td>11933 a</td>
<td>6.18 a</td>
</tr>
<tr>
<td>3% SL/SD/PB³</td>
<td>1.96 a</td>
<td>11899 a</td>
<td>6.22 a</td>
</tr>
<tr>
<td>6% SL/SD/PB⁴</td>
<td>1.97 a</td>
<td>11933 a</td>
<td>6.20 a</td>
</tr>
<tr>
<td>3% SD/PB⁵</td>
<td>1.95 a</td>
<td>11827 a</td>
<td>6.20 a</td>
</tr>
<tr>
<td>6% SD/PB⁶</td>
<td>1.97 a</td>
<td>11963 a</td>
<td>6.22 a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01</td>
<td>58</td>
<td>0.10</td>
</tr>
<tr>
<td>Month 1</td>
<td>1.96 a</td>
<td>11906 a</td>
<td>6.19 a</td>
</tr>
<tr>
<td>Month 2</td>
<td>1.96 a</td>
<td>11913 a</td>
<td>6.22 a</td>
</tr>
<tr>
<td>Month 3</td>
<td>1.96 a</td>
<td>11915 a</td>
<td>6.20 a</td>
</tr>
<tr>
<td>Month 4</td>
<td>1.97 a</td>
<td>11928 a</td>
<td>6.20 a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01</td>
<td>42</td>
<td>0.10</td>
</tr>
</tbody>
</table>

¹ 3% solution of sodium diacetate  
² 6% solution of sodium diacetate  
³ 3% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination  
⁴ 6% solution of sodium diacetate, sodium lactate, and potassium benzoate in a 1:1:1 combination  
⁵ 3% solution of sodium diacetate, and potassium benzoate in a 1:1 combination  
⁶ 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination  

a Values in the same column with same letters are not significantly different ($P > 0.05$)
References


CHAPTER 6. RISK ASSESSMENT OF LISTERIA MONOCYTOGENES ON FRANKFURTERS WITH ORGANIC ACID SALTS SURFACE TREATMENTS

A paper to be submitted to The Journal of Food Protection

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Keywords: food safety, organic acid salts, Listeria monocytogenes, frankfurters

Introduction

Risk assessment of microbiological hazards in foods has gained increased attention in food microbiology in recent years, and has become a priority area of work for the Codex Alimentarius Commission (CAC) since 1999 (Nauta, 2002). The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have developed an international strategy for identifying and assessing microbiological risks, food safety policies and sanitary measures to achieve specific food safety goals, and to provide standards for food (FAO and WHO, 2000). Microbiological risk assessment is a “third wave” of food safety tools, with the first being good hygienic practices and the second being hazard analysis and critical control point (HACCP) system. The current efforts in microbiological risk assessment are at the early stage of development and implementation, similar to HACCP in the late 1980’s (Forsythe, 2002). A proper approach to growth modeling can enhance food safety by assessing the safety of food products under a variety of conditions and predicting the effects of intervention measures in food production processes (Nauta, 2002). Because food usually contains lower numbers of microbial pathogens than samples tested in laboratory trials, growth modeling is used to extrapolate low dose responses from high dose-response data. Consequently, it is necessary to build a mathematical model that describes the
relationship between ingestion of a certain number of a given pathogen and the possible outcomes (Forsythe, 2002).

In the 1990's, two major bacterial growth curve prediction-modeling packages were generated, based on bacterial responses to food environments: one was Food Micro-Model (FMM), a commercial package supported by the Ministry of Agriculture, Fisheries and Food (MAFF) and subsequently, the Food Standards Agency (FSA) in the UK, and the other was the Pathogen Modeling Program (PMP) of the Eastern Regional Research Center (ERRC), Agricultural Research Service (ARS), US Department of Agriculture (USDA). These two independent food microbiology modeling packages constitute thousands of microbial growth and survival curves that are the basis for numerous microbial models used by industry, academia and government regulatory agencies (ComBase, 2003).

The PMP was designed as a research and instructional tool for estimating the effects of multiple variables on the growth, inactivation or survival of food-borne pathogens. Compared with FMM, PMP has an advantage in that it can be easy accessed and downloaded without cost from http://www.arserrc.gov/mfs/PMP6_Start.htm. However, there is no guarantee that predicted values will match those that would occur in any specific food system, because most of the models in PMP are based on extensive experimental data from microbial behavior in liquid microbiological media. Therefore, PMP models should be validated for each specific food product by users before the models can be used effectively (ERRC, 2004).

The PMP provides:

- growth models for *A. hydrophila*, *B. cereus*, *C. perfringens*, *E. coli O157:H7*, *L. monocytogenes*, *Salmonella*, *S. flexneri*, *S. aureus*, and *Y. enterocolitica*.
- non-thermal survival models for *E. coli O157:H7*, *L. monocytogenes*, *Salmonella spp.*, and *S. aureus*.
- thermal inactivation models for: *C. botulinum*, *E. coli O157:H7* and *L. monocytogenes*.
- gamma irradiation models for *S. typhimurium*, and *E. coli O157:H7*.
- cooling/growth models for *C. botulinum* and *C. perfringens* in simulated meat gravy, *C. perfringens* in cured beef and cured chicken.
- time-to-toxigenesis model for *C. botulinum* on fish.
• time-to-turbidity models for *C. botulinum*.

The sigmoidal equations, e.g. logistic, and Gompertz equation, are widely used to describe bacterial growth (Buchanan et al., 1994; Lu, Z., 2003). The form of a growth curve is sometimes chosen by simply looking at some plots of the data, however, it is better to select or construct a function that have biological interpretation and meaningful parameters. The Gompertz model generally provided a better fit to the microbiology growth data compared with logistic models (Lu, Z., 2003).

With the increased food safety concern that has developed in the meat industry, more and more of the industry has begun to use organic acid salts for treatment of ready-to-eat (RTE) meat products to inhibit *L. monocytogenes* growth. The USDA issued a "zero" tolerance policy for *L. monocytogenes* in RTE meats, and has give processors three options to control this pathogen. The first option is to employ a post-lethality treatment process and an antimicrobial agent or process to limit the growth of *Listeria*. The post-lethality process must be identified as a critical control point (CCP) in the company’s HACCP plan. The second option is to employ either a post-lethality process or an antimicrobial application. The third option allows companies to use sanitation measures only, but the sanitation measures must include five conditions, i.e. 1) testing of food contact surfaces for *L. monocytogenes*, 2) identification of procedures to be used when a facility will implement hold-and-test procedures following a positive test for *L. monocytogenes*, 3) description of the frequency when the testing will be done, 4) identification of the size and location of the sites sampled, and 5) an explanation of why the testing frequency is considered sufficient (Chilton, J., 2003).

Organic acid salts such as sodium lactate (SL), sodium diacetate (SD), potassium benzoate (PB), potassium sorbate (PS) and their combinations are recognized as potential inhibitions of *L. monocytogenes* growth on frankfurters. A proper *L. monocytogenes* growth curve model, based on frankfurters treated with organic acids salts, would avoid unnecessary repetition of experiments, increase the efficiency of research efforts, and improve food safety. There is no predictive growth curve, however, for *L. monocytogenes* on frankfurters with surface organic acid salt treatments available in either of the two modeling packages. Therefore, it is necessary to find a proper mathematical model that will predict *L.*
monocytogenes growth on RTE meats after surface organic acid salt treatments, to fill in a gap in the existing pathogen modeling programs.

The objective of this study was to select a proper L. monocytogenes growth curve model for frankfurters treated with organic acid salts and to provide a means of risk assessment for L. monocytogenes in RTE meats, treated with organic acid salts. This information is expected to improve the practical guidelines and methodology for hazard characterization of microbial pathogens on RTE meat products.

Materials and Methods

Manufacturing process

Meat trimmings, consisting of boneless beef (80/20 lean/fat ratio), lean pork (50/50 lean/fat ratio) and pork fat (20/80 lean/fat ratio), were formulated into frankfurters. The three sources of meat were singly ground using a 0.79 cm grinder plate and mixed prior to sampling for measurement of actual fat content. Frankfurters were formulated on a 25% fat basis for the meat block. Beef trim, salt, sodium erythorbate, sodium nitrite, seasonings and one-half of the total water (ice) were chopped for approximately 3 minutes in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA), until temperature reached 40 °F. The pork trim, pork fat and remaining one-half of the ice were then added and this composite was chopped for an additional 3 minutes, until temperature reached 55 °F. The meat batter was placed into a vacuum stuffer (Risco® Model RS 4003- 165; Stoughton, MA) and stuffed into 22 mm diameter, peelable cellulose casings (Devra Teepak™Wienie-Pak® Coastal Corrugated Inc., N. Charleston, SC) and linked (Poly-clip® GmbH & Co. KG, Germany) at 8.5 cm in length. A conventional cooking-smoking cycle of 2.5 hours for frankfurters was used in a humidity-controlled smoke house (Alkar, DEC International Inc., Lodi, WI) to achieve a cooked product internal temperature of 71.1 °C. The franks were showered and then chilled for approximately 16-18 hours in a 4 °C cooler.

Experimental design

This study included seven different surface treatments, including a control with no surface dip solution for the frankfurters, sodium diacetate alone at 3 or 6% (total wt/vol, SD), sodium diacetate + potassium benzoate at 3 or 6% (total wt/vol, SD/PB), and lactate + sodium diacetate + potassium benzoate at 3 or 6% (total wt/vol, SL/SD/PB). Preliminary
experiments showed that dipping for 3 min resulted in an approximately 0.08% pickup by the frankfurters. Surface-treated products were placed in bags (2 frankfurters per package) and inoculated with a 5-strain cocktail of *L. monocytogenes*, to reach a final level of $10^4$ cells per package (90.8 g). Each of the treatments also included a treated but uninoculated control. The 7 treatments utilized for both inoculated and non-inoculated frankfurters are shown in Table 1.

Frankfurters were stored at −2.2, 1.1, 4.4, 10.0 or 12.8°C for up to 90 days. Packages were analyzed for *L. monocytogenes* counts immediately after inoculation and then every 48 hours during 90 days of storage. Background bacteria, i.e. mesophilic aerobic plate counts (APC), lactic acid bacteria and yeast/mold counts were also enumerated.

**Microorganisms**

The inoculum included 5 *L. monocytogenes* strains: Scott A (human isolate), H7764 (serotype 1/2a, deli meat isolate), H7762 (serotype 4b, hotdog isolate), H7962 (serotype 4b, hotdog isolate), and H7969 (serotype 4b, hotdog isolate). Scott A strain was obtained from National Animal Disease Center (NADC), and the other four strains were obtained from the Centers for Disease Control and Prevention, Atlanta, GA (CDC), as clinical isolates from the Bil Mar Foods outbreak of 1998-1999.

The *L. monocytogenes* cultures were individually grown in Trypticase Soy Broth containing 0.6% Yeast Extract (TSB-YE broth, Difco Laboratories, Becton Dickinson and Company, Sparks, Maryland) in a rotating platform incubator at 35°C for 24 hours. Then, 1 ml of culture from each individual stain was combined to give a 5-ml mixed culture of *L. monocytogenes*, and the mixed culture was transferred to 500-ml TSB-YE broth and incubated at 35°C for 24 hours to reach the stationary phase. The final concentration of the original 5-strain mixture of *L. monocytogenes* was 9.30 log CFU/ml, and the culture was serially diluted to achieve $10^5$ cells/ml in the inoculum. The number of viable cells in the inoculum was verified by plate count methods. Typical colonies were gram-stained and then analyzed by using API *Listeria* kits (bioMerieux, Inc., Hazelwood, MO).

Frankfurters was surface inoculated with the 5-strain mixture of *L. monocytogenes* by adding 1 ml of the inoculum ($10^4$ cells/ml) into each package. Average package weight was 90.8g. The packages were hand-massaged 5-10 s to evenly distribute the inoculum on the
surface of each frankfurter immediately after inoculation. After vacuum-packaging, all frankfurters were placed in U.N. approved biosafety shippers (AirSea-Atlanta, GA) with cold packs. A temperature recorder was placed in one of the biosafety shippers to check for assurance of refrigeration, and the samples were shipped overnight to Silliker Laboratories for microbial analysis.

Survival and growth data for *L. monocytogenes* on frankfurters treated with organic acid salts were analyzed to determine the impact of both storage temperature and various organic acid salts on the growth of *L. monocytogenes* (Lu et al., 2004a; and Lu et al., 2004b).

**Predictive Modeling**

Scatter plots of *L. monocytogenes* counts (CFU/gm) versus time for the 7 treatments at 5 temperatures (-2.2, 1.1, 4.4, 10.0, and 12.8 °C) were developed to compare the effects of treatments and temperatures.

Primary models that relate *L. monocytogenes* counts to time were fitted first to the data generated for samples receiving the same treatment and stored at the same temperature. Secondary models were then used to relate the growth parameter estimates derived from the primary models with temperature.

The non-linear regression module of SYSTAT® Version 10.0 was used to fit both the primary model and the secondary models to the data. A least-square loss function and the Gauss-Newton estimation algorithm were used, and the Wald confidence intervals were calculated.

**Results and Discussion**

**Scatter Plots**

Scatter plots of *L. monocytogenes* counts (CFU/gm) versus time for the 7 treatments at 5 temperatures (-2.2, 1.1, 4.4, 10.0, and 12.8 °C) were made to examine the effect of temperature and organic acid salts on growth of the *L. monocytogenes* (Plots are shown in the Appendix to this thesis). The scatter plots show that the effect of temperature on *L. monocytogenes* growth was very significant. Regardless of treatment, *L. monocytogenes* counts did not change with time at -2.2 °C or 1.1°C, but showed steady increases at 4.4, 10.0 and 12.8 °C after a variable lag period.

**Primary Models**
Several mathematical models relating bacterial counts to time have been reviewed in previous publications (Buchanan et al., 1994; McNab, 1998; van Gerwen and Zwietering, 1998; Whiting, 1993; Wijtzes et al., 1995). These models were tested as potential primary growth mathematical models, and are summarized in Table 2. The Gompertz model generally provided a good fit to the microbiology growth data collected in this study compared with logistic and exponential models (Lu, Z., 2003). Several forms of the Gompertz model as listed in Table 2 were tested by Novigen Sciences, Inc. (Barraj, L. M., 2001), and the reparametrized Gompertz model was selected as the primary mathematical model presenting *L. monocytogenes* growth on frankfurters, because its parameters are biologically interpretable, where:

\[ t: \text{time in days,} \]
\[ \log (n): \text{natural logarithm of the number of organisms at time } t, \]
\[ \log (n_0): \text{natural logarithm of the estimated initial number of organisms,} \]
\[ \log (n_{\max}): \text{natural logarithm of the estimated maximum number of organisms,} \]
\[ \mu_{\max}: \text{maximum specific growth rate,} \]
\[ \lambda: \text{lag time.} \]

Therefore, the primary model selected in this study is of the form:

\[ \log(n) = \log(n_0) + \left[ \log(n_{\max}) - \log(n_0) \right] \times \exp \left[ -e^{\frac{\mu_{\max} \times x \times (t-1)}{2 \log(n_{\max}) - \log(n_0)}} + 1 \right] \]

The initial number of organisms at time 0, \( n_0 \), was assumed constant for all models and set at the level of \( 10^5 \, \text{CFU / 247g} \). Rather than using a fixed value for the maximum number of organisms, the model allows this quantity to be estimated from the data, in order to determine the impact, if any, of the treatments on maximum population density.

Results of the selected primary model parameter estimates are presented in Table 3, including the treatments, temperatures, estimated parameters in the model and the associated confidence intervals for each of the estimates. Several models did not converge due to the extremely large variation in the collected *L. monocytogenes* data, including the 6% SD treatment at 1.1 °C, the 3% SD/PB treatment at 4.4 °C, and the 3% SL/SD/PB treatment at 10 °C. In addition, some of the estimates have wide confidence interval coverage. Therefore, it is necessary to note that the models that did not converge and the wide confidence intervals...
both indicated poor fitting of the data which would not provide correct predictions. In general, however, these results are consistent with Koseki and Itoh (2001) who reported that estimates of log \( n_{\text{max}} \) and \( \mu_{\text{max}} \) for psychrotrophic bacteria showed an increasing trend as the temperature increased, while the estimates of \( \lambda \) showed a decreasing trend as temperature increased.

A one-way analysis of covariance using treatment groups as factors and temperature as a covariate was conducted on the parameter estimates derived from the primary models. The relationships between temperature and the three parameters, \( \mu_{\text{max}} \), log \( n_{\text{max}} \), and \( \lambda \) were shown in Figure 1-3, respectively. Treatment was significantly associated with only one of the parameters, \( \lambda \), i.e. the estimate of lag time. Specifically, samples treated with 3% SD or 6% SD were associated with the longer lag times. Although no significant association was observed between treatment and \( \mu_{\text{max}} \) (maximum specific growth rate), samples treated with SD or SD/PB solutions at either 3 or 6% were associated with lower estimated \( \mu_{\text{max}} \).

There have been many attempts at fitting bacterial growth data to mathematical models, however, most have been performed using data from specific bacteria in controlled environments, such as modified nutrient broth or agar (ERRC, 2004), and it is reported that the bacteriocin activity in meat was less effective than in broth (Stiles and Hastings, 1991). The Pathogen Modeling Program of USDA provides numerous growth curves for \( L. \) monocytogenes, but none of them use \( L. \) monocytogenes growth data on frankfurters with organic acid salt surface treatments. On the other hand, Novigen fitted the mathematical \( L. \) monocytogenes growth curve model (Gompertz) by using experimental data collected from \( L. \) monocytogenes inoculated frankfurters. Therefore, it is more meaningful to predict \( L. \) monocytogenes growth on frankfurters with organic acid salts using the Gompertz mathematical growth curve model provided by Novigen and than use the \( L. \) monocytogenes growth curve model provided by USDA. From the present study, it is apparent that \( L. \) monocytogenes growth on frankfurters treated with organic acid salts can be fitted to the Gompertz curve, and the parameters obtained from the Gompertz function can be used to calculate lag time and growth rate. Therefore, increases in the \( L. \) monocytogenes population can be predicted under these conditions on the basis of storage temperature, storage period, and initial population density.
Secondary Models

Secondary models (Marks et al., 1998; Ross and McMeekin, 1994) relating the estimates of the three parameters, growth rate, lag time and maximum number of organisms, from the primary model were subsequently modeled against temperature.

The following models were considered for describing the temperature effects on the various parameters:

- **The Béhrádek-type model**, known as the Ratkowsky equation (Ross and McMeekin, 1994; Witjzes et al., 1995): \( \mu_{\text{max}} = b_2 \times (T - T_{\text{min}})^2 \), where
  
  \( T \) is the temperature,
  
  \( b_2 \) is a parameter to be estimated, and
  
  \( T_{\text{min}} \) is the suboptimal temperature, assumed to be \(-1.2 \, ^\circ\text{C}\) (FDA, 2001).

- **The Arrhenius-type model** (Ross and McMeekin, 1994):
  
  \[ \log (\mu_{\text{max}}) = \log (A) - \frac{E_a}{RT} \]
  
  where
  
  \( T \) is the temperature,
  
  \( A \) is a parameter to be estimated,
  
  \( E_a \) is interpreted as the activation energy of the growth-rate-limiting reaction, and
  
  \( R \) is the gas constant.

- **Linear models** (Marks et al, 1998):
  
  \[ \log (\mu_{\text{max}}) = a + b \times \log (T) \]
  
  \[ \log (\lambda) = c + d \times \log (T) \]
  
  \[ \log (n_{\text{max}}) = e + f \times T \]
  
  Where \( a, b, c, d, e, \) and \( f \) are parameters to be estimated.

Because the number of data points in this study was limited, the more parsimonious model, the Béhrádek-type model, was adopted to express the relationship between the growth rate and temperature. In addition, this model provided a better fit for the data than the Arrhenius-type model and the linear models described above. The estimates of the parameter \( (b_2) \) in the Béhrádek-type model and its confidence interval were summarized in Table 4.

**Risk Rank**

The estimates for *L. monocytogenes* populations calculated by the given microbiological risk assessment model up to 70 days are shown in Table 5, assuming an
initial contamination of 2-log (CFU/g) of *L. monocytogenes* on the frankfurter surface. According to the risk assessment model, temperature is the most important factor affecting *L. monocytogenes* growth, and the population of *L. monocytogenes* is lower than 3-log for 14 days of storage at 1.1 and 4.4 °C without any organic acid salt treatment. Treatment with SD at 3% would keep the *L. monocytogenes* population as low as 2.09-log at 10.0 and 12.8 °C, and 6% SD, or 3% or 6% SL/SD/PB treatments would inhibit *L. monocytogenes* growth to levels lower than 2.06-log at 12.8 °C for one week.

Ranking of *L. monocytogenes* risk for frankfurters with organic acid salt treatments over 70 days at 1.1, 4.4, 10.0 and 12.8 °C is shown in Table 6. A rank of “1” indicates the lowest risk and a rank of “24” indicates the highest risk in terms of food safety concerns at each point in time. Treatments that did not converge in the predictive model or prediction of unreasonable *L. monocytogenes* population numbers, were not included in the ranking. These treatments included 6% SD at 1.1 °C, 3% SD/PB at 4.4 °C, and 3% SL/SD/PB at 10.0 °C, each of which failed to converge in their predictive models, and 6% SD at 10.0 °C, which predicted a very unlikely *L. monocytogenes* population of more than 1000-log within 7 days of the initial 2-log CFU/g. According to the ranking in Table 6, the 6% SL/SD/PB treatment at 1.1 °C would be the most effective treatment for control of *L. monocytogenes* growth, followed by 3% SD at 1.1°C, 6% SD/PB at 1.1°C, 6% SD at 4.4°C and 3% SD at 4.4°C.

Conclusions

According to these comparisons, predicting *L. monocytogenes* growth on frankfurters treated with organic acid salts was more reasonable using the Gompertz mathematical growth curve model provided by Novigen than the *L. monocytogenes* growth curve model provided by USDA. It was more meaningful to compare *L. monocytogenes* inhibition effects of treatments using the population density rather than the lag phase. Therefore, it may be practical to do risk assessments for *L. monocytogenes* on frankfurters by using a log linear model which incorporates frankfurter surface treatments, storage temperatures and their interactions.

It is a disadvantage that the *L. monocytogenes* growth curve model from Novingen can not converge or is unable to predict accurately due to that large variation of the confidence interval of the parameters, but it should have better predictability than the USDA
PMP growth curve for *L. monocytogenes* on organic acid salt treated frankfurters. While there are limitations associated with either the USDA PMP model or the selected mathematical primary and secondary models used by the Novingen for *L. monocytogenes* predictions, predictive models provide useful comparisons for developing relative risk assessments for *L. monocytogenes* on frankfurters.
TABLE 1. Organic acid salt treatments for frankfurters at -2.2, 1.1, 4.4, 10.0 or 12.8 °C

<table>
<thead>
<tr>
<th>Treatment number</th>
<th>Uninoculated</th>
<th>Inoculated with $10^4 L. monocytogenes$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>3% SD$^1$</td>
<td>3% SD</td>
</tr>
<tr>
<td>3</td>
<td>6% SD$^2$</td>
<td>6% SD</td>
</tr>
<tr>
<td>4</td>
<td>3% SD/PB$^3$ (1:1)</td>
<td>3% SD/PB (1:1)</td>
</tr>
<tr>
<td>5</td>
<td>6% SD/PB$^4$ (1:1)</td>
<td>6% SD/PB (1:1)</td>
</tr>
<tr>
<td>6</td>
<td>3% SL/SD/PB$^5$ (1:1:1)</td>
<td>3% SL/SD/PB (1:1:1)</td>
</tr>
<tr>
<td>7</td>
<td>6% SL/SD/PB$^6$ (1:1:1)</td>
<td>6% SL/SD/PB (1:1:1)</td>
</tr>
</tbody>
</table>

$^1$ 3% solution of sodium diacetate
$^2$ 6% solution of sodium diacetate
$^3$ 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination
$^4$ 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination
$^5$ 3% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination
$^6$ 6% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination
TABLE 2. Primary growth mathematical models used to compare the fit of the \textit{L. monocytogenes} growth data

<table>
<thead>
<tr>
<th>Model</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exponential</td>
<td>$\ln(n) = \ln(n_0) + \mu t$</td>
</tr>
<tr>
<td>Lag-exponential</td>
<td>$\ln(n) = \ln(n_0) \text{ for } t &lt; \lambda$</td>
</tr>
<tr>
<td></td>
<td>$\ln(n) = \ln(n_0) + \mu(t - \lambda) \text{ for } t \geq \lambda$</td>
</tr>
<tr>
<td>Gompertz (Buchanan)</td>
<td>$\log(n) = \log(n_0) + [\text{MPD} - \log(n_0)] \times \exp[-e^{-B(t-M)}]$</td>
</tr>
<tr>
<td>Gompertz</td>
<td>$\log(n) = A + C \times \exp[-e^{-B(t-M)}]$</td>
</tr>
<tr>
<td>Reparametrized Gompertz</td>
<td>$\log(n) = \log(n_0) + [\log(n_{\text{max}}) - \log(n_0)] \times \exp[-e^{\frac{\mu_{\text{max}} \times e \times (A-I)}{\log(n_{\text{max}}) - \log(n_0)} + 1}]$</td>
</tr>
<tr>
<td>Logistic model for inactivation/survival</td>
<td>$\log \left( \frac{n}{n_0} \right) = \log \left[ F_1 \times \frac{1 + e^{\beta \lambda t_l}}{1 + e^{b \times (t - t_l)}} + (1 - F_1) \times \frac{1 + e^{-b \times t_l}}{1 + e^{b \times (t - t_l)}} \right]$</td>
</tr>
<tr>
<td>Logistic</td>
<td>$n = n_0 \text{ for } t &lt; \lambda$</td>
</tr>
<tr>
<td></td>
<td>$n = \frac{n_{\text{max}}}{1 + \frac{n_{\text{max}}}{n_0 - 1} \times e^{-\mu t (t - \lambda)}} \text{ for } t \geq \lambda$</td>
</tr>
</tbody>
</table>

- $n$: bacterial count at time $t$
- $n_0$: initial bacterial count
- $\mu$: growth rate
- $\lambda$: lag-time (hours)
- MPD: Logarithm of the maximum population density, assumed fixed at 9.
- B: Constant
- $n_{\text{max}}$: maximum number of microorganisms
- $\mu_{\text{max}}$: maximum specific growth rate
TABLE 3. Primary models parameter estimates for growth of *L. monocytogenes* on frankfurters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Log ($n_{max}$)</th>
<th>95% CI</th>
<th>μ</th>
<th>95% CI</th>
<th>λ</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Control</td>
<td>1.1</td>
<td>5.875</td>
<td>4.322</td>
<td>7.428</td>
<td>0.220</td>
<td>-0.093</td>
<td>0.533</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>18.725</td>
<td>10.020</td>
<td>27.431</td>
<td>0.363</td>
<td>0.271</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>15.931</td>
<td>14.246</td>
<td>17.615</td>
<td>1.735</td>
<td>1.225</td>
<td>2.245</td>
</tr>
<tr>
<td></td>
<td>12.8</td>
<td>15.748</td>
<td>12.862</td>
<td>18.634</td>
<td>1.782</td>
<td>0.973</td>
<td>2.591</td>
</tr>
<tr>
<td>3% SD^1</td>
<td>1.1</td>
<td>6.955</td>
<td>4.311</td>
<td>9.600</td>
<td>0.251</td>
<td>-0.255</td>
<td>0.757</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>11.660</td>
<td>2.315</td>
<td>21.004</td>
<td>0.154</td>
<td>0.031</td>
<td>0.277</td>
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<tr>
<td></td>
<td>10.0</td>
<td>17.311</td>
<td>14.370</td>
<td>20.251</td>
<td>0.846</td>
<td>0.550</td>
<td>1.143</td>
</tr>
<tr>
<td>6% SD^2</td>
<td>1.1</td>
<td>2.674</td>
<td>1.676</td>
<td>3.671</td>
<td>0.125</td>
<td>-1.033</td>
<td>1.283</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>20.749</td>
<td>-33.335</td>
<td>74.833</td>
<td>0.161</td>
<td>0.017</td>
<td>0.305</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>16.783</td>
<td>13.578</td>
<td>19.988</td>
<td>0.747</td>
<td>0.426</td>
<td>1.068</td>
</tr>
<tr>
<td>3% SD/PB^3</td>
<td>12.8</td>
<td>13.007</td>
<td>10.852</td>
<td>15.161</td>
<td>0.861</td>
<td>0.135</td>
<td>1.586</td>
</tr>
<tr>
<td>4.4</td>
<td>2.674</td>
<td>1.676</td>
<td>3.671</td>
<td>0.125</td>
<td>-1.033</td>
<td>1.283</td>
<td>1.743</td>
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<tr>
<td></td>
<td>10.0</td>
<td>17.077</td>
<td>8.074</td>
<td>26.081</td>
<td>0.686</td>
<td>0.038</td>
<td>1.333</td>
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<tr>
<td></td>
<td>12.8</td>
<td>13.939</td>
<td>11.506</td>
<td>16.372</td>
<td>0.954</td>
<td>0.290</td>
<td>1.618</td>
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</table>
### TABLE 3. (continued)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature</th>
<th>Log (n&lt;sub&gt;max&lt;/sub&gt;)</th>
<th>95% CI</th>
<th>μ</th>
<th>95% CI</th>
<th>λ</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(°C)</td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>6% SD/PB&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.1</td>
<td>4.888</td>
<td>3.554</td>
<td>6.222</td>
<td>0.458</td>
<td>-1.786</td>
<td>2.703</td>
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1 3% solution of sodium diacetate  
2 6% solution of sodium diacetate  
3 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination  
4 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination  
5 3% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination  
6 6% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination  

n<sub>max</sub>: maximum number of microorganisms  
μ: growth rate  
λ: lag-time (hours)
TABLE 4. Parameter estimates of secondary model \( \mu = b_2 \times (T - T_{\text{min}})^2 \) for growth of *L. monocytogenes* on frankfurters

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<th>Upper</th>
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- \( \mu \): growth rate
- \( b_2 \): a parameter to be estimated
- \( T \): temperature
- \( T_{\text{min}} \): the suboptimal temperature, assumed to be \(-1.2\) °C
- \(^1\) 3% solution of sodium diacetate
- \(^2\) 6% solution of sodium diacetate
- \(^3\) 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination
- \(^4\) 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination
- \(^5\) 3% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination
- \(^6\) 6% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination
TABLE 5. *L. monocytogenes* population estimates using a microbiological risk assessment model (log (n₀) = 2)

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<th>28 day</th>
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n₀: initial number of organisms
1 3% solution of sodium diacetate
2 6% solution of sodium diacetate
3 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination
4 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination
5 3% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination
6 6% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination
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13% solution of sodium diacetate
2 6% solution of sodium diacetate
3 3% solution of sodium diacetate and potassium benzoate in a 1:1 combination
4 6% solution of sodium diacetate and potassium benzoate in a 1:1 combination
5 3% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination
6 6% solution of sodium lactate, sodium diacetate, and potassium benzoate in a 1:1:1 combination
FIGURE 1. Primary model parameter estimates for treatment groups using $\mu$ (maximum specific growth rate) vs. temperature ($^\circ$C) for *L. monocytogenes* on frankfurters
FIGURE 2. Primary model parameter estimates for treatment groups using log \( (n_{\text{max}}) \) (natural logarithm of the estimated maximum number of organisms) vs. temperature \(^{\circ}\text{C}\) for \(L.\) monocytogenes growth on frankfurters.
FIGURE 3. Primary model parameter estimates for treatment groups using $\lambda$ (log time) vs. temperature ($^\circ$C) for *L. monocytogenes* growth on frankfurters.
References


CHAPTER 7. CONCLUSIONS

Listeriosis is a foodborne illness primarily caused by *L. monocytogenes*, a gram-positive, non-spore-forming, non-acid-fast, facultative anaerobic, psychrotrophic rod that can tolerate acids, sanitizers and antibiotics, and exists ubiquitously. The reported average mortality of foodborne listeriosis is approximately 30%, which is much higher than the mortality caused by E. coli O157:H7 or Salmonella spp. Such high mortality and the increased frequency of outbreaks of listeriosis involving meat products has made *L. monocytogenes* a major food safety concern in the meat industry over the last two decades.

RTE meat products have been reported to be the source of listeriosis in susceptible populations, e.g., organ transplant patients, patients with AIDS, HIV-infected individuals, pregnant women, patients with cancer and the elderly. Handling, storage, processing and associated food supply systems must be carefully managed by food producers and processors to control the growth of *L. monocytogenes* in food products, particularly in RTE meats. To assume safety of food products, *L. monocytogenes* must be reduced from potentially harmful levels to 0 CFU/ml.

Research has contributed greatly to understanding conditions and factors that affect *L. monocytogenes* growth and survival, including factors such as nutrients, pH, temperature, and water activity ($a_w$). Chemical preservatives, irradiation, bacteriocins, low temperature, pasteurization, and MAP packaging systems have also each been shown to impact growth of *L. monocytogenes*. However, further research to determine how those inhibitory factors interact with each other is necessary to achieve further improvements in control of *L. monocytogenes* in meat products.

Recent research has reported that organic acids, such as diacetate, lactate, sorbate and benzoate or their salts affect the growth of microorganisms such as *L. monocytogenes* by acting on the cell wall and the cell membrane, destroying their structure and subsequently the transport mechanism of nutrients to the cell. Recent research has also shown that efficiency of those organic acids is governed by a dose-effect relationship. Synergistic effects were observed between benzoate and other organic acids, such as acetate, lactic acids and citric acid. Combined with nitrite or nisin, the antilisterial activity of both lactate and sorbate may be enhanced. Also, addition of polyphosphate to lactate and nisin combinations further
increased the antilisterial activity. On the other hand, there was no synergistic antilisterial effect observed for sorbate with 4% sordium lactate. Therefore, use of organic acid or their salts in combinations may increase the degree of anti-microbial action and lower the necessary concentration of individual substances but each must be considered on a case-by-case basis. Moreover, surface treatment of RTE meats with organic acids or their salts is more efficient than using organic acids or their salts as a product ingredient because *L. monocytogenes* contamination on these products is typically on the surface as a result of post-heating contamination. The storage temperature, pH, a_w, NaCl and nitrite concentration of RTE meats, and the type and level of organic acids used are major factors affecting the antilisterial activities of organic acids and their salts.

Because of the large number of variables that exist in processed meat products, more research is needed to determine the most effective inhibitory treatments for *L. monocytogenes* in specific RTE meat products.

Based on the results of this study, the maximum population density was the best criteria to use for selection of treatments with greatest potential impact on *L. monocytogenes*. Consequently, treatments with SD, SD/PB, or SL/SD/PB appear to have the most potential for surface application on frankfurters to achieve *L. monocytogenes* inhibition. Storage of contaminated frankfurters at 10 °C did not suppress *L. monocytogenes* in any treatments for longer than 2 weeks, thus, lower temperature is necessary for frankfurter storage even if these inhibitors are included. To determine long-term effectiveness of these compounds, it is necessary to conduct a more complete study of *L. monocytogenes* inhibition by SD, SD/PB, or SL/SD/PB with a range of storage temperatures and an extended storage time.

The Gompertz equation may be used to calculate growth curve parameters of *L. monocytogenes* on frankfurters, providing typical growth curves are obtained. Therefore, with more complete growth curve data, *L. monocytogenes* population on frankfurters with various inhibitory compounds may be predicted on the basis of storage temperature, storage period, and initial population.

Surface treatments with 6% SD, 6% SL/SD/PB, 3% SD/PB and 6% SD/PB effectively inhibited *L. monocytogenes* growth at 1.1 °C compared to the control group, and the treatment 6% SD was the most effective.
However, the results of this study also suggest that, under the conditions used for this study, *L. monocytogenes* is capable of surviving refrigerated storage even in the presence of the additives tested, and storage temperatures lower than 4.4 °C are necessary for frankfurters even with surface treatments utilizing organic acid salts.

The 6% SD/PB treatment increased meaty flavor, but the 3% SL/SD/PB decreased smoke flavor of the frankfurters. However, there was no significant difference between control group and the other surface-treated frankfurters in salty, sour, and pepper flavors in sensory evaluation. Color was significantly affected by storage time and treatments, but not the treatment-time interaction. Texture of the frankfurters was affected by the storage time, but not the treatments. No difference was observed between the control group and the other treatments for pH value, nitrite or sodium content.

In order to predict *L. monocytogenes* growth on frankfurters with organic acid salts treatments, the model built by Novigen should be much more reasonable to use when comparing the Gompertz mathematics growth curve model provided by Novigen and the *L. monocytogenes* growth curve model provided by USDA according to my experiment. Because the lag phase is not as standard as standard growth curve, it is more meaningful to compare the *L. monocytogenes* inhibition effect of each treatment according to their population density but not lag phase. Therefore, it is practical to do risk assessment of *L. monocytogenes* on frankfurters by a log linear model associated with frankfurters surface treatments, the storage temperature and their interactions.

While there are limitations associated with either USDA PMP models or the Novingen mathematics models for *L. monocytogenes* evaluated, it can be concluded that there are some predictive models that could be useful in developing risk assessments for *Listeria monocytogenes* on frankfurters with organic acid salts surface treatments.
APPENDIX: SCATTER PLOTS OF *L. monocytogenes* COUNTS (LOGCFU/G) VERSUS TIME (DAYS)

Control group at -2.2 °C

Control group at 10.0°C

Control group at 1.1 °C

Control group at 12.8°C

Control group at 4.4°C
3% SD at -2.2°C

3% SD at 10.0°C

3% SD at 1.1°C

3% SD at 12.8°C

3% SD at 4.4°C
3% SD/PB at -2.2°C

3% SD/PB at 10.0°C

3% SD/PB at 1.1°C

3% SD/PB at 12.8°C

3% SD/PB at 4.4°C
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