EFFECTS OF ORGANIC ACIDS, HOP ACIDS AND THEIR MIXTURES ON THE INHIBITION OF *LISTERIA MONOCYTOGENES*

By

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ABSTRACT

EFFECTS OF ORGANIC ACIDS, HOP ACIDS AND THEIR MIXTURES ON THE INHIBITION OF *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes is responsible for an infectious disease called listeriosis, which occurred after the consumption of foods. Among various foods, meat products such as RTE meats, frankfurters, deli meat, and pate have been ranked first as the types of food vehicles involved in listeriosis outbreaks due to the extended storage and frequent consumption with no additional heat. The overall goal of this study was to investigate the antilisterial effects of different organic acid salts, hop acid extracts, and their combinations in liquid media and processed meats. To achieve the overall goal, four separated studies were conducted.

In study I, nine different organic acid salts were investigated for *Listeria* inhibition, physicochemical changes and organoleptic characteristics in full- and low-sodium frankfurters. Potassium acetate and potassium diacetate (PAPD) out of the nine organic acid mixtures was the most effective in inhibiting *Listeria* in full- and low-sodium frankfurters during storage at 4, 7 or 10^{o}C. The sensory characteristics of all formulations were similar except a low score was seen for flavor and overall acceptability in low-sodium frankfurters containing PAPD.

In study II, eight different hop acid extracts were investigated for *Listeria* inhibition with/without PAPD in trypticase soy broth containing yeast extract (TSBYE). Five hop acid extracts (α-acid, β-acid, acid-tetra, K-tetra, and K-hexa) out of the eight including acid-iso, K-iso, and K-rho significantly inhibited *Listeria* in liquid media at 25 and 50 ppm at 37^{o}C. The combinations of these five hop acids at 25 or 50 ppm with 0.5% PAPD led to better inhibition of *Listeria* than any single hop acid or 0.5% PAPD alone. After 30 min exposure at 85^{o}C, all of the

five hop acids were heat stable with the best inhibitory activity seen for the α-acid, regardless of heating time. At 7°C in liquid media, the mixture of 5 ppm hop acid/0.5% PAPD was listeristatic, whereas none of the single hop acids showed any *Listeria* inhibition except β-acid.

In study III, the antilisterial activity of hop α- and β-acids at 5 ppm with/without 0.5% PAPD were investigated in deli-style turkey meats during storage at 4 and 7°C. Both α- and β-acids at 5 ppm did not inhibit *L. monocytogenes* during storage at 4 and 7°C, while the hop/PAPD combinations were listeristatic, regardless of the hop acid type. Similar results of no inhibition were observed in skim milk and 2% milk containing α- or β-acid at 5 ppm.

In study IV, antilisterial activities of hop α- and β-acids at various concentrations were investigated in turkey slurry at 7 and 37°C. Hop α- and β-acids exhibited antilisterial activity at the concentration $\geq$ 750 ppm at 37°C and $\geq$ 500 ppm at 7°C, respectively. These results indicate that formulation of single hop acid required > 500 ppm at 7°C to inhibit *Listeria* in meat products, which is 100 or more times greater than the 5 ppm hop acid in liquid media.

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INTRODUCTION

Listeria monocytogenes is readily destroyed during normal cooking (Zaika et al., 1990), but the pathogen is of greatest concern as a post-thermal contaminant especially in meats due to its ability to grow during extended refrigerated storage (James et al., 1985; Sauders and Weidmann, 2007; Adam and Moss, 2008; Tompkin, 2002; USDA-FSIS, 1999a,b). As a result, the consumption of *Listeria*-contaminated foods such as ready-to-eat (RTE) meat could cause hospitalizations with high rates of death (Stacy et al., 2014; Cartwright et al., 2013). Among 23 food categories, both deli meats and frankfurters (non-reheated) are associated with greater risk of listeriosis (FDA/CFSAN and USDA/FSIS, 2003). Consumption of contaminated turkey frankfurters was linked to a massive multi-state outbreak during 1998-1999 that involved 108 cases, including 14 fatalities and 4 miscarriages or stillbirths (Mead et al., 2006). Consumption of non-reheated frankfurters ranked second and fourth in terms of risk on a per serving basis and an annual basis, respectively, according to the 2003 *Listeria* risk assessment of ready-to-eat (RTE) foods (USDA/FSIS, 2003c). Based on the recent report from the Centers for Disease Control and Prevention (CDC), the incidence of listeriosis in 2013 has not decreased, compared with 2010 – 2012, indicating a gap between the current food safety system and the need for better food safety interventions (Crim et al., 2014).

To control and minimize both presence and levels of *L. monocytogenes* in RTE meat and poultry products, U.S. Department of Agriculture, Food Safety and Inspection Service (USDA/FSIS) issued an interim final rule requiring one of three alternatives: (i) apply both a post-lethality treatment and an antimicrobial agent; (ii) apply either a post-lethality treatment or antimicrobial agent; or (iii) use of sanitation control measures to prevent recontamination after

processing (USDA/FSIS, 2003a). Many *Listeria* control strategies have been assessed, including the addition of sodium lactate and/or diacetate to the product formulation (Bedie et al., 2001; Glass et al, 2002; Hwang and Tamplin, 2007; Legan et al,2004; Lianou et al, 2007a,b; Luchansky et al, 2006; Mbandi and Shelef, 2002; Pradhan et al, 2009; Samelis, et al, 2005; Seman, et al, 2002; Stekelenburg, 2003; Stekelenburg, 2001; Uhart, et al, 2004) application of steam or/and hot water pasteurization (Lecompte et al, 2008; Murphy et al, 2003; Murphy et al,2002; Murphy et al, 2006;Murphy et al, 2005; Sommers et al, 2002), irradiation (Chun et al, 2009; Foong et al, 2004; Gursel and Gurakan, 1997; Jin et al, 2009; Zhu et al, 2009), and high pressure processing (Basaran-Akgul et al, 2010; Bowman et al, 2008; Gudbjomsdottir et al, 2010). Some of these approaches have marginal success because of organoleptic concerns about sodium lactate-diacetate (Blom et al, 1997; Islam et al, 2002), quality, cost and consumer acceptability issues about food irradiation (Foong et al, 2004), high capital investment and low throughput for high-pressure processing (Balasubramaniam and Farkas, 2008), and fat smearing and/or purging for steam and hot water pasteurization (personal observation). Hence, additional research is still needed to develop new inhibitors or improve current food additives for the inhibition of *L. monocytogenes.*

Currently, antilisterial agents are commonly used by manufacturers of RTE meat products with a wide range of choices (Aureli et al., 1992; Tassou et al., 1995; Thongson et al., 2005; Lucus and Were, 2009). According to the Interagency Risk Assessment for *L. monocytogenes* in retail delicatessens, the predicted risk of listeriosis from the consumption of RTE deli products could be reduced by approximately 96% if those products contain antimicrobial agents (Akingbade et al., 2013). The application of various organic acids and their salts to RTE meats is effective on inhibiting *Listeria* growth (Barmpalia et al., 2004; Bedie et al.,

2001; Blom et al., 1997; Glass et al., 2002; Hwang et al., 2007; Islam et al., 2002; Mbandi and Shelef, 2002; Samelis et al., 2005; Seman et al., 2008; Stekelenburg, 2003). However, most organic acids negatively affect both flavor and organoleptic taste (Blom et al, 1997; Islam et al, 2002). Hence, the reduction of negative impacts from organic acids while maintaining or improving the antilisterial activity have been evaluated based on the combined effect of *Listeria* inhibition (Barmpalia et al., 2004; Glass et al., 2002; Mbandi and Shelef, 2001; Stekelenburg, 2003).

Hop acids, used in beer industry, have been known to possess antimicrobial activities against gram-positive bacteria (Haas and Barsoumian, 1994; Bhattacharya et al., 2003; Sakamoto and Konings, 2003). Both hop α- (humulone) and β- (lupulone) acids are major hop resin components, which give damage to bacterial cytoplasmic membrane, interfere with active transport of sugar and amino acids, and reduce intracellular pH by dissociating into protons and hop anions (Teuber and Schmalreck, 1973; Simpson and Hammond 1991; Blanco et al. 2006). Hop extracts are more popular than the traditionally dried hops due to the convenience, stability, economic cost, and good quality (Wilson et al., 2003). Until today, however, only a few studies have been conducted to evaluate the effects of hop/organic acid mixtures on *Listeria* inhibition in meat products. Therefore, the overall goal of this study was to evaluate the antilisterial activity of different organic acids and hop acids, and the mixture of the best organic acid and hop acid components.

In this study, we hypothesized that combinations of hop acid/organic acid effectively inhibit the growth of *L. monocytogenes* in RTE meat products. To achieve this goal, the specific objectives of this study were to: (1) assess *L. monocytogenes* inhibition and the physicochemical and organoleptic characteristics of full- and low-sodium frankfurters prepared with nine different

organic acid salts, (2) assess the antilisterial effect of eight hop acid extracts with/without the best organic acid salts in TSBYE, (3) assess the antilisterial activity of the best hop acid and the best organic acid in deli-style turkey meats during storage at 4 and 7^{o}C, and (4) assess the antilisterial activities of the best hop and organic acids in turkey slurry at 7 and 37^{o}C.

CHAPTER 1: LITERATURE REVIEW

1.1 *Listeria monocytogenes*

Listeria monocytogenes is one of most dangerous pathogens transmitted through food (Stacy et al., 2014) causing human illness and death (Painter and Slutsker, 2007). The growth ability of *L. monocytogenes* under refrigerated condition poses a particular concern for ready-to-eat (RTE) meat products since these products are usually kept refrigerated and consumed without reheating (USDA-FSIS, 2014). Consequently, robust methods to control the pathogen in RTE meat products are needed to ensure the safety of RTE consumers.

1.1.1 Characteristics of *Listeria monocytogenes*

L. monocytogenes is a Gram-positive, short rod-shaped, non-spore forming, facultative anaerobic bacteria (Bell and Kyriakides, 2005). *L. monocytogenes* is a major foodborne human pathogen and one of eight important species (*L. monocytogenes, L. ivanovii, L. seeligeri, L.innocua, L.welshimeri, L. marthii, L. grayi, and L. rocourtiae*) currently known within the genus *Listeria* (Rocourt and Buchrieser, 2007). At present, 13 serotypes of *Listeria* are known, with the serotypes 1/2a, 1/2b, and 4b causing most foodborne infections in humans (CDC, 2013). *Listeria* can grow and survive at temperatures of 0-42°C, pH 4.0-9.6, a_w 0.90, and 10% NaCl (Adam and Moss, 2008; Bell and Kyriakides, 2005; Sauders and Weidmann, 2007). The concern of this organism in foods is its psychrotrophic characteristics, acid and salt tolerance, and ubiquitous presence in nature including food processing plants and home-kitchen (Cox et al., 1989). Although *L. monocytogenes* is eliminated during normal cooking, it can contaminate foods during post-thermal processing such as peeling, slicing, and re-packaging. Thus, it is not

surprising that several intervention strategies have been evaluated and implemented to control *L. monocytogenes* in food products.

1.1.2 Listeriosis and manifestation

L. monocytogenes is responsible for an infectious disease called listeriosis (Painter and Slutsker, 2007). The organism is typically transmitted via contaminated food (Norton and Braden, 2007). Listeriosis frequently occurs in susceptible persons such as neonates, the elderly, pregnant women, and immunocompromised persons. Symptoms of listeriosis include fever, vomiting, and diarrhea, which can lead to septicemia, meningitis, and central nervous system infections. The infection is severe in pregnant women and can result in spontaneous abortion, premature birth, and death of the infant (Bell and Kyriakides, 2005; Fsihi et al., 2001; Painter and Slutsker, 2007). Although the numbers of listeriosis cases are not high compared to other foodborne pathogens, *L. monocytogenes* ranks 2nd among other foodborne pathogens in terms of fatalities and induces an annual loss of $2.6 billion due to illness in the United States (Scallan et al., 2011; Hoffmann et al., 2012). These concerns underline the importance of controlling this pathogen in foods.

1.1.3 Listeriosis outbreaks and incidences of *Listeria monocytogenes* in RTE meat products

L. monocytogenes was recognized as a foodborne pathogen in 1981, when it was linked to consumption of contaminated coleslaw in Canada (Schlech et al., 1983). The first listeriosis outbreak in the U.S. occurred after consuming pasteurized milk in 1983 (Fleming et. al., 1985). After that, listeriosis has been continuously reported from a variety of food products such as

cheese, milk, processed meat, and fresh produce including sprouts, celery, and cantaloupe (Cartwright et al., 2003; Norton and Braden, 2007; CDC, 2014b). The first listeriosis outbreak from processed meat (hot dog) was occurred in the U.S. in 1988, with the numerous reported outbreaks from consumption of processed meats, thereafter (CDC, 2014b). In 1998, a multistate outbreak of listeriosis in which hot dogs manufactured by Bil Mar Foods, Michigan were implicated, resulted in 101 hospitalizations, 15 deaths, and 6 stillbirths or miscarriages, and recalls of 35 million pounds of hot dogs and deli meats (CDC, 1999). In 2000, a multistate outbreak of listeriosis associated to the consumption of slice deli meat manufactured by Cargill Turkey Products, Inc., Texas resulted in 29 cases with 4 deaths and 3 miscarriages, and recalls of processed turkey and chicken deli meats from the company (CDC, 2000). In 2002, a multistate outbreak of listeriosis linked to the consumption of slice turkey deli meat manufactured by Pilgrim's Pride Foods, Pennsylvania resulted in 46 cases with 7 deaths and 3 stillbirths or miscarriages, and recalls of 27.4 million pounds of fresh and frozen ready-to-eat turkey and chicken products (CDC, 2002). The foodborne outbreaks associated to the consumption of RTE meat products contaminated with *L. monocytogenes* are summarized in Table 1.1. Several outbreaks of listeriosis associated to the consumption of RTE meat products led the USDA-FSIS to issue their interim final rule (*Listeria* Rule) for the control of *L. monocytogenes* in RTE meat and poultry products (USDA-FSIS, 2003a).

Table 1.1 Listeriosis outbreaks in the U.S. associated with RTE meat products (CDC, 2014b).

Year	State	Food Vehicle	No. of Hospitalization	No. of Death
1998	Colorado	Hot dog	4	-
1998	Multistate	Hot dog	101	21
1999	Florida	Deli meat	2	1
1999	New York	Hot dog	4	-
1999	Minnesota	Deli meat	5	1
1999	Multistate	Pate	11	-
2000	Multistate	Deli meat (sliced turkey)	29	7
2002	Multistate	Deli meat (sliced turkey)	46	10
2005	Multistate	Deli meat (sliced turkey)	13	1
2006	Ohio	Ham	3	-

Many major listeriosis outbreaks have been traced to the consumption of RTE meats contaminated after thermal processing (Cartwright et al., 2013). Among various food products, meat products such as RTE meats, frankfurters, deli meat, and pate have been ranked first as the types of food vehicles involved in listeriosis outbreaks due to the extended storage and frequent consumption with no additional heating (Cartwright et al., 2013; FDA/CFSAN and USDA-FSIS, 2003b; Farber et al., 2007).

Up to 2013, the incidence of listeriosis had not changed significantly since 2006 with 0.26 cases reported per 100,000 population, 91% of these cases led to hospitalization, and 19.5% resulted in death (CDC, 2014a). From 1999 to present, several RTE meat products were documented as food vehicles responsible for listeriosis including hot dogs, pate, and deli meat-

sliced turkey (CDC, 2014b). Among those food categories, deli meats and hot dogs were reported to be responsible for 70% of listeriosis cases in the U.S. during 1998-2008 (Cartwright et al., 2013). These data indicate that processed RTE meat product is the primary food vehicle for human listeriosis (FDA/CFSAN and USDA-FSIS, 2003b).

1.1.4 USDA guidelines to control *Listeria monocytogenes* in RTE meat products

The emergence of problem from *L. monocytogenes* in processed meat and poultry products began during the 1980's (USDA-FSIS, 2003a). In 1987, USDA-FSIS developed a monitoring and verification program for *L. monocytogenes* in meat products including beef jerky, roast beef, cooked beef, cooked corned beef, sliced ham, luncheon meat, small-diameter sausage, large-diameter sausage, cooked/uncured poultry, salads and spreads, and dry and semi-dry fermented sausages (USDA-FSIS, 2014). USDA-FSIS also established a "zero tolerance" policy for *L. monocytogenes* in RTE foods in 1989, indicating that any amount of *L. monocytogenes* in RTE meat or poultry products renders it adulterated and subject to a voluntary recall (USDA-FSIS, 2003a,b). This program resulted in decreasing the rate of illness from *L. monocytogenes* for 44% and the rate of death by 48% during 1989-1993 (Tappero et al., 1995; USDA-FSIS, 2003a).

In 2003, USDA-FSIS issued an interim final rule (*Listeria* Rule) for the control of *L. monocytogenes* in RTE meat and poultry products (USDA-FSIS, 2003a). According to the Rule, three alternative methods were established to control *L. monocytogenes* contamination in RTE products: Alternative 1 - apply both a post-lethality treatment and an antimicrobial agent or process to suppress growth of *L. monocytogenes*, Alternative 2 – apply either a post-lethality

treatment or antimicrobial agent to control growth of *L. monocytogenes*, Alternative 3 - use of sanitation control measures to prevent recontamination after processing (USDA-FSIS, 2012).

In 2004, the report of the assessment of effectiveness of *L. monocytogenes* interim final rule showed that this rule had positive impact in addressing *L. monocytogenes* (USDA-FSIS, 2004). After 10 years since USDA-FSIS issued the interim final rule, the data from the USDA-FSIS monitoring and sampling program indicated that the percent positive in testing for *L.monocytogenes* in RTE products has decreased from 0.76% in 2003 to 0.34% in 2013 (USDA-FSIS, 2015).

In response to the *Listeria* rule, several post-lethality treatments and antimicrobial agents to control *L. monocytogenes* have been studied. For example, Bowman et al. (2008) found that the application of high hydrostatic pressure processing (HPP) at 400-600 MPa prevented *Listeria* growth due to cell structure and gene damage. Chun et al. (2009) reported that the UV-C irradiation (1000-8000 J/m^2) effectively decreased *L. monocytogenes* populations on RTE sliced ham. Foong et al. (2004) showed that the exposure of various RTE meat products to irradiation reduced the populations of *L. monocytogenes* depending on the dose of irradiation.

1.2 Organic acids

1.2.1 Nature of organic acids

Organic acids are the compounds containing carbon in the structure with acidic properties. They can be found as natural constituents or additives in foods. The most common functional group of organic acids is the carboxyl group (-COOH), however alcohol with a hydroxyl group (-OH) and the organic compounds containing thiol (-SH), enol, or phenol groups are also referred to as organic acids. Organic acids are weak acids since they do not fully dissociate in water. The two basic forms of organic acids are pure acids such as lactic acid, propionic acid, acetic acid, and benzoic acid, as well as buffered acids which are the calcium or sodium salts of pure organic acids (Theron and Lues, 2011). The buffered form has advantages over the pure form since it does not significantly change the pH of the food, is safe to handle, and is less corrosive to the machines (Theron and Lues, 2007). Chemical structures of some organic acids are shown in Fig. 1.1.

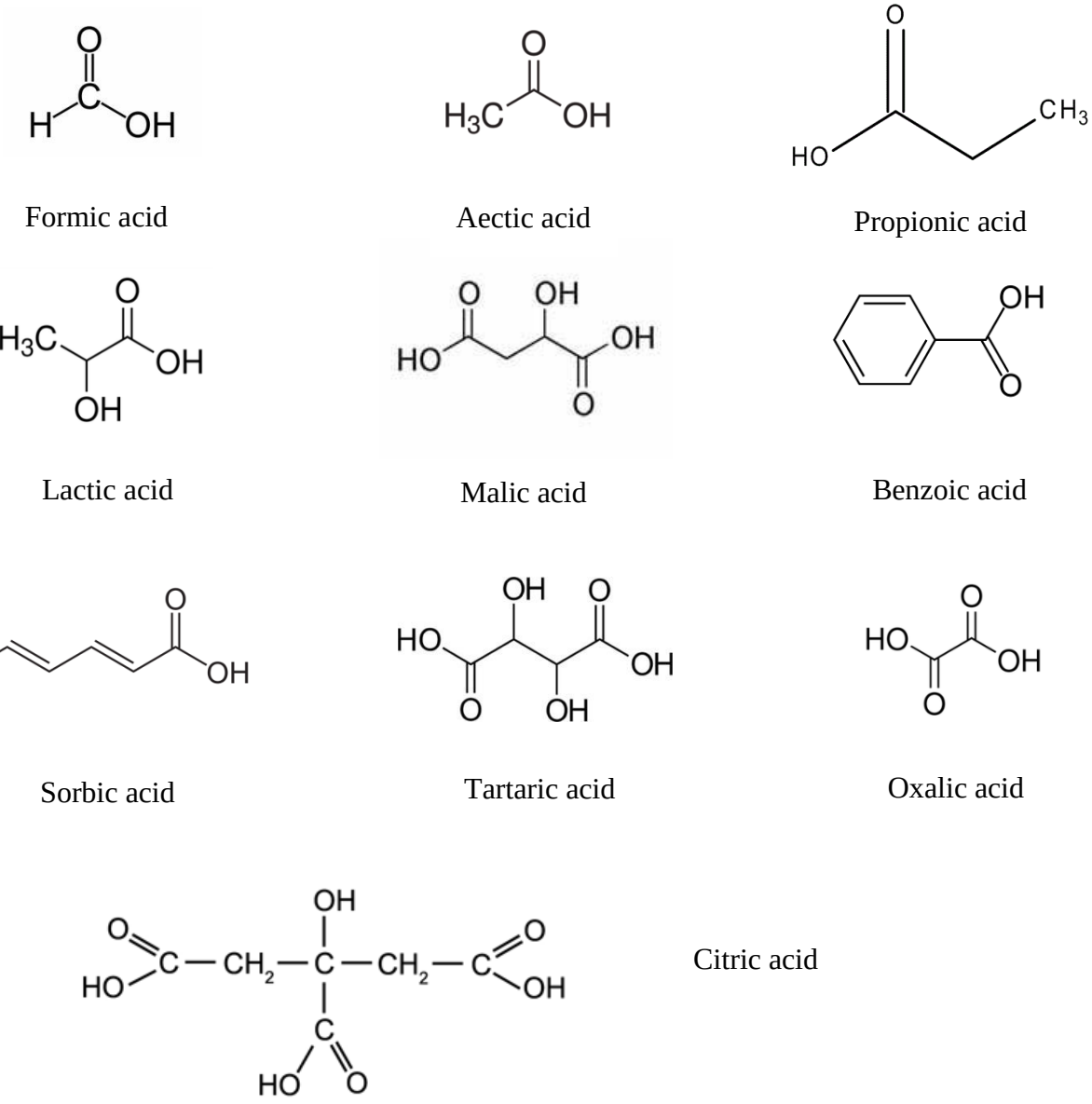

Figure 1.1 Chemical structures of some organic acids frequently used in food.

1.2.2 Organic acids as antimicrobial agents

Due to several outbreaks from consumption of RTE meat products contaminated with *L. monocytogenes*, various strategies have been investigated and implemented to control *L. monocytogenes*. The use of antimicrobial agents is one method recommended by USDA-FSIS (USDA-FSIS, 2003b). An antimicrobial agent is defined as a substance that effectively reduces or eliminates microorganisms or suppresses growth to no more than 2 log units throughout the shelf life of the product (USDA-FSIS, 2003b). These antimicrobial substances adversely impact microbial protein synthesis, enzyme activity, cell membrane and/or cell wall, and/or transport mechanisms for nutrients (Lück and Jager, 1997). Organic acids have been widely used as antimicrobials due to their effectiveness and low cost in minimizing microbial growth (Theron and Lues, 2007; Mani-López et al., 2012).

The undissociated form of an organic acid is responsible for antimicrobial activity due to its hydrophobicity, enable penetration through the microbial cell membrane and subsequent inhibitory action (Lück and Jager, 1997). Once penetrate, the organic acids lead to cytoplasmic acidification, toxic anion accumulation, and disruption of essential metabolic reactions (Theron and Lues, 2011; Lopez et al., 2012) (Fig 1.2).

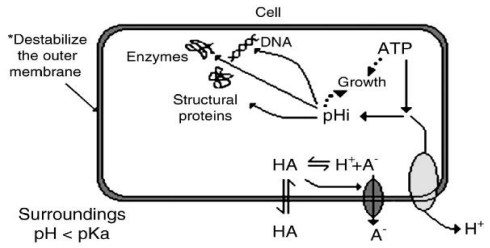

Figure 1.2 Mechanism of antimicrobial action of organic acids in a microbial cell (Lopez et al., 2012).

1.2.3 Applications of organic acids to food products

Organic acids are applied to a wide variety of foods including RTE meat products to control *Listeria* spp. Several organic acids and their application methods have been significantly improved to control *Listeria* including spraying, dipping, and incorporating into product formulation, or antimicrobial packaging (Stekelenburg, 2003; Barmpalia et al., 2004; Uhart et al., 2004; Stopforth et al., 2010). Moreover, several studies showed that various organic acids in combination with other antimicrobial compounds or post-lethality treatments are more effective than a single organic acid. However, organic acids or their salts reportedly impart strong acid odor in the product (Blom et al., 1997; Islam et al., 2002; Stekelenburg and Kant-Muermans, 2001).

According to Blom et al. (1997), the mixture of 2.5% lactate and 0.25% acetate inhibited *L. monocytogenes* in servelat and cooked ham throughout 5 weeks of storage at 4°C but the inhibition was not maintained in the cooked ham after 3 weeks of storage at 9°C. Additionally, consumer acceptance of servelat formulated with a lactate/acetate mixture was less than that formulated without an acid mixture due to the sour taste related to the lactate/acetate mixture.

Bedie et al. (2001) reported that higher organic acid concentrations provided better of *L. monocytogenes* inhibition on frankfurters. Using 3% sodium lactate currently allowed by USDA-FSIS inhibited the growth of *L. monocytogenes* on frankfurters during storage at 4°C for 70 days and using 0.25% sodium diacetate provided the inhibition for 35-50 days. When using twice the concentration of both organic acids, they showed the complete inhibition for 120 days at 4°C.

Islam et al. (2002) found that dipping frankfurters in up to 25% sodium benzoate, sodium propionate, potassium sorbate, or sodium diacetate solution alone (yield < 0.3% residue of

inhibitor to frankfurter) was not sufficient to control *L. monocytogenes* growth during abusive temperature storage at 13 and 22°C. They also reported that the flavor and overall acceptability scores for frankfurters treated with sodium diacetate were lower compared to the non-diacetate treatments.

Glass et al. (2002) reported that treating wieners with 6% lactate or 3% diacetate and dipping of wieners into the combined solution did not delay the growth of *L. monocytogenes* during refrigerated storage. While the inclusion of lactate and diacetate mixture in the weiner formulation can inhibit the growth of *L. monocytogenes* at 4.5°C storage for 60 days, this treatment was less effective at 7°C.

According to Barmpalia et al. (2004), incorporating 1.8% sodium lactate or 0.25% sodium diacetate into a frankfurter formulation retarded *L. monocytogenes* growth during storage at 10°C. When both organic acids were used in combination or combined with dipping in either 2.5% lactic acid or 2.5% acetic acid after processing, *Listeria* inhibition was improved.

Hwang and Tamplin (2007) reported a model to predict the lag phase and growth of *L. monocytogenes* in ground ham as affected by the level of sodium lactate (1.0 – 4.2%) and sodium diacetate (0.05-0.2%) at various temperatures (0-45°C). The model showed that higher lactate and diacetate concentrations extended the lag phase at low (≤ 15°C) but not at high storage temperatures.

Organic acids and their salts show significant antilisterial activity and are frequently sprayed, dipped, or incorporated into product formulations (Bedie et al., 2001; Glass et al., 2002; Samelis et al., 2005). Moreover, some organic acids have been used to create antimicrobial packaging materials (Guo et al., 2014). Currently, organic acid salts are allowed in meat and poultry products to inhibit microbial growth, with the limit of 4.8%, 0.25%, and 4.8% for

potassium lactate, sodium diacetate, and sodium lactate, respectively, in product formulations by weight (Code of Federal Regulations, 2011).

The antilisterial efficacy of these acid salts is affected by many factors including pH, water activity, nitrite, salt content, and storage conditions (Samelis et al., 2003; Seman et al., 2002). Extensive research has been conducted to determine the survival of *L. monocytogenes* in RTE meat products by dipping in solutions of sodium diacetate or sodium lactate alone (Islam et al., 2002; Uhart et al., 2004). These acid salts were more effective (Bampalia et al., 2004) when used together rather than alone (Glass et al., 2002; Samelis et al., 2002; Stekelenburg, 2003); however, they usually impart strong acidic odor (Blom et al., 1997; Islam et al., 2002; Stekelenburg and Kant-Muermans, 2001).

1.3 Hop and hop extracts

1.3.1 Hop plant

Hop plant (*Humulus lupulus*) belongs to the family *Cannabinaceae* and only female hop flowers are used for commercial purposes (Verzele and Keukeleire, 1991). Germany and the USA are the two largest hop growers with about 70% of the total world production in 2013 (IHGC, 2014). The hop plant has long been recognized as a food as early as the first century A.D., as a medicinal additive in the 8^{th} and 9^{th} century (Verzele and Keukeleire, 1991), and as a food ingredient in beer production from the 12^{th} century (Hass & Barsoumian, 1994; Hoffman, 1956). Only female hop cones containing small yellow granules called lupulin glands are used to flavor and preserve beer (Verzele and Keukeleire, 1991; Tim, 2003). In beer processing, dried hops are usually added during wort boiling. However, hop extracts have become more popular than the traditionally dried hops due to the convenience, uniformity, stability, economic cost, and good quality (Wilson et al., 2003).

1.3.2 Hop extracts

When extracted from hop cones with an organic solvent (hexane or ethyl alcohol) or carbon dioxide, the resulting fractions contain mainly alpha- and beta-acids, with the remainder consisting of oils, waxes and uncharacterized resins (Wilson et al., 2011). The composition of hops is shown in Table 1.2. The constituents with differing side chains for hop alpha-acids are humulone, cohumulone, and adhumulone and those of hops beta-acids are lupulone, colupulone, and adlupulone (Fig. 1.3) (FDA 2001; Srinivasan et al, 2004). In beer processing, only hop alpha-acids undergo the isomerization process during the wort boiling stage with the result of iso-alpha-acids providing the bitter taste to beer (Keukeleire, 2000; Sakamoto and Konings, 2003). In order to maintain the desirable level bitterness in beer, iso-alpha-acids are currently produced off-line to facilitate their addition at any stage during beer processing (Keukeleire, 2000). To prevent beer off-flavor due to the decomposition of iso-alpha-acids when exposed to light, hop acids are reduced and formulated as potassium salts in concentrated aqueous solutions (Keukeleire, 2000) (Fig. 1.3). These reduced iso-alpha-acid compounds are stable to light and stabilize beer foam (Tim, 2003).

Table 1.2 Composition of hops (Verzele and Keukeleire, 1991).

Component	% by weight
Alpha acids	2 – 12
Beta acids	1 – 10
Amino acids	0.1
Cellulose	40 – 50
Essential oil	0.5 – 5
Monosaccharides	2
Oils and fatty acids	Trace to 25%
Pectins	2
Polyphenols (Tannins)	2 – 5
Proteins	1
Salts (ash)	10
Water	8 – 12

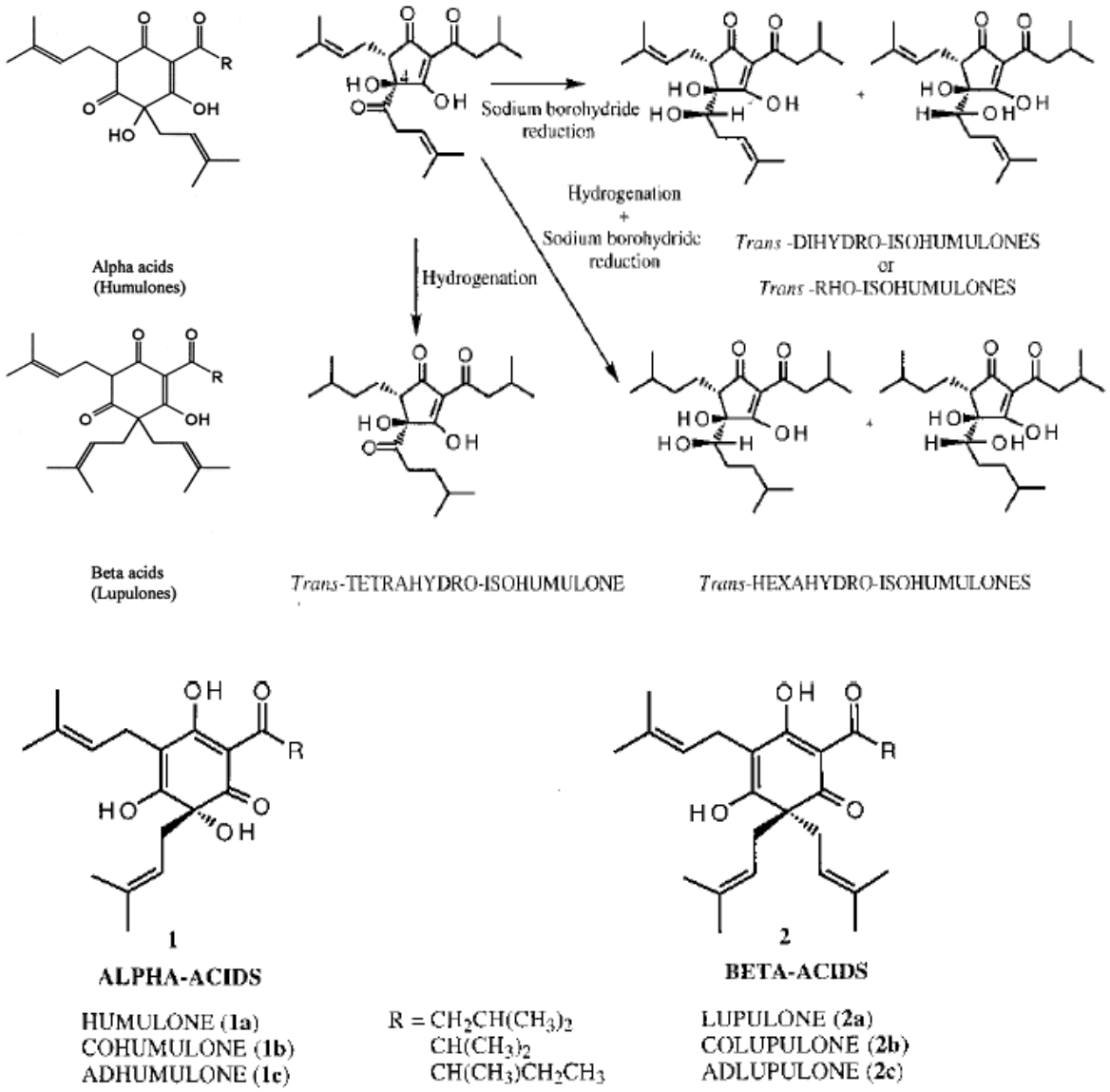

Figure 1.3 Some hop compounds and reduced iso-alpha-acids (Adapted from Keukeleire, 2000).

1.3.3 Hops as antimicrobial agents

Hop compounds have received increasing attention due to their antimicrobial activity against beer spoilage microorganisms, especially Gram-positive bacteria (Haas and Barsoumian, 1994; Bhattacharya et al., 2003; Sakamoto and Konings, 2003). The main target of hop acids in bacterial inhibition is the microbial cell membrane (Teuber and Schmalreck, 1973). In Gram-negative bacteria, however, serumphosphatides presented in the outer membrane of bacteria cells inactivate both lupulones and humulones, and minimize the bactericidal activity of the hop components (Teuber and Schmalrek, 1973). However, some studies have shown that hops in combination with other antimicrobial agents are effective in controlling some Gram-negative bacteria. Fukao et al. (2000) found that the combination of 100 ppm hop resin with 0.5% sodium hexametaphosphate inhibited the growth of *E. coli* K-12 IFQ3301 in broth and mashed potatoes, whereas using either hop resin or sodium hexametaphosphate alone did not inhibit *E. coli*. Natarajan et al. (2008) also showed that lupulone (beta-acid) in combination with polymyxin B sulfate inhibited *Proteus vulgaris*, *Serratia marcescens*, and *Proteus mirabilis*.

Hop acids are weak acids and the inhibition of bacterial growth is mainly attributed to the undissociated form (Simpson and Smith, 1992; Sakamoto and Konings, 2003). The undissociated form of hop acids can invade the microbial cell and dissociate into protons and hop anions, resulting in lowering of the intracellular pH and diffusion of divalent cations out of the cell (Fig. 1.4) (Sakamoto and Konings, 2003). Inhibition of bacterial growth also has been attributed by the prenyl group on the side chain of hop acid which causes cell membrane leakage (Teuber and Schmalreck, 1973; Schmalreck and Teuber, 1975; Keukeleire, 2000).

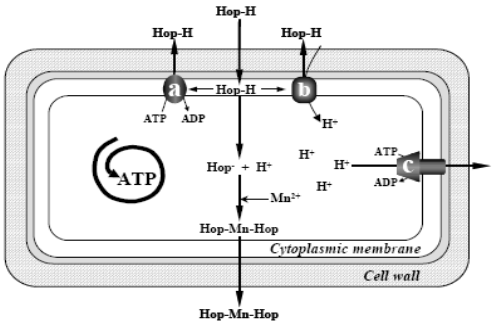

Figure 1.4 Hop mechanisms in a microbial cell (Sakamoto and Konings, 2003).

Numerous studies have demonstrated the antimicrobial activity of hop acids. Hop compounds showed inhibition against *Bacillus subtilis* (Schmalreck and Teuber, 1975), fungi (Mizobuchi and Sato, 1985), *Lactobacillus acidophilus* (Todd et al., 1992), *Streptococcus mutans* (Bhattacharya et al.,2003), protozoa (Srinivasan et al., 2004), and lactobacilli (Rückle and Senn, 2005). Millis et al. (1994) showed that 6 ppm beta acid completely inhibited *L. monocytogenes* growth in brain heart infusion broth after incubation at 35^{o}C for 24 h. Larson et al. (1996) showed that 10 μg/mL of hop extract contained 41% beta acid and 12% alpha acid and the 10 μg/mL of hop extract containing 94.7% beta acid completely inhibited *L. monocytogenes* in trypticase soy broth and brain heart infusion broth, respectively, after incubation at 37^{o}C for 24 h. The antilisterial activity of hop beta acids (1.0-5.0 μg/ml) was further improved when mixed with other antimicrobial agents (Shen and Sofos, 2008; Shen et al., 2009).

For practical application in RTE meat products, the USDA-FSIS approved hop beta acids as generally recognized as safe (GRAS) for frankfurter casings and cooked ready-to-eat meat and poultry products (US/FDA GRAS Notice Nr 000063) (USDA-FSIS, 2013). Currently, several hops extracts are produced by fractionation and chemical conversion to serve the brewers' demand (Mahaffee et al, 2009; Wilson et al., 2011). However, almost no studies have been

conducted to evaluate the antilisterial activity of various hop extracts, including isomerized or reduced forms in liquid media and meat products with/without organic acid salts.

CHAPTER 2: INHIBITION OF *LISTERIA MONOCYTOGENES* IN FULL AND LOW SODIUM FRANKFURTERS AT 4, 7, OR 10ºC USING SPRAY-DRIED MIXTURES OF ORGANIC ACIDS SALTS

2.1 Introduction

Various organic acid salts have been used as antimicrobial agents singly or jointly against *L. monocytogenes* in RTE meat products to improve the product safety with no quality loss. Recently, several powder forms of organic salts have been developed as *Listeria* growth inhibitors but scientific studies on their efficacy of inhibition in RTE meat products have not been conducted enough. Hence, the purpose of this research was to evaluate the impact of five powdered (the mixtures of sodium lactate, sodium acetate, sodium diacetate, potassium acetate, and/or potassium diacetate) and four liquid inhibitors (the mixtures of sodium lactate, sodium diacetate, and/or potassium lactate) on *Listeria* inhibition, organoleptic quality, and physicochemical properties of frankfurters. The hypothesis of this research is that the powdered organic acid salts exhibit antilisterial activities as same as or better than the current commercial inhibitors in liquid for full- and low-sodium frankfurters without significant impacts on physicochemical and organoleptic characteristics.

2.2 Material and methods

Two sets of experiments were conducted. Initially, 10 full-sodium frankfurter formulations containing organic acid salt or their mixtures (5 powdered, 4 liquid and 1 control) were assessed for *Listeria* inhibition, organoleptic quality, and physicochemical properties. Thereafter, five-low sodium frankfurters containing three best powdered inhibitors, one liquid control, and one inhibitor-free control were similarly evaluated.

2.2.1 Full sodium frankfurter preparation using powdered or liquid inhibitors

Full sodium (1.8% salt) frankfurters were manufactured in the Michigan State University Meat Laboratory (East Lansing, MI). Raw meat (boneless pork butt, pork back fat, and 80% ground beef) and nonmeat ingredients were purchased locally, and the powdered and liquid inhibitors were obtained from Niacet b.v. (Tiel, The Netherlands). Both pork butt and back fat were coarsely ground using a 0.95 cm plate (Model 4146, Hobart Corporation, Troy, OH) and premixed for 3 min (Model Butcher Boy 250F, Lasar Mfg Co. Inc., Los Angeles, CA).

In each of three replications, 10 different frankfurter formulations (22.7 kg/batch) were randomly prepared by adding water (no inhibitor) or one of the inhibitors (powdered or liquid) (Tables 2.1 through 2.3) by weight (wt/wt) as follows: (1) no inhibitor (water) control: CTR; (2) 0.25% sodium lactate (SL) + 0.25% sodium acetate (SA): powdered inhibitor (PI-1); (3) 0.5% SL + 0.5% SA: PI-2; (4) 0.25% SL + 0.25% SA + 0.16% sodium diacetate (SD): PI-3; (5) 0.6% potassium acetate (PA) + 0.15% potassium diacetate (PD): PI-4; (6) 0.8% PA + 0.2% PD: PI-5; (7) 1.5% SL + 1.0% water: liquid inhibitor (LI-1); (8) 1.4% SL + 0.1% SD + 1.0% water: LI-2; (9) 1.5% potassium lactate (PL) + 1.0% water: LI-3; and (10) 1.4% PL + 0.1% SD + 1.0% water: LI-4. The inhibitor weight differences, due to different amounts in organic salts and liquid, were adjusted with pork meat and water, respectively.

Meat batter prepared on a different day for each replication was blended with spices and preservatives in a bowl chopper (model K64-Va, Maschinenfabrik Seydelmann KG, Aalen, Germany) to a final batch temperature of 12^{o}C. The resulting emulsion was then stuffed into cellulose casing (24 mm, Viscofan USA, Inc., Montgomery, AL), which was linked (model 500, VEMAG Maschinenbau GmbH, Verden, Germany) into 9- to 10-cm length and cooked to an internal temperature of 70^{o}C in a smoke-free smokehouse (model A28, CGI Processing Equip.

Cicero, IL). The end-cooking temperature was confirmed with a calibrated digital thermometer and logger (model 800024, Sper Scientific Ltd., Scottsdale, AZ). To assess cooking yield, four frankfurter links per treatment (10 frankfurters per link) were randomly selected, labeled, and weighed before cooking. After cooking, rinsing and surface drying, the labeled frankfurters were reweighed to determine cooking yield. The remaining frankfurters were stored overnight at 2^{o}C, manually peeled, placed in pouches (product # 75001979, Koch Supplies Inc, Kansas City, MO), and vacuum packed (Multivac Sepp Haggenmueller GmbH & Co. KG., Wolfertschwenden, Germany). Three groups of samples were prepared for physicochemical, microbial, and consumer sensory analyses.

2.2.2 Low sodium frankfurter preparation with powdered or liquid inhibitors

Low sodium frankfurters (1% salt) were manufactured as previously described for the full-sodium frankfurter except using the following five formulations: (1) no inhibitor (water) control (CTR); (2) 0.5% SL + 0.5% SA: PI-2; (3) 0.247% SL + 0.247% SA + 0.156% SD: PI-3; (4) 0.8% PA + 0.2% PD: PI-5; and (5) 1.4% PL + 0.1% SD + 1.0% water: LI-4 (Tables 2.1 through 2.3).

Table 2.1 Base formulation of frankfurters.

Meat	Amount (%)
Beef trim 85/15	31.56
[1]Pork boneless butt	39.3 – 40.7
Pork back fat	4.6 – 4.8
Ingredient	
[1]Water/Ice	18.54 – 19.5
[2] Seasoning blend (no salt)	1.17
[3] Powdered or liquid inhibitors	0 – 2.5
Salt	1.8 or 1.0
Phosphate	0.31
Curing salt (6.25% nitrite)	0.18
Total	100

[1] Additional pork and water were used to adjust the batch due to the different amounts of organic salts and liquid in inhibitors.
[2] Components of seasoning blend are described in Table 2.2
[3]Components of powdered or liquid inhibitors are described in Table 2.3

Table 2.2 Components of seasoning blend (no salt) added to frankfurter formulations.

Components	Amount (%)
Dextrose	80.06
Monosodium glutamate	6.67
Onion powder	3.33
Garlic powder	2.50
Sodium erythorbate	3.50
Spice extractive	1.44
Tricalcium phosphate	2.50
Total	100

Table 2.3 Percent of powdered inhibitors (PI) and liquid inhibitors (LI) added to frankfurter formulations.

Inhibitors	Sodium lactate	Sodium acetate	Sodium diacetate	Potassium acetate	Potassium lactate	Potassium diacetate	Water	Total amount
Control	0	0	0	0	0	0	2.5	2.5
PI-1	0.25	0.25						0.5
PI-2	0.5	0.5						1.0
PI-3	0.25	0.25	0.15					0.65
PI-4				0.6		0.15		0.75
PI-5				0.8		0.2		1.0
LI-1	1.5						1.0	2.5
LI-2	1.4		0.1				1.0	2.5
LI-3					1.5		1.0	2.5
LI-4			0.1		1.4		1.0	2.5

2.2.3 Physicochemical analysis

Seven physicochemical parameters were assessed: pH, protein, fat, moisture, water activity (a_w), sodium, and cooking yield. For pH, a 5-g sample was homogenized in 25 ml of deionized water, and the pH was measured with a meter (Accumet AR15, Fisher Scientific Inc., Pittsburgh, PA) equipped with a pH electrode (model 13-620-631, Fisher Scientific Inc., Houston, TX). Protein, fat, and moisture contents were determined with a nitrogen analyzer (model FP-2000 Nitrogen Analyzer, Leco Corp. St. Joseph, MI), fat extractor (Soxtec System HT6, Tecator AB, Höganäs, Sweden), and drying oven (model Yamato DX 400, Yamato Scientific. Ltd., Tokyo, Japan), respectively, according to AOAC International (2005) methods 992.15, 991.36 and 950.46B, respectively. The a_w was determined with an AquaLab meter (Decagon Devices, Inc., Pullman, WA). Sodium content was determined with a pH-ion analyzer (model 123, Omnion, Inc., Rockland, MA) equipped with a sodium-specific electrode (model A230T, Omnion, Inc., Rockland, MA) calibrated with standard sodium solutions. Cooking yield for each frankfurter formulation was based on the weight difference before and after cooking in the smokehouse.

2.2.4 *Listeria monocytogenes* strains and frankfurter inoculation

The following six *L. monocytogenes* strains of different pulsed-field gel electrophoresis types were selected for use: Lm-10-s11 (serotype 1/2a, delicatessen isolate), Lm-12-s11 (serotype 1/2b, delicatessen isolate), Lm-12-s8 (serotype 1/2b, delicatessen isolate), R3-031 (serotype 1/2a, food isolate from a hot dog outbreak), N1-227 (serotype 4b, food isolate from a deli meat outbreak), and R2-763 (serotype 4b, food isolate from a deli meat outbreak), all of which were obtained from Dr. Martin Wiedmann (Cornell University, Ithaca, NY). Each strain

had been preserved at -80^{o}C in Trypticase Soy Broth (TSB) containing 0.6% (w/v) yeast extract (YE) (Difco, Becton Dickinson, Sparks, MD) and 20% glycerin. For the experiments, strains were subjected to two consecutive cultures in TSBYE for 24 h at 37°C, pelleted by centrifugation at 3,100 x g for 15 min at 4°C, and then resuspended in sterile phosphate buffered saline (PBS; pH 7.4). The optical density (OD) of each cell suspension was measured at 600 nm, suspensions were adjusted to the same OD value, and 5 mL of each suspension was added to 3 liters of PBS to obtain a six-strain *L. monocytogenes* cocktail containing ~1 x 10^6 CFU/mL. The *L. monocytogenes* population in the inoculum was confirmed by plating appropriate dilutions on Trypticase soy agar (TSA) (Difco, BD) with YE and incubating 22 to 24 h at 37°C.

Fifty frankfurters from each formulation were aseptically transferred to a mesh bag, immersed in the six-strain *L. monocytogenes* cocktail, and gently stirred for 1 min. The mesh bag with the frankfurters was then removed, drained for 1 min, and placed in a biosafety hood for 25 min for the inoculum to absorb. Two frankfurters were aseptically transferred to each of 10 Shanvac vacuum bags (10 by 15 cm outside dimensions; Shannon Packaging, Chino, CA), vacuum sealed, and stored at 4, 7, and 10°C for up to 90 days. Uninoculated frankfurters from each formulation were used to quantify the background bacteria.

2.2.5 Microbial analysis

Immediately after packaging and after 15, 30, 45, 60, 75 and 90 days of storage at 4, 7, and 10°C, one inoculated and one uninoculated bag per treatment was randomly selected to quantify *L. monocytogenes* and mesophilic aerobic bacteria (MAB), respectively. All samples (25 g) were diluted 1:10 in PBS and homogenized in a stomacher (NEUTEC Group Inc, Farmingdale, NY) for 1 min. Appropriate serial dilutions in PBS were then plated on modified

Oxford agar (Difco, MD) and TSAYE to enumerate *L. monocytogenes* and MAB, respectively, after 48 h of incubation at 35°C.

The methods for physicochemical analyses, *Listeria* inoculation, and quantification of *Listeria* and mesophilic aerobic bacteria (MAB) were the same as used for both full-sodium and low-sodium frankfurters.

2.2.6 Consumer sensory analysis, full-sodium frankfurters

For sensory analysis of full sodium frankfurters, the following two sets of samples were manufactured in three separate batches for evaluation on separate days: (1) four sodium-based frankfurter formulations with a control, and (2) four potassium-based frankfurter formulations with a control. Frankfurters containing the 1% powdered mixture of 0.5% SL and 0.5% SA were not included because the USDA does not permit SA concentrations above 0.25% (US-FDA, 2011). A total of 330 frankfurter consumers (110 consumers per replication) were recruited from students, staff, and faculty members at Michigan State University to evaluate the full sodium frankfurter formulations on three different days (55 panelists each for the sodium- and potassium-based formulations plus the controls).

On the day of evaluation, 20 frankfurters of each formulation were gently heated with agitation in separate pots of boiling water to achieve an internal temperature of 72^{o}C. The heated frankfurters were then placed in sealable bags, which were immersed in 63^{o}C water until given to the panelists. These boiling and warming procedures were repeated until the 2- to 2.5-h sensory evaluation was finished. Upon serving, each frankfurter was cross-cut to a length of 4 cm, placed in a randomly coded 4-oz (120-ml) soufflé cup, and covered. Trays containing samples of five different formulations along with a glass of filtered water were randomly presented to each

panelist in individual booths equipped with a touch-screen computer and controlled lighting. All samples were evaluated for appearance, texture, flavor, and overall acceptability using a 9-point hedonic scale (9 = like extremely and 1 = dislike extremely). Data were collected using the Sensory Information Management Systems (Sensory Computer Systems, Morristown, NJ) and included any written comments concerning the samples.

2.2.7 Consumer sensory analysis, low-sodium frankfurters

For sensory analysis of low-sodium frankfurters, 210 frankfurter consumers (105 consumers per replication) were similarly recruited with 20 frankfurters per treatment prepared as previously described in full-sodium frankfurters. On the day of evaluation for appearance, texture, flavor, and overall acceptability, trays containing samples of four different formulations along with a glass of filtered water were randomly presented as previously described.

2.2.8 Statistical analysis

Physicochemical and microbial data were subjected to the general linear model procedure of SAS (SAS Institute, 2002). To better assess the effect of treatment on *Listeria* inhibition, the area under the graph of *Listeria* population in frankfurters during storage for each treatment was calculated. The higher area under graph, the better growth of *L. monocytogenes.* Means were compared with Tukey's Test at $\alpha = 0.05$ level. For sensory analysis, a mixed model analysis of variance was used for comparison of means with Tukey's Test at $\alpha = 0.05$.

2.3 Results and discussion

2.3.1 Physicochemical analysis

Ten full-sodium frankfurter formulations (CTR, PI-1 through PI-5, and LI-1 through LI-4) and five low-sodium frankfurter formulations (CTR, PI-2, PI-3, PI-5, and LI-4) were assessed for seven physicochemical parameters: sodium, pH, a_w, cook yield, moisture, protein, and fat (Tables 2.4 and 2.5). Sodium concentrations were correlated with the amount of sodium present in the *Listeria* inhibitors. Full sodium frankfurter formulations PI-2, LI-1, and LI-2 were highest in sodium (1,279 – 1,345 mg/100g of sample), followed by PI-1, PI-3 (1,172-1,178 mg/100g), and PI-4, 5, LI-3, LI-4, and CTR (950 – 1,035 mg/100g), and none of these formulations contained sodium-based *Listeria* inhibitors except for LI-4 (0.1% SD). Similarly, low-sodium frankfurter formulation PI-2 was higher in sodium ($P < 0.05$) (926 mg/100g) than PI-5, LI-4, and CTR (750 to 763 mg/100g), and PI-3 was intermediate (839 mg/100g).

Frankfurter pH was influenced by the presence and amount of SA (pH 8.9), SL (pH 6.3) and SD (pH 4.5 – 5.0) in the formulation. Full-sodium frankfurters containing none or trace (≤ 0.1%) amounts of diacetate (PI-1, PI-2, LI-1, LI-3, and LI-4) were significantly less acidic (pH 6.39 - 6.41) than those formulations containing diacetate (PI-3, PI-4, PI-5, and LI-2, pH 6.21-6.26, $P < 0.05$), and CTR had an intermediate pH of 6.35. For low-sodium frankfurters, formulations PI-2, LI-4, and CTR were less acidic (pH 6.29 – 6.31) than the remaining two formulations (PI-3 and PI-5; pH 6.13 to 6.15; $P < 0.05$) which contained > 0.156% PD. Similar to these findings, Pal et al. (2008) found that the pH of frankfurters decreased from 6.17 to 6.02 as the SD and PL concentrations increased. Fat and moisture content in full- and low-frankfurters were within 2% and the remaining parameters (a_w, cooking yield, and protein) differed by <1% (Tables 2.4 and 2.5).

Table 2.4 Impact[1] of formulations containing powdered and liquid inhibitors[2] on the physicochemical properties of full sodium frankfurters.

Parameters[3]	CTR (0%)[4]	PI-1 (0.5%)	PI-2 (1.00%)	PI-3 (0.65%)	PI-4 (0.75%)	PI-5 (1.00%)	LI-1 (2.50%)	LI-2 (2.50%)	LI-3 (2.50%)	LI-4 (2.50%)	Standard Error
Sodium (mg/100g)	1031^{c}	1178^{b}	1279^{ab}	1172^{b}	1035^{c}	1021^{c}	1345^{a}	1328^{a}	950^{c}	992^{c}	17.93
pH	6.35^{c}	6.39^{b}	6.41^{a}	6.21^{e}	6.26 ^{d}	6.22^{e}	6.39^{ab}	6.26^{d}	6.40^{ab}	6.40^{ab}	0.004
a_w	0.956^{a}	0.955^{a}	0.950^{a}	0.956^{a}	0.950^{a}	0.955^{a}	0.951^{a}	0.949^{a}	0.951^{a}	0.953^{a}	0.002
Cooking yield (%)	89.50^{b}	88.51^{c}	90.03^{ab}	90.69^{a}	88.07^{c}	90.08^{ab}	88.03^{c}	88.01^{c}	90.16^{ab}	90.04^{ab}	0.12
Moisture (%)	60.79^{ab}	60.02^{de}	60.50^{abc}	60.61^{ab}	60.50^{abcd}	60.84^{a}	60.07^{cde}	59.71^{e}	60.33^{bcd}	60.11^{cde}	0.10
Protein (%)	14.86^{ab}	15.15^{a}	14.44^{bc}	14.58^{bc}	14.62^{abc}	14.48^{bc}	14.18^{c}	14.65^{abc}	14.32^{c}	14.16^{c}	0.20
Fat (%)	18.67^{ab}	18.59^{ab}	18.84^{a}	18.47^{ab}	17.78^{bc}	18.82^{a}	17.03^{cd}	17.42^{cd}	17.71^{bc}	16.55^{d}	0.21

[1]Mean values with same letters in the same row were not significantly different ($P \geq 0.05$).
[2]Inhibitors in the formulation as in Table 2.3.
[3]Least square means of $n = 9$ to 36 observations.
[4]Amount of inhibitor.

Table 2.5 Impact[1] of formulations containing powdered and liquid inhibitors[2] on the physicochemical properties of low sodium frankfurters.

Parameters[3]	CTR (0%)[4]	PI-2 (1.00%)	PI-3 (0.65%)	PI-5 (1.00%)	LI-4 (2.50%)	Standard Error
Sodium (mg/100g)	750^{b}	926^{a}	839^{ab}	754.89^{b}	763^{b}	19
pH	6.30^{a}	6.31^{a}	6.15^{b}	6.13^{b}	6.29^{a}	0.02
a_w	0.971^{a}	0.950^{a}	0.956^{a}	0.955^{a}	0.966^{a}	0.002
Cooking yield (%)	89.57^{b}	90.03^{ab}	90.69^{a}	90.08^{ab}	89.65^{a}	0.40
Moisture (%)	62.74^{ab}	60.50^{abc}	60.61^{ab}	60.84^{a}	62.09^{a}	0.36
Protein (%)	14.37^{ab}	14.44^{bc}	14.58^{bc}	14.48^{bc}	14.35^{a}	0.14
Fat (%)	18.06^{ab}	18.84^{a}	18.47^{ab}	18.82^{a}	18.12^{a}	0.34

[1]Mean values with same letters in the same row were not significantly different ($P \geq 0.05$).
[2]Inhibitors in the formulation as in Table 2.3.
[3]Least square means of $n = 9$ to 36 observations.
[4]Amount of inhibitor.

2.3.2 Microbial growth

Dip inoculation yielded average *L. monocytogenes* populations of 4.6 and 4.7 log CFU/g of sample in full- and low sodium frankfurters, respectively (Fig. 2.1 and 2.2). After storing the vacuum-packed full-sodium frankfurters at 4^{o}C for up to 90 days, all formulations showed better *Listeria* inhibition than the inhibitor-free CTR (Fig 2.1 and Table A.2). *Listeria* populations in the four diacetate-containing formulations (PI-3, PI-4, PI-5, and LI-2) continuously decreased to 4.02 (PI-4), and to 4.23 (LI-2) log CFU/g at the end of storage (Fig. 2.1 and Table A.1). In low sodium frankfurters, PI-5 was the only formulation in which *Listeria* decreased to 4.15 log CFU/g after 90 days of storage at 4^{o}C (Fig. 2.2 and Table A.3). Full sodium PI-2, LI-4 and low-

sodium PI-3 formulations suppressed *Listeria* growth at 4°C for 45 and 30 days, respectively, while low-sodium PI-2 and full-sodium PI-1 (containing half the inhibitor concentrations found in PI-2) allowed continuous growth to 6.51 and 6.90 log CFU/g, respectively, by the end of storage.

Two single organic salt formulations (LI-1 and LI-3) extended the initial *Listeria* lag phase in full-sodium frankfurters but then allowed populations to increase by 1.2 to 1.4 log CFU/g during storage at 4°C, whereas addition of SD to the same formulations either decreased *Listeria* populations by 0.33 log CFU/g or maintained the initial level with almost no growth, respectively. These results agree with several other studies in which a greater combined efficacy was found for SD than for SL, and SD plus SL was the most effective combination for inhibiting growth of *Listeria* (Barmpalia et al., 2004; Glass et al., 2002; Mbandi and Shelef, 2001; Schlyter et al., 1993; Stekelenburg, 2003).

Listeria populations in the low- and full-sodium inhibitor-free CTR exceeded 7.0 log CFU/g after 30 to 45 days of storage at 4°C, and 15 to 30 days of storage at 7 and 10°C, respectively. Of the 10 full-sodium formulations, only PI-4 and PI-5 had listericidal activity (-0.54 to -0.55 log CFU/g) at 7°C during 90 days of storage, whereas PI-5 in low-sodium frankfurters was listericidal (-0.24 log CFU/g) at 7°C and listeristatic (+0.02 log CFU/g) at 10°C during storage. *Listeria* inhibition also was reported by Barmpalia et al. (2004) when frankfurters were manufactured with SL plus SD, dipped in an organic acid solution, and stored for 40 days at 10°C. For the remaining seven full-sodium formulations, those containing two or three organic salts were more inhibitory than those containing a single organic salt.

In low-sodium formulations PI-2, PI-3, and LI-4, *Listeria* populations increased less than 2 log CFU/g during 90 days at 4°C and 30 days at 7°C days, with virtually no inhibition seen for

any of the formulations at 10°C (Fig. 2.2). In contrast, Barmpalia et al. (2004) found significant *Listeria* reductions in frankfurters containing 1.8% SL + 0.125% SD during 40 days of storage at 10°C. These differences might be due to the combined use of high organic acid concentrations plus smoke which was not used in our study. In low-sodium and uncured products, the effect of SL and SD against *Listeria* growth was reduced (Legan et al., 2004; Mbandi and Shelef, 2002; Seman et al., 2002). *Listeria* was suppressed for 28 days in unsmoked and uncured bratwurst and up to 84 days in smoked and cured bratwurst (Glass et al., 2002). Legan et al. (2004) found that their predictive growth model for *Listeria* worked better for cured than uncured and low-sodium products.

Uninoculated frankfurters had initial MAB background counts of 1.25 and 2.57 CFU/g in full- and low-sodium formulations, respectively, (Fig. 2.3 and 2.4). MAB populations in low- and full-sodium uninoculated CTR reached ≥ log 7.0 CFU/g after 30 and 45 days at 4 °C, respectively, which were 1.7 to 2.7 log CFU/g higher than those formulations containing *Listeria* growth inhibitors.

At elevated temperatures, MAB rapidly grew in the uninoculated full-sodium frankfurters to > 7.0 log CFU/g during 30 days at 10°C and 45 days at 7°C compared to 15 days at 10°C for the low sodium formulations. All nine full-sodium frankfurter formulations containing inhibitors yielded maximum MAB populations of 5.1 – 6.6 log CFU/g at 7°C and 7.0 to 7.4 log CFU/g at 10°C. All four low sodium frankfurter formulations allowed MAB populations to increase > 7.0 log CFU/g at 7°C and 10°C during storage. Barmpalia et al. (2004) reported that the total microbial count in control frankfurters reached 6.1 log CFU/cm^2 after 40 days of storage at 10°C. In contrast, MAB populations in our nonsmoked control increased to 6.8 log CFU/g for full-sodium formulations and 7.3 log CFU/g for low-sodium formulations after 15 days at 10°C.

These variations in growth are again expected based on the differences in formulation, smoking, and salt content. In general, similar growth trends were seen for MAB and *Listeria* on frankfurters regardless of the formulation, confirming the findings of Patel et al. (2009).

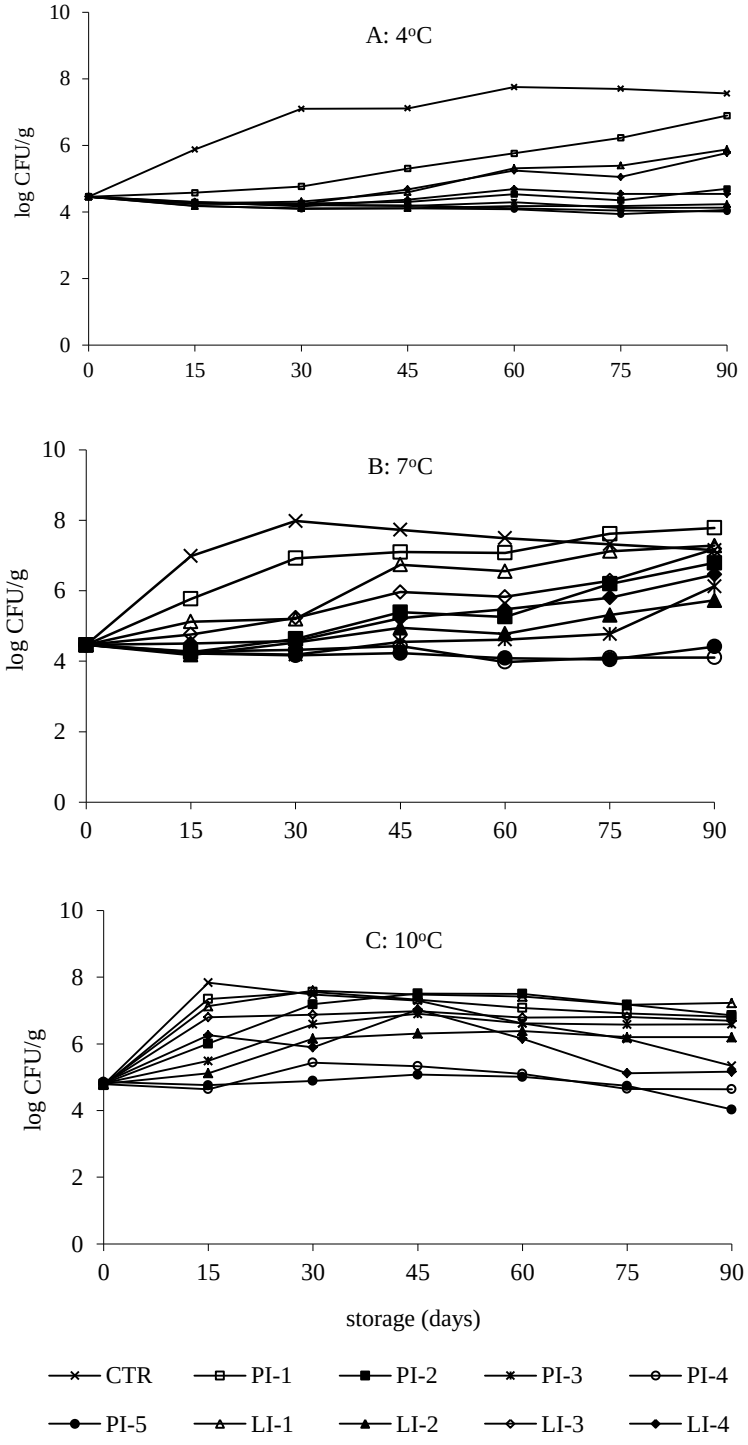

Figure 2.1 Population of *L. monocytogenes* on vacuum-packaged full-sodium frankfurters formulated with powdered or liquid inhibitors[a] during 90 days of storage at 4 (A), 7 (B) and 10°C (C).

[a] Inhibitors in the formulations as in Table 2.3.

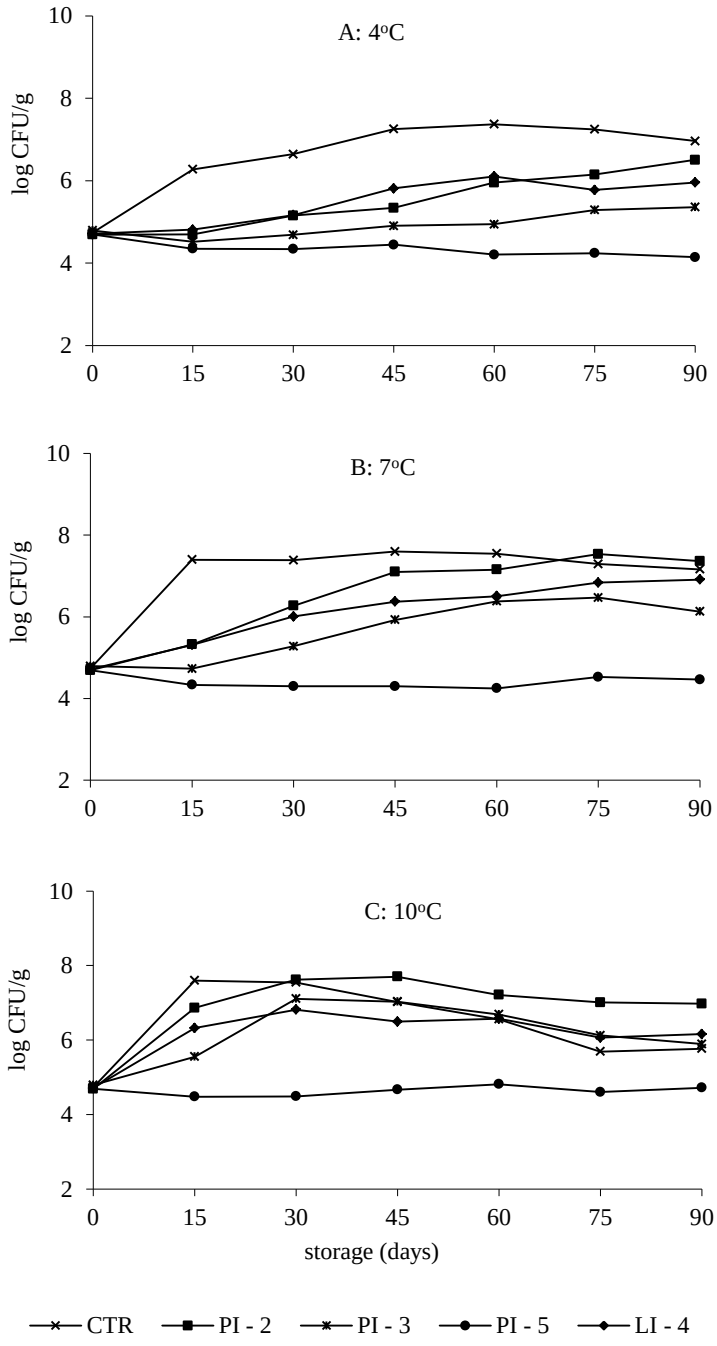

Figure 2.2 Population of *L. monocytogenes* on vacuum-packaged low-sodium frankfurters formulated with powdered or liquid inhibitors[a] during 90 days of storage at 4 (A), 7 (B) and 10°C (C).

[a] Inhibitors in the formulations as in Table 2.3.

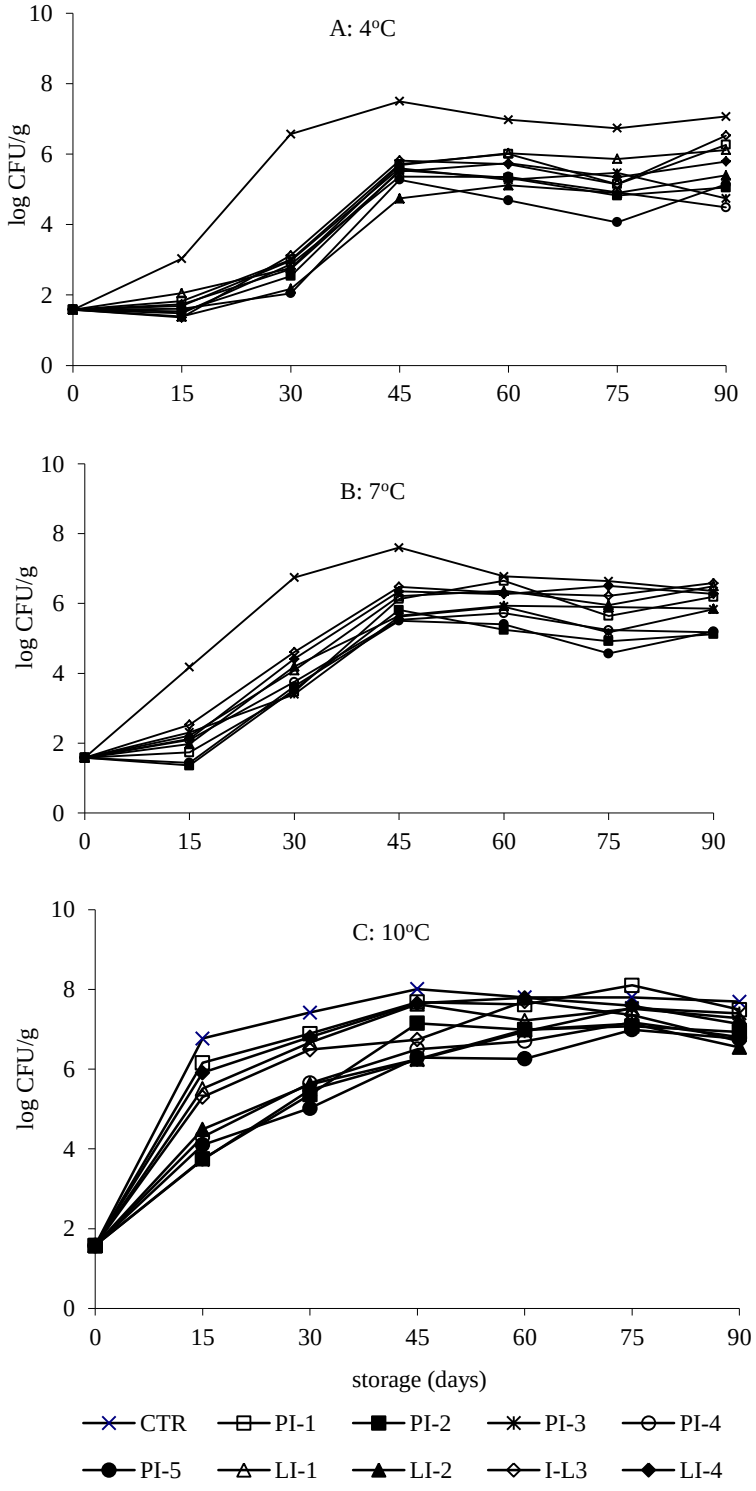

Figure 2.3 Population of mesophilic aerobic bacteria on vacuum-packaged full-sodium frankfurters formulated with powdered or liquid inhibitors[a] during 90 days of storage at 4 (A), 7 (B) and 10°C (C).

[a] Inhibitors in the formulations as in Table 2.3.

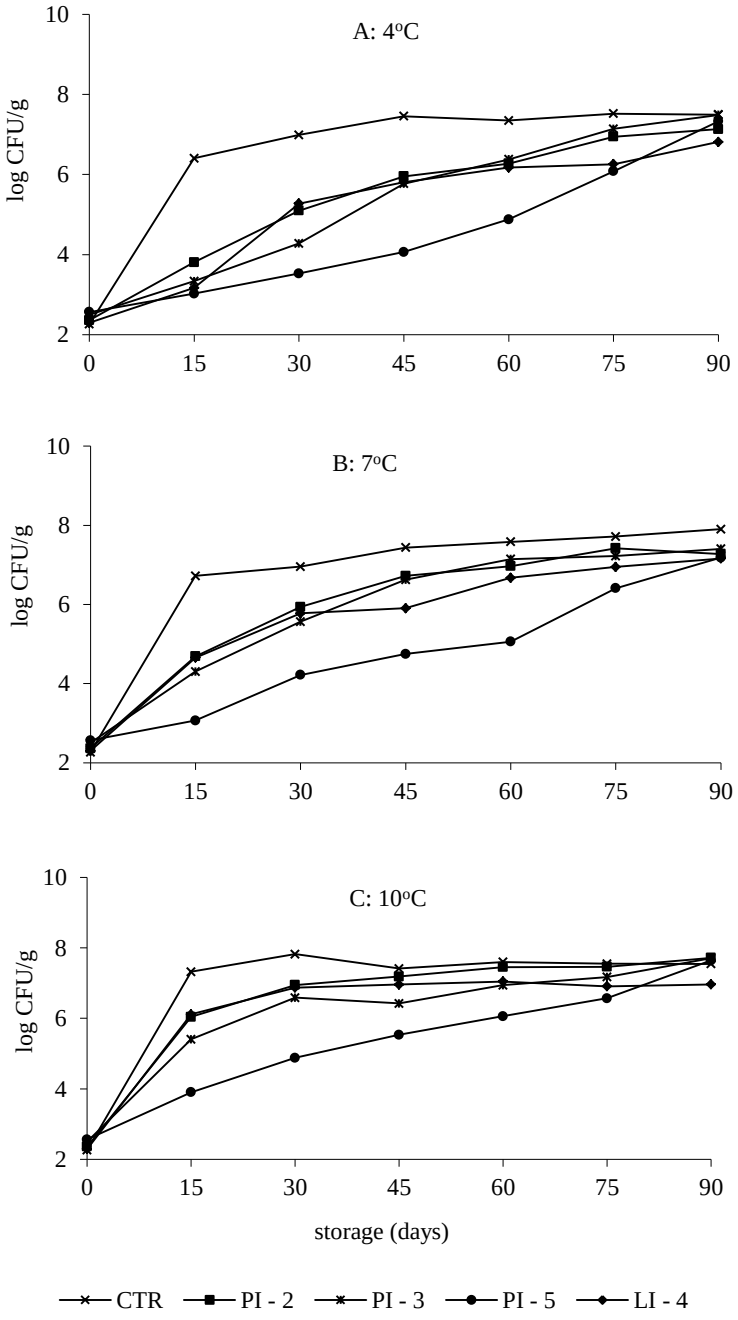

Figure 2.4 Population of mesophilic aerobic bacteria on vacuum-packaged low-sodium frankfurters formulated with powdered or liquid inhibitors[a] during 90 days of storage at 4 (A), 7 (B) and 10°C (C).

[a] Inhibitors in the formulations as in Table 2.3.

2.3.3 Sensory analysis

Most frankfurter formulations were evaluated for consumer acceptance, with the exception of PI-2 (0.5% SL + 0.5% SA) in which the SA concentration exceeded the USDA allowable maximum limit of 0.25% (Cox et al., 1989). No significant differences in appearance, texture, flavor, and overall consumer acceptability were seen for full-sodium frankfurters containing sodium- or potassium-based inhibitors ($P > 0.05$) (Table 2.6); these findings agree with those from three previous studies (Barmpalia et al., 2004; Blom et al., 1997; Lu et al., 2005). Islam et al. (2002) reported significantly lower consumer acceptance scores for frankfurters that were dipped in a 25% SD solution for 1 min (0.3% SD pick-up). However, the strong initial acetic acid odor diminished after 3 days of storage, suggesting that no differences in acceptance scores would be expected thereafter. Using a trained panel, Stekelenburg and Kant-Muermans (2001) found that hams containing 0.2% SD had significantly lower scores for odor and taste than did hams containing lower concentrations of SD (0.1%), SL (3.3%), buffered sodium citrate (1%), or SD (0.1%). Lu et al. (2005) did not observe any sour or meaty off-flavor for frankfurters after 3 min of immersion in a 6% SD solution (0.08% SD pick-up). The SD and PD concentrations used in our study were ≤ 0.156% and ≤ 0.2, respectively, which were at or below the SD pick-up concentration (0.2 to 0.3%), reported to adversely impact sensory attributes.

In low-sodium frankfurters, formulation PI-5 (0.2% PD + 0.8% PA) received a significantly lower score ($P < 0.05$) for flavor and overall acceptability than did the CTR, with a similar texture score (Table 2.7). Unlike full-sodium frankfurters, the lower scores for low-sodium frankfurters were expected because of weaker masking of acetic acid from PD. Similarly, Stekelenburg and Kant-Muermans (2001) reported no adverse sensory attribute for

ham containing 0.1% but not 0.2% SD. However, the potassium salt may have a different flavor. A trained sensory panel noted a significant increase in bitterness when 40 and 50% KCl was used as a substitution for NaCl in fermented sausage (Glass et al., 2002) and marinated chicken breasts (Lee et al., 2012), respectively.

To control post-thermal *Listeria* growth, meat and poultry manufacturers are increasingly incorporating organic salts (e.g., SL and SD) into product formulations. These salts were originally developed in liquid form (60% solute) because they are highly hygroscopic. In the present study, three full-sodium frankfurter formulations developed for *Listeria* inhibitors and containing organic salts as powders with diacetate had properties similar to those of formulations containing liquid inhibitors. Formulations in which organic acids were combined as liquids or powders were more effective against *Listeria* than were formulations with single organic salts, particularly at lower temperatures.

Low-sodium RTE meat products represent a greater risk of listeriosis for consumers due to decreased antilisterial efficacy of organic salts (Glass et al., 2002; Mbandi and Shelef, 2002; Seman et al., 2002). In this study, two low-sodium frankfurter formulations containing powdered inhibitors (PI-3 and PI-5) had similar or greater antilisterial activity than did those containing liquid inhibitors, including 2.5% PL plus SD, when stored at 4, 7, or 10^{o}C for 90 days. Strong inhibition of *Listeria* and MAB was achieved using PA and PD regardless of storage time and temperature. Compared with the low-sodium CTR, the powder-based formulation containing 0.2% PD (PI-5) was similar in appearance and texture but had lower scores for flavor and overall acceptability. Given these findings, powdered organic salts bacterial inhibitors based on PA and PD should provide an attractive alternative to liquid inhibitors for both full- and low-sodium frankfurters.

Table 2.6 Impact[1] of powdered and liquid inhibitors[2] on sensory properties of full sodium frankfurters.

Sodium-based frankfurters[3]	CTR (0%)[4]	PI-1 (0.5%)	PI-3 (0.65%)	LI-1 (2.5%)	LI-2 (2.5%)	Standard Error
Appearance	6.35[a]	6.50[a]	6.44[a]	6.44[a]	6.35[a]	0.19
Texture	6.52[a]	6.49[a]	6.76[a]	6.49[a]	6.37[a]	0.21
Flavor	6.51[a]	6.52[a]	6.80[a]	6.38[a]	6.26[a]	0.21
Overall	6.51[a]	6.43[a]	6.74[a]	6.30[a]	6.15[a]	0.20

Potassium-based Frankfurters[3]	CTR (0%)[4]	PI-4 (0.75%)	PI-5 (1.0%)	LI-3 (2.5%)	LI-4 (2.5%)	Standard Error
Appearance	6.23[a]	6.13[a]	6.27[a]	6.38[a]	6.34[a]	0.12
Texture	6.41[a]	6.29[a]	6.30[a]	6.37[a]	6.35[a]	0.13
Flavor	6.42[a]	6.30[a]	6.26[a]	6.12[a]	6.39[a]	0.14
Overall	6.30[a]	6.12[a]	6.19[a]	6.10[a]	6.33[a]	0.13

[1]Mean values with same letters in the same row were not significantly different ($P \geq 0.05$).
[2]Inhibitors as in Table 2.3.
[3]n = 330 observations.
[4]Amount of inhibitor.

Table 2.7 Impact[1] of powdered and liquid inhibitors[2] on consumer acceptance scores in low sodium frankfurters.

Frankfurters[3]	CTR (0%)[4]	PI-3 (0.5%)	PI-5 (0.65%)	LI-4 (2.5%)	Standard Error
Appearance	6.33[b]	6.52[ab]	6.60[a]	6.56[ab]	0.11
Texture	6.64[a]	6.53[a]	6.36[a]	6.55[a]	0.11
Flavor	6.51[a]	6.50[ab]	6.09[b]	6.41[ab]	0.12
Overall	6.50[a]	6.47[a]	6.07[b]	6.40[ab]	0.12

[1]Mean values with same letters in the same row were not significantly different ($P \geq 0.05$).
[2]Inhibitors as in Table 2.3.
[3]n = 206 observations.
[4]Amount of inhibitor.

2.4 Conclusion

Overall findings in the study demonstrate that various powdered organic acid salts showed either bactericidal or bacteriostatic effects on frankfurters during storage depending on types of inhibitor combinations and the salt concentration. In full-sodium frankfurters, three powdered formulations containing diacetate were equivalent or superior to four liquid formulations for *Listeria* inhibition, especially when potassium acetate was combined with potassium diacetate. Multiple organic salts in the formulations were more effective in *Listeria* inhibition than those containing a single organic salt. In low-sodium frankfurters, the formulation with potassium acetate and potassium diacetate showed the better inhibition against *Listeria* and MAB growth, regardless of storage day and temperature. Given these findings, powdered organic salts based on potassium acetate and potassium diacetate would be an alternative inhibitor to current liquid inhibitors used in RTE meat products. Further research is needed to improve the sensory properties of the applied products while retaining or improving the antimicrobial efficacy of the applied organic acid salts.

CHAPTER 3: ANTILISTERIAL EFFECTS OF DIFFERENT HOP ACIDS IN COMBINATION WITH POTASSIUM ACETATE AND POTASSIUM DIACETATE AT 7 AND 37ºC

3.1 Introduction

In the previous study (Chapter 2), nine organic acid salts from Niacet b.v. (Tiel, The Netherlands) were evaluated for their antilisterial activities in frankfurters and the results indicated that the mixture of 80% potassium acetate and 20% potassium diacetate (PAPD) showed the best antilisterial activity (Sansawat et al., 2013). However, the product containing PAPD had low sensory score compared to that of the control due to its acid flavor. Hop extracts have been reported to possess antimicrobial activity against *L. monocytogenes* (Schmalreck and Teuber, 1975; Todd et al., 1992; Millis et al., 1994; Larson et al., 1996; Bhattacharya et al.,2003; Shen and Sofos, 2008; Shen et al., 2009). As a result, it will be ideal if the negative impact of PAPD is decreased by combining with hop acid, especially through product formulation. Hence, the purpose of this study was to evaluate antilisterial effect and thermal stability of eight hop acid extracts available from Kalsec® Inc. with/without PAPD in trypticase soy broth with yeast extract (TSBYE). The hypothesis of this research is that the combination of hop extracts and organic acid salts will bring synergistic effects in *L. monocytogenes* inhibition.

3.2 Material and methods

3.2.1 Hop acids and potassium acetate/potassium diacetate (PAPD)

Eight different hop acid extracts and one potassium acetate/potassium diacetate (80:20, PAPD) powder were obtained from Kalsec® Inc. (Kalamazoo, WI) and Niacet b.v. (The Netherlands), respectively (Table 3.1). The average concentrations of hop α-acid, β-acid, acid-iso and acid-tetra in the extracts were 67, 96, 78 and 76%, while the concentrations of hop

potassium salts were 55, 38, 30, and 10% for K-rho, K-hexa, K-iso, and K-tetra, respectively (Table 3.1). For the assessment of antilisterial activity, the hop acid extracts were dissolved in 100% ethanol and PAPD was dissolved in sterile distilled water and filter-sterilized (0.22 μm Millex® GS Filter Unit, Carrigiwohill Co., Cork, Ireland).

Table 3.1 Concentrations of eight hop acid extracts and potassium acetate/potassium diacetate.

Materials	Concentration (%)[1]
Alpha acid (α-acid) – Humulone[2]	67.2
Beta acid (β-acid) – Lupulone[2]	96.0
Acid form of isomerized alpha acid (acid-iso)[2]	77.5
Acid form of tetrahydroisoalpha acid (acid-tetra)[2]	75.8
Potassium salt of isomerized alpha acid (K-iso)[3]	30.3
Potassium salt of hexahydroisoalpha acid (K-hexa)[3]	37.8
Potassium salt of tetrahydroisoalpha acid (K-tetra)[3]	9.6
Potassium salt of dihydroisoalpha acid (K-rho)[3]	54.6
Potassium acetate/Potassium diaceate	80/20[4]

[1]The concentrations of hop extracts specified by Kalsec® Inc.
[2]The remainder (or carrier) is primarily non-characterized resinous material including tannins, fats, polymers, hop acid by-products, hydrophobic substances, and moisture due to the removal of the solvent to FDA trace limits.
[3]The remainder (or carrier) is mostly water due to the extraction into aqueous solutions.
[4]The mixture of 80% potassium acetate and 20% potassium diacetate prepared by Niacet b.v.

3.2.2 *L. monocytogenes* strains and inoculum preparation

The six-strain *L. monocytogenes* cocktail containing ~1 x 10^8 CFU/mL was prepared and confirmed the *L. monocytogenes* population in the inoculum as explained in Chapter 2.

3.2.3 Antilisterial activity of eight hop extracts at 37°C

All of eight hop extracts were individually dissolved in 100% ethanol and added to TSBYE to achieve a concentration of 50 ppm (w/v). For control, the same amount of ethanol was added without hop acid and PAPD. After inoculating with the six-strain *L. monocytogenes* cocktail at 37°C for 24 h, appropriate serial dilutions in PBS were plated on TSAYE to enumerate *L. monocytogenes*.

3.2.4 Determination of synergistic effect of hop acid/PAPD mixtures on inhibition of *L. monocytogenes* at 37°C

Hop acids (25 ppm) with/without 0.5% PAPD were separately added to TSBYE in test tubes (16 x 150 mm Pyrex® Culture Disposable Tube, Corning Incorporated, Corning, NY). For control, the same amount of ethanol was added without hop acid and PAPD. All tubes were inoculated with *Listeria* for approximately 5.0 – 6.0 log CFU/mL. The resulting solutions as well as the 0.5, 1, and 0 (control) % PAPD were incubated at 37°C for 24 h. The *Listeria* counts were enumerated by plating appropriate dilutions in PBS on TSAYE and incubating at 37°C for 22 - 24 h. The synergistic effect of hop/PAPD combination was determined by calculating the combination index (CI) (adapted from Chou and Talalay, 1983), which was the result of sum of log-reduction (comparing to initial inoculation) from individual treatment dividing by log-reduction from combination treatment. The results were interpreted as synergistic (CI < 1), additive (CI = 1), and antagonistic (CI > 1).

3.2.5 Antilisterial activity of heated hop extracts

Hop acids at 25 ppm with/without 0.5% PAPD were separately added to TSBYE in test tubes. During 30 min of heating in a 85^{o}C water bath, these tubes were removed at 5 min intervals, immediately placed in ice slurry for chilling to 37^{o}C and inoculated with *Listeria* for approximately 5.0 – 6.0 log CFU/mL. The resulting solutions as well as the 1, 0.5, and 0 (control) % PAPD were incubated with the same amount of ethanol that was used to solubilize hop acids. For antilisterial activity, appropriate dilutions were plated on TSAYE followed by 22 - 24 h incubation at 37°C.

3.2.6 Minimal inhibitory concentrations (MIC) of hop extractions in TSBYE

Different concentrations of the five hop acids (α-acid, β-acid, acid-tetra, K-tetra, and K-hexa) ranging from 0 to 25 ppm were added to TSBYE. The uninoculated control and five hop extracts inoculated with *L. monocytogenes* at 5.0 – 6.0 log CFU/mL were incubated, after which optical density (O.D.) was measured at 600 nm to determine the MIC.

3.2.7 Antilisterial activity of hop extracts and PAPD mixtures at 7^{o}C

To assess antilisterial activity at 7^{o}C for 6 days, TSBYE was prepared to contain 5 ppm of each of the five hop acids (α-acid, β-acid, acid-tetra, K-tetra, and K-hexa) with/without 0.5% PAPD, in addition to 1% PAPD, 0.5% PAPD and an inhibitor-free control. The temperature of 7^{o}C was chosen to represent the condition of temperature abuse during storage of foods including meats. After inoculating with *L. monocytogenes* (3.0 – 4.0 log CFU/mL), the samples were plated daily for 6 days on TSAYE agar to assess antilisterial activity.

3.2.8 Statistical analysis

The microbiological data from triplicate experiments were converted to log CFU/mL. An analysis of variance (ANOVA) was performed using the mixed procedure of SAS software (SAS Institute, 2002). The slope of graph of *Listeria* population during storage at 7^{o}C for 6 days for each treatment (growth rate) was calculated to better assess the effect of treatment on *Listeria* inhibition. Statistically significant differences between the treatments were determined using Tukey's Test at $\alpha = 0.05$.

3.3 Results and discussion

3.3.1 Inhibitory activity of hop acids against *L. monocytogenes* at 37°C

When TSBYE containing each of eight hop extracts at 50 ppm was inoculated with the six-strain *L. monocytogenes* cocktail at 5.9 log CFU/mL and incubated at 37°C for 24 h, the pathogen was below the detection limit of 1 log CFU/mL in hop α-acid, with significantly lower populations (3.6 ~ 3.8 log CFU/mL) seen for β-acid, acid-tetra, K-tetra, and K-hexa. However, *Listeria* grew to 8.6 to 8.9 log CFU/mL in TSBYE containing hop acid-iso, K-iso and K-rho, which was similar to the control ($P > 0.05$) (Fig. 3.1). King and Ming (2002) also reported listericidal effect at 50 ppm hop β-acids in trypticase soy broth (TSB) with the population decreased by 4 logs. In addition, Larson et al. (1996) and Milles and Schendel (1994) reported that *Listeria* was completely inhibited by ≥ 10 ppm hop β-acids in TSB and brain-heart broth, respectively, whereas iso-α-acids showed little inhibition.

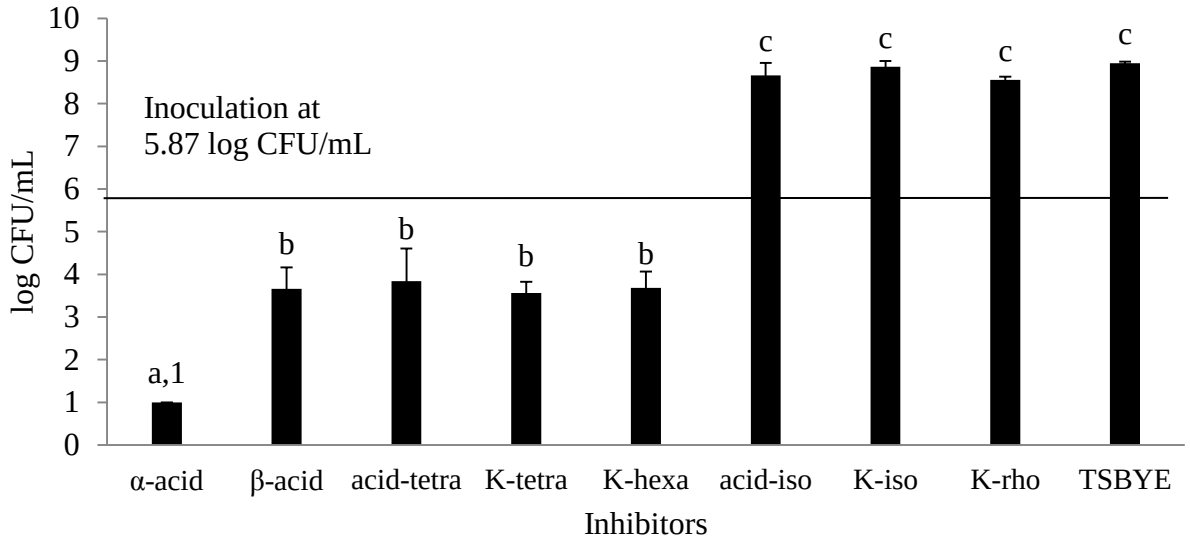

Figure 3.1 Effect of 50 ppm hop acid extracts on *Listeria monocytogenes* counts in TSBYE broth after incubation at 37°C for 24 h.
[a-c] Bars with same letters were not significantly different ($P \geq 0.05$).
[1] No viable *L. monocytogenes* detection was marked as 1.0 log CFU/mL.
(The minimum detectability of the methodology was > 10 cells per milliliter).

3.3.2 Synergistic effect of hop acid/PAPD mixtures on inhibition of *L. monocytogenes* at 37°C

To evaluate the potential synergistic effects of hop acid/PAPD mixtures, five hop extracts (α-acid, β-acid, acid-tetra, K-tetra, and K-hexa) were selected based on the test results at 50 ppm (Fig. 3.1). When the mixtures of 25 ppm hop acid/0.5% PAPD were incubated at 37°C with *Listeria* at 5.7 log CFU/mL, the pathogen was no longer detected (below detection limit of 1 log CFU/mL) in α-acid/PAPD, β-acid/PAPD, and acid-tetra/PAPD after 24 h, whereas trace levels (1.4 – 2.0 log CFU/mL) of *Listeria* were seen in K-tetra/PAPD and K-hexa/PAPD (Fig. 3.2). In case of single addition, a significant listericidal effect was observed in α-acid by reducing the *Listeria* populations from 5.7 to 1.8 log CFU/mL, with reduction to 3.6 – 4.2 log CFU/mL in β-acid, acid-tetra, K-tetra, K-hexa, 0.5% PAPD and 1% PAPD (Fig. 3.2). No inhibitor control allowed the *Listeria* to grow to 9.2 log CFU/mL. The synergistic effect in *Listeria* inhibition was found when hop acid was used in combination with PAPD except α-acid/PAPD (Table 3.2) (See CI calculation in appendix B). However, no synergism from α-acid/PAPD combination was observed potentially due to the limit detection of the method.

These results support previous findings of complete *Listeria* inhibition in TSBYE at 4°C when 3 ppm hop β-acid was combined with 1.0% potassium lactate, 0.25% sodium diacetate, or 0.1% acetic acid (Shen and Sofos, 2008). Seman et al. (2004) also reported that *Listeria* populations decreased from 4.3 log CFU/package to undetectable when hot dogs were dipped in an antibacterial solution containing 20,000 ppm hop β-acid, 0.3 M potassium lactate, and 0.3% lactic acid in polypropylene glycol.

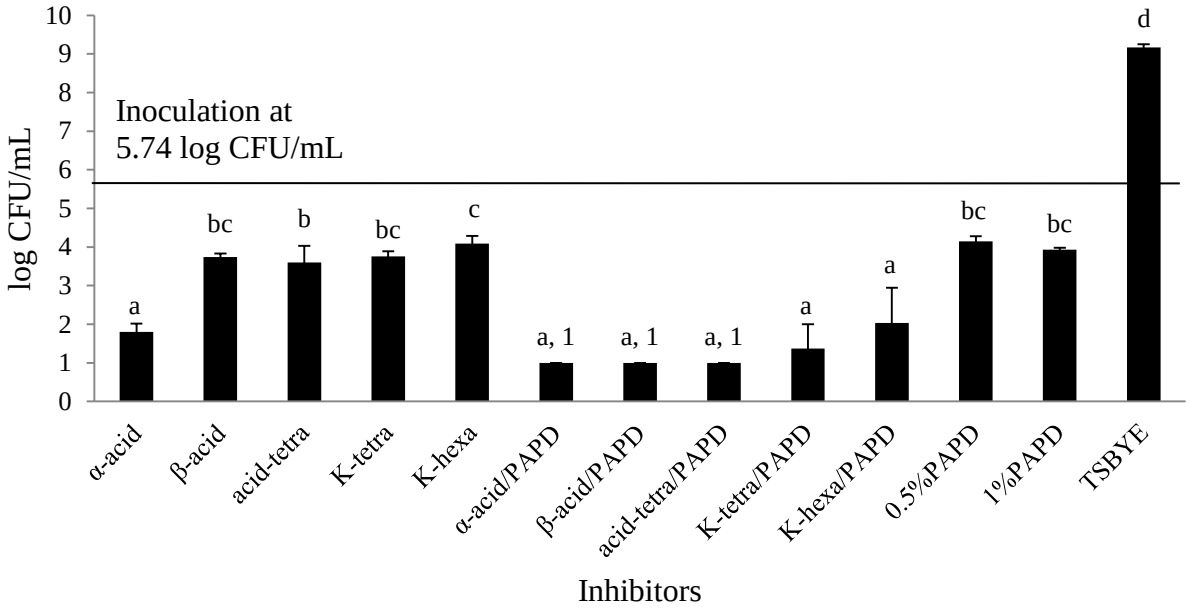

Figure 3.2 Effect of 25 ppm hop acid extracts, 0.5 and 1% PAPD[2], and mixtures of 25 ppm hop acid extracts/0.5% PAPD on *Listeria monocytogenes* counts in TSBYE broth after incubation at 37°C for 24 h.

[a-d]Bars with same letters were not significantly different ($P \geq 0.05$).

[1]No viable *L. monocytogenes* detection was marked as 1.0 log CFU/mL. (The minimum detectability of the methodology was > 10 cells per milliliter).

[2]The mixture of 80% potassium acetate and 20% potassium diacetate.

Table 3.2 Interpretation[1] effects of 25 ppm hop acid extract/0.5% PAPD[2] mixtures on *Listeria monocytogenes* counts in TSBYE broth after incubation at 37°C for 24 h.

Treatment	Log-reduction from hop acid or PAPD alone	Log-reduction from hop/PAPD	Sum of log-reduction from hop acid alone and PAPD alone	CI[3]	Interpretation
α acid	3.94	4.74	5.53	1.17	Antagonistic
β acid	2.00	4.74	3.59	0.76	Synergistic
Acid-tetra	2.14	4.74	3.73	0.79	Synergistic
K-tetra	1.98	4.37	3.57	0.82	Synergistic
K-hexa	1.65	3.70	3.24	0.86	Synergistic
PAPD	1.59	-	-	-	-

[1]Data was from Fig. 3.2.
[2]The mixture of 80% potassium acetate and 20% potassium diacetate.
[3]Combination index.

3.3.3 Thermal stability of hop acid with/without PAPD at 85°C

Thermal stability of hop acids is critically important if the hop inhibitor is formulated to meat batters to prevent *Listeria* growth after cooking. To evaluate the heat stability, α-acid, β-acid, acid-tetra, K-tetra, and K-hexa were dissolved in 100% ethanol, diluted to 25 ppm with/without 0.5% PAPD, and submerged to a water bath at 85°C for up to 30 min.

Initially and at 5 min interval, tubes were removed, immediately cooled to 37°C, and inoculated with *Listeria* at 5.0 log CFU/mL. When the resulting samples were incubated overnight at 37°C, *Listeria* populations decreased to 2.0 and 2.9 log CFU/mL in α-acid when heated for 10 and 30 min at 85°C, respectively. *Listeria* populations in the remaining hop acids decreased to 3.5 – 3.8 log CFU/mL, regardless of the heating time (Table 3.2). When PAPD and hop acid were mixed, *L. monocytogenes* was not detected regardless of the heating time, except K-tetra/PAPD and K-hexa/PAPD, which reduced the pathogen to 1.2 – 2.1 log CFU/mL (Table

3.2). Number of *Listeria* inoculum was decreased to 3.9 – 4.0 and 3.5 – 3.6 log CFU/mL in 0.5 and 1% PAPD, respectively, regardless of the heating time, whereas the control allowed *Listeria* to grow to 9.4 log CFU/mL after incubation at 37^{o}C for 24 h.

Table 3.3 Population of *L. monocytogenes*[1] in TSBYE[2] containing 25 ppm hop acid extracts with/without 0.5% PAPD[3] after heating at 85°C.

Treatment	Populations of *L. monocytogenes*[4] (log CFU/mL) in TSBYE cooked various time (min)						
	Min 0	Min 5	Min 10	Min 15	Min 20	Min 25	Min 30
α acid	1.92 ± 0.24 [a]	1.84 ± 0.16 [a]	1.96 ± 0.36 [ab]	2.19 ± 0.28 [abc]	2.76 ± 0.51 [bc]	3.01 ± 0.03 [c]	2.87 ± 0.20 [c]
β acid	3.54 ± 0.38	3.50 ± 0.32	3.59 ± 0.24	3.67 ± 0.19	3.81 ± 0.18	3.83 ± 0.11	3.75 ± 0.17
Acid-tetra	3.46 ± 0.27	3.50 ± 0.17	3.57 ± 0.11	3.51 ± 0.17	3.46 ± 0.13	3.63 ± 0.13	3.58 ± 0.13
K-tetra	3.64 ± 0.07	3.65 ± 0.08	3.65 ± 0.08	3.63 ± 0.07	3.63 ± 0.09	3.55 ± 0.09	3.61 ± 0.04
K-hexa	3.58 ± 0.06	3.56 ± 0.09	3.56 ± 0.01	3.53 ± 0.02	3.51 ± 0.05	3.51 ± 0.14	3.50 ± 0.13
α acid + PAPD	ND[5]	ND	ND	ND	ND	ND	ND
β acid + PAPD	ND	ND	ND	ND	ND	ND	ND
Acid tetra + PAPD	ND	ND	ND	ND	ND	ND	ND
K-tetra + PAPD	1.20 ± 0.07	1.38 ± 0.35	1.53 ± 0.10	1.37 ± 0.14	1.39 ± 0.16	1.48 ± 0.03	1.66 ± 0.04
K-hexa + PAPD	1.35 ± 0.33	1.69 ± 0.27	1.76 ± 0.19	1.70 ± 0.43	1.99 ± 0.36	2.06 ± 0.66	2.08 ± 0.68
0.5%PAPD	3.88 ± 0.20	3.94 ± 0.16	3.94 ± 0.10	3.94 ± 0.08	3.94 ± 0.18	3.99 ± 0.18	3.97 ± 0.16
1%PAPD	3.48 ± 0.06	3.43 ± 0.10	3.43 ± 0.10	3.48 ± 0.14	3.54 ± 0.12	3.49 ± 0.12	3.61 ± 0.13
TSBYE	9.41 ± 0.14						

[1]*Listeria* inoculated with 5.01 ± 0.53 log CFU/mL. [2]TSBYE, Trypticase soy broth with yeast extract.
[3]PAPD, Potassium acetate and potassium diacetate. [4]Means ± standard deviation of $n = 6$ observations for each reading. [5]ND, Not detected.
[a-c]Mean values with same letters in the same row were not significantly different ($P \geq 0.05$).

3.3.4 Minimal inhibitory concentrations of hop acids against *Listeria* growth at 37°C

When *Listeria* inoculum (5.1 log CFU/mL) was incubated at 37°C for 24 h, optimal density (O.D.) at 600 nm increased to 0.8. Using the method, minimal inhibitory concentrations (MIC) against *Listeria* were determined with no increase of O.D. The MIC was 6.3 ppm for α-acid, β-acid, acid-tetra, with 12.5 ppm seen for K-tetra and K-hexa (Table 3.4). These results are consistent with a previously reported MIC of ~ 6 ppm for hop β-acid (Millis and Schendel, 1994; Barney et al., 1995).

Table 3.4 Minimal inhibitory concentrations (ppm) of hop acid extracts on *L. monocytogenes*[1] growth.

Treatment	Growth of *L. monocytogenes* (O.D.)[2]						
	Hop acid concentration (ppm)						
	25	12.5	6.3	3.1	1.6	0.8	0
α-acid	0.000	0.000	0.000	0.005	0.432	0.530	0.799
β-acid	0.000	0.000	0.000	0.004	0.008	0.392	0.799
acid-tetra	0.000	0.000	0.000	0.004	0.547	0.716	0.799
K-tetra	0.000	0.000	0.010	0.546	0.712	0.748	0.799
K-hexa	0.000	0.000	0.336	0.704	0.755	0.761	0.799

[1] Initial inoculation of *L. monocytogenes* was 5.11 ± 0.28 log CFU/mL.
[2] O.D. Optical density at 600 nm: the growth of *Listeria* in TSBYE after incubation at 37°C for 24 h.

3.3.5 Effect of hop acid/PAPD mixtures on inhibition of *L. monocytogenes* at 7°C

In study 3.3.2, antilisterial activities of hop acids at 25 ppm, PAPD and their combinations were evaluated in TSBYE at 37°C. To meet the USDA allowance for hop acid (USDA-FSIS, 2013) and to simulate the condition of temperature abuse during food storage, the antilisterial activity of hop acids at 5 ppm were evaluated with/without 0.5% PAPD during six days of storage at 7°C. In addition to the hop acids and hop acid/PAPD mixtures, 1% PAPD, 0.5% PAPD and inhibitor-free control were included. All mixtures of 0.5% PAPD/5 ppm hop acid and single addition of 1% PAPD, 0.5% PAPD, and 5 ppm β-acid showed listeristatic effect with similar slope of *Listeria* growth curve ($P \geq 0.05$) (Fig. 3.3). However, the remaining hop acids and control allowed the pathogen to grow from 3.8 log CFU/mL to 5.6 – 7.3 log CFU/mL at the end of storage (Fig. 3.3 and Table A.7).

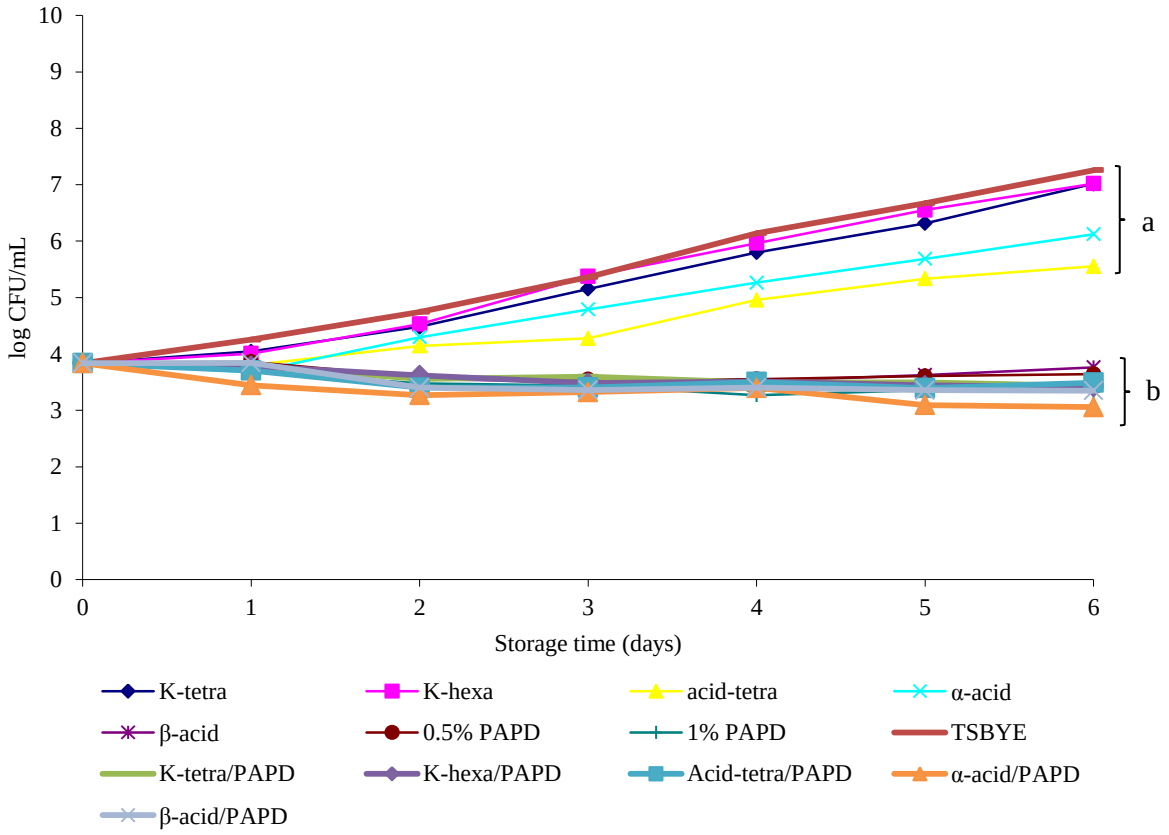

Figure 3.3 Population of *L. monocytogenes* in TSBYE with or without different hop acid extracts at 5 ppm, 0.5 or 1% PAPD, and mixtures of 5 ppm hop acid extracts/0.5% PAPD during 6 days of storage at 7°C.
[a-b] Slope of graphs with same letters were not significantly different ($P \geq 0.05$).

The comparison of antilisterial effects between hop acids at 25 ppm/37°C and hop acids at 5 ppm/7°C, with/without 0.5% PAPD, led to two interesting observations. Firstly, β-acid was less effective than α-acid at 37°C while the opposite was true at 7°C. The different activity at two different temperatures can be explained by the shorter shelf-life of hop β-acid at elevated temperatures, presumably due to oxidation. Using HPLC and well diffusion assay, Seman et al. (2004) demonstrated that photo-oxidation decreased the antilisterial activity of hop β-acid compared to that with antioxidants. Using chelating or antioxidant agents, King and Ming (2002) also reported stronger antilisterial activity (more than 2 logs) of hop β-acid compared to

hop β-acid alone at 30°C for 48 h in trypticase soy broth. Regardless of incubation temperature, however, the mixtures of hop acid and PAPD showed consistent listericidal effect.

Secondly, the combination of 0.5% PAPD /25 ppm hop acid showed stronger listericidal activity at 37°C compared to single application of hop acids or PAPD except α-acid (Fig. 3.2). Interestingly, no synergistic effect was found using the combination of 0.5% PAPD/5 ppm hop acid at 7°C (Fig. 3.4). These results could be explained if the amount (5 ppm) of hop acids was not sufficient to generate inhibition or synergistic effects at 7°C. Larson et al. (1996) reported that *Listeria* growth was similar in the skim milk control and the milk containing 1 and 10 ppm hop β-acid during 30 days of storage at 4°C. A listericidal effect was seen only when the addition of hop β-acid was increased to 100 and 1,000 ppm.

3.4 Conclusion

In evaluation of *Listeria* inhibition, five (α-acid, β-acid, acid-tetra, K-tetra, and K-hexa) out of 8 hop extracts were more antilisterial than the remaining three (acid-iso, K-iso, and K-rho). The minimal inhibitory concentration of hop acids was < 6.3 ppm for α-acid, β-acid, and acid-tetra, with < 12.5 ppm seen for K-tetra, and K-hexa. Among the five hop extracts, α-acid was most inhibitory against *L. monocytogenes* at 37^{o}C regardless of heating at 85^{o}C, whereas β-acid demonstrated the best antilisterial activity at 7^{o}C. PAPD alone inhibited *Listeria* more effectively at 7 than at 37^{o}C, probably due to the high growth rate of *Listeria* at 37^{o}C. Regardless of incubation temperature and addition amount, the mixtures of hop acid/PAPD resulted in more robust inhibition than did any single addition. At 25 ppm, five hop acids (α-acid, β-acid, acid-tetra, K-tetra, and K-hexa) showed listericidal activity at 37^{o}C, whereas the hop acids at 5 ppm allowed *Listeria* to grow at 7^{o}C, except β-acid, potentially due to insufficient amount. Based on this findings, the single addition of hop acids at 5 ppm appears to be not sufficient, except β-acid, to inhibit *Listeria* at 7^{o}C while the mixture of hop/PAPD provide a better inhibition.

CHAPTER 4: INHIBITION OF *LISTERIA MONOCYTOGENES* IN DELI-STYLE TURKEY AND MILK USING HOP ACID EXTRACTS WITH OR WITHOUT POTASSIUM ACETATE AND POTASSIUM DIACETATE

4.1 Introduction

Hop acids have long been known for antimicrobial activity against Gram positive bacteria (Sakamoto and Konings, 2003). Previously, the antimicrobial activity of hop acids has predominantly been assessed in liquid media or on the foods after surface application rather than after product formulaiton. More specifically, no research has been conducted to evaluate antimicrobial activity of hop or hop/organic acid combinations in processed meat formulation. Although RTE meat products are fully cooked, the additional contamination may occur during post-thermal handling and storage. Therefore, this study was designed to assess antilisterial activity of hop or hop/organic acid combinations in the practical situation at manufacture plants and at deli stores or at homes (Fig. 4.1). After manufacture of product, the time required for deli meat distribution to retail store is about 10 to 30 days (USDA-FSIS, 2003c). After delivered to retail stores, the deli meat is usually displayed for 5 to 30 days depending on sale (Personal interview). After purchase, more than 75% of consumers are likely consuming deli meats within 1 to 10 days (AMI, 2000).

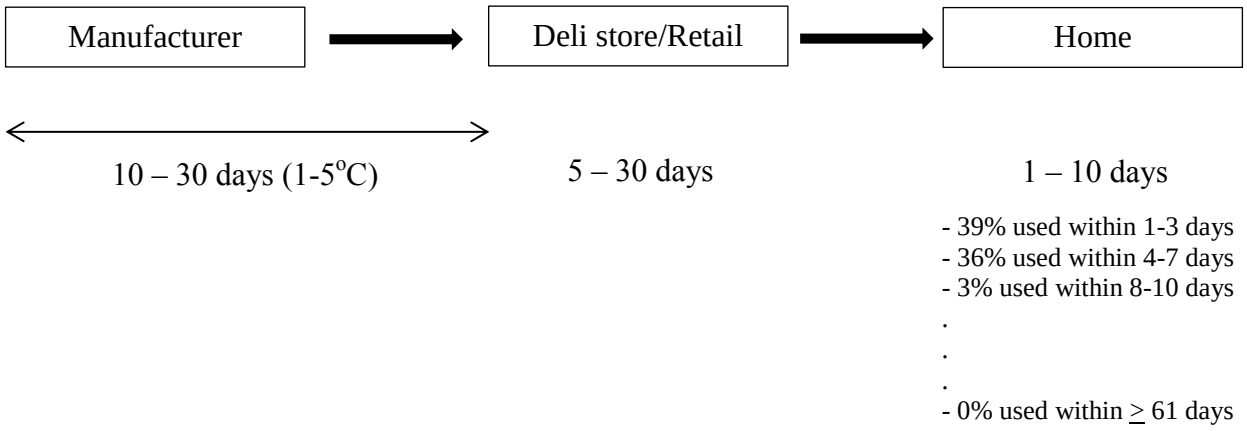

Figure 4.1 Expected times for production, distribution, and consumption of delicatessen meats.

In our previous study (chapter 3), the combination of hop acid and PAPD demonstrated additive or synergistic effects on *Listeria* inhibition in liquid media. Therefore, the objective of this study was to evaluate the antilisterial activity of hop extracts with/without PAPD in deli-style turkey meat. The hypothesis of this research is that that the mixture of hop acid and PAPD effectively inhibits *Listeria* in deli-style turkey meat not only upon production but also during the distribution and storage.

4.2 Material and methods

4.2.1 Deli-style turkey preparation with/without *Listeria* inhibitors

Turkey breasts and ingredients required for deli-style turkey were obtained locally. Hop acid extracts and PAPD were obtained from Kalsec Inc. (Kalamazoo, MI) and Niacet b.v. (Tiel, The Netherlands), respectively, while OptiForm was purchased from PURAC America, Inc (Lincolnshire, IL). Deli turkey meat was traditionally manufactured at the Michigan State University (MSU) Meat Laboratory (East Lansing, MI), using the following formuation: turkey breast (71.84%), water (20.44-21.94%), salt (1.68%), phosphate (0.36%), starch (2.50%), sugar

(1.44%), sodium nitrite (0.18%), erythorbate (0.0578%) and inhibitor (0-2.5%). Seven *Listeria* inhibitors were prepared as previously described and tested as follow: (1) inhibitor-free control (CTR); (2) 2.5 % OptiForm® solution of 56% potassium lactate, 4% sodium diacetate, and 40% water (PLSD); (3) 0.5% powdered mixture of potassium acetate (80%) and potassium diacetate (20%) (PAPD); (4) 5 ppm hop alpha acid (α-acid, containing 67.2% α-acid); (5) 5 ppm α-acid and 0.5% PAPD (α-acid/PAPD); (6) 5 ppm hop beta acid (β-acid, containing 96.0% β-acid); and (7) 5 ppm β-acid and 0.5% PAPD (β-acid/PAPD).

Turkey breast meat was ground and mixed with the required ingredients in a bowl chopper (model K64-Va, Maschinenfabrik Seydelmann KG, Aalen, Germany) under vacuum for 8 min. The meat batter was stuffed into fibrous casings (90 mm; Devro-Teepak Inc., Danville, IL) and cooked to an internal temperature of 74^{o}C in a smoke-free smokehouse. After cooking and showering, the deli turkey chubs were stored overnight at 2^{o}C and sliced for physicochemical and microbial analyses.

4.2.2 Physicochemical analysis of deli turkey meat

Deli turkey meat was analyzed for pH, water activity (a_w), moisture, fat, and cooking yield. For pH, a 5-g sample was homogenized in 25 mL of deionized water and then pH was measured with a meter (Accumet AR15, Fisher Scientific, Pittsburgh, PA) equipped with a pH electrode (model 13-620-631, Fisher Scientific, Houston, TX). Water activity (a_w) was determined with an AquaLab meter (Decagon Devices, Pullman, WA). Moisture and fat contents were determined with drying oven (model Yamato DX 400, Yamato Scientific. Ltd., Tokyo, Japan), and fat extractor (Soxtec System HT6, Tecator AB, Höganäs, Sweden), respectively, according to the AOAC International official methods 950.46B and 991.36,

respectively (AOAC, 2005). The cooking yield of deli turkey was determined based on the weight difference before and after cooking.

4.2.3 *Listeria monocytogenes* strains and inoculum preparation

The cocktail of six *L. monocytogenes* strains was prepared to contain ~1 x 10^8 CFU/mL as explained in Chapter 3. The cocktail was then serially diluted in sterile phosphate-buffered saline (PBS; pH 7.4) to a level of approximately 10^5 CFU/mL of *L. monocytogenes* for deli meat inoculation. The *L. monocytogenes* population in the inoculum was enumerated by plating appropriate dilutions on Trypticase soy agar with yeast extract (TSAYE) followed by 24 h incubation at 37°C.

4.2.4 Deli turkey meat inoculation

Listeria inoculation of the deli turkey meat was conducted in two different ways to simulate contamination in the plant during manufacture and at retail delis or in the home. For contamination during manufacture, the deli meats were sliced (approximately 1.5 mm thick and 25 $\pm$ 1 g weight) with a mechanical delicatessen slicer (model 410, Hobart, Troy, OH) and spot inoculated at several locations on one side with 0.1 mL to obtain 2-3 log CFU/g. The slices were then placed in a biological safety cabinet for 20 min to allow the inoculum absorb. Four slices were placed in each bag (18 by 30 cm; VacMaster, Kansas city, MO), vacuum packaged (Multivac, Sepp Haggenmueller GmbH & Co. KG., Wolfertschwenden, Germany), and stored at 4 and 7^{o}C. For contamination at retail delis or at home, the cooked chubs were first stored for 30 and 60 days 4^{o}C, and then sliced and inoculated as above. Four slices were placed on a piece of

delicatessen paper (20 x 27 cm; Brown Paper Goods, Waukegan, IL), aseptically transferred to a zip lock delicatessen bag (20 x 25 cm; Elkay Plastics), and stored at 4 and 7°C for 10 days.

4.2.5 Microbiological analysis

Turkey slices from the day of manufacture were assessed for initial populations of *L. monocytogenes*, and then tested at 15 day intervals for up to 60 days. Slices prepared after 30 and 60 days of storage were analyzed for the pathogen initially and every 2 days up to 10 days. For each treatment, duplicate 25-g samples were diluted 1:10 in PBS and homogenized in a stomacher (NEUTEC Group, Farmingdale, NY) for 2 min. Appropriate serial dilutions in PBS were plated on modified Oxford agar (MOX) (Difco, BD) to enumerate *L. monocytogenes* after incubation for 48 h at 37°C.

4.2.6 Antilisterial activity of hop extracts and PAPD mixtures in milk at 7°C

The antilisterial activity of hop acids was assessed in skim milk and 2% milk. Both skim milk and 2% milk (Meijer, Grand Rapids, MI) were purchased locally, to which hop α-acid, β-acid, acid-tetra, K-tetra, and K-hexa were added separately with/without 0.5% PAPD as well as 0% (control) and 0.5% PAPD. These samples were inoculated with the six-strain *L. monocytogenes* cocktail so as to contain approximately 3.0 – 4.0 log CFU/mL and incubated at 7°C for 6 days. The *L. monocytogenes* populations were then enumerated daily by plating an appropriate serial dilution in PBS on MOX and incubating at 37°C for 48 h.

4.2.7 Statistical analysis

All experiments were conducted in triplicates. The microbiological data were converted to log CFU/g (for deli meat) or log CFU/mL (for milk) and analyzed using the mixed procedure of SAS software (SAS Institute, 2002). To better assess the effect of treatment on *Listeria* inhibition, the slope of graph of *Listeria* population during storage for each treatment (growth rate) was calculated. Mean differences of *L. monocytogenes* population or slope of the graph between treatments were determined using Tukey's Test at $\alpha = 0.05$ level. For the comparison of skim milk and 2% milk, the student *t* test was used for a paired-wise comparison for *Listeria* growth.

4.3 Results and discussion

4.3.1 Physicochemical properties of deli turkey meat

Deli-style turkeys containing 7 inhibitors including the control were assessed for pH, a_w, moisture, fat, and cooking yield. No significant differences were found among the seven inhibitor treatments regardless of the evaluation parameter ($P \geq 0.05$, Table 4.1). The pH and a_w ranged from 6.26 to 6.30 and from 0.966 to 0.972, respectively, which were similar to the values found previously in deli turkey meats prepared with/without common organic acid mixture (Zhang et al., 2012). Shen et al. (2009) also reported that dipping frankfurters in 0.03 – 0.10% hop β-acid solutions did not significantly change the pH and a_w values compared to undipped control. In general, our results indicate that the six antimicrobial agents did not affect ($P \geq 0.05$) physicochemical properties of deli-style turkey.

Table 4.1 Physicochemical properties of deli-style turkey meat

Inhibitor \Parameter [1]	pH	a_w	Moisture (%)	Fat (%)	Cooking yield (%)
CTR	6.30 ± 0.04	0.968 ± 0.007	72.96 ± 0.91	0.97 ± 0.37	85.93 ± 2.42
PLSD	6.30 ± 0.04	0.966 ± 0.005	71.34 ± 1.23	1.01 ± 0.41	87.02 ± 3.48
PAPD	6.27 ± 0.05	0.970 ± 0.003	71.69 ± 1.61	0.97 ± 0.47	85.04 ± 3.33
α-acid	6.30 ± 0.06	0.971 ± 0.005	71.71 ± 0.45	0.97 ± 0.41	83.90 ± 0.92
α-acid/PAPD	6.29 ± 0.04	0.970 ± 0.001	72.31 ± 1.66	0.99 ± 0.44	84.78 ± 0.44
β-acid	6.30 ± 0.06	0.972 ± 0.004	72.94 ± 1.33	0.99 ± 0.38	86.47 ± 0.03
β-acid/PAPD	6.26 ± 0.07	0.972 ± 0.005	72.82 ± 1.09	1.01 ± 0.36	85.63 ± 0.91

[1]Number of observations for each parameter per inhibitor, n = 9 except for cooking yield (n = 2).
CTR: Inhibitor-free control.
PLSD: 2.5% of potassium lactate (56%)/sodium diacetate (4%)/water (40%).
PAPD: 0.5% of potassium acetate (80%)/potassium diacetate (20%).
α-acid: 5 ppm of hop α-acid.
α-acid/PAPD: 5 ppm α-acid/0.5% PAPD.
β-acid: 5 ppm of hop β-acid.
β-acid/PAPD: 5 ppm β-acid/0.5% PAPD.

4.3.2 *L. monocytogenes* on deli turkey meat and in milk

Spot inoculation resulted in *L. monocytogenes* populations of 2.28 to 2.59 log CFU/g in deli-style turkey (Fig. 4.2 and Table A.8). Deli-style turkey formulated with PLSD, PAPD, β-acid/PAPD, or α-acid/PAPD showed similar trends for *Listeria* growth ($P \geq 0.05$), inhibiting the growth of *L. monocytogenes* for 60 days during storage at 4°C (Fig. 4.2). However, the addition of α- or β-acid allowed the pathogen populations to increase > 4.5 log CFU/g, which was not significantly different from the control ($P < 0.05$) (Fig. 4.2 and Table A.8).

When stored at 7°C for 60 days, again, the trends of *Listeria* growth for PLSD, PAPD, β-acid/PAPD, or α-acid/PAPD were similar ($P \geq 0.05$) (Fig. 4.2). Both β-acid/PAPD and α-acid/PAPD allowed *Listeria* to grow < 2.0 log CFU/g, whereas increase of 2.2 to 3.2 and 5.6 to 5.8 log were seen for two organic acid mixtures (PAPD and PLSD) and two hop acid extracts (α- and β-acid), respectively, with a 5.9 log increase seen for the inhibitor-free control (Fig. 4.2 and Table A.8). When used individually, Bedie et al. (2001) reported that 6% sodium lactate and 0.5% sodium diacetate were listeristatic or listericidal in frankfurters during 120 days of storage, while half the concentration prevented growth of *L. monocytogenes* for 50 to 70 days. When used organic acid salts, our previous research showed that five of nine organic acid mixtures were listericidal on frankfurters during 90 days of storage at 4°C, whereas only one (PAPD) of five organic acid mixtures maintained listericidal activity when the storage temperature was increased to 7°C (Fig. 2.1). Similarly, Blom et al (1997) demonstrated that a mixture of 2.5% sodium lactate and 0.25% sodium acetate inhibited the growth of *L. monocytogenes* in sliced cooked ham throughout 5 weeks of storage at 4°C, but only 2 to 3 weeks at 9°C.

According to the USDA-FSIS definition for an antimicrobial agent (USDA-FSIS, 2003), such an antimicrobial agent is a substance that effectively reduces, eliminates, or suppresses

microbial growth throughout the shelf life of the products. As a result, the agent should allow no more than 2 logs of growth during the product's shelf life. Therefore, our results suggest that the combination of 5 ppm α-acid/0.5% PAPD and 5 ppm β-acid/0.5% PAPD could be used under USDA-FSIS alternatives 1 or 2 to inhibit *L. monocytogenes* in ready-to-eat meat products during the storage at 7^{o}C. One of the interesting results found in this research is that both PLSD and PAPD were very effective in inhibiting *Listeria* at 4^{o}C but not at 7^{o}C. At 7^{o}C, the mixture of β-acid/0.5% PAPD was the most effective with the intermediate seen for α-acid/0.5% PAPD, PAPD, and PLSD, followed by no inhibition in α-acid, β-acid, and control (Fig. 4.2).

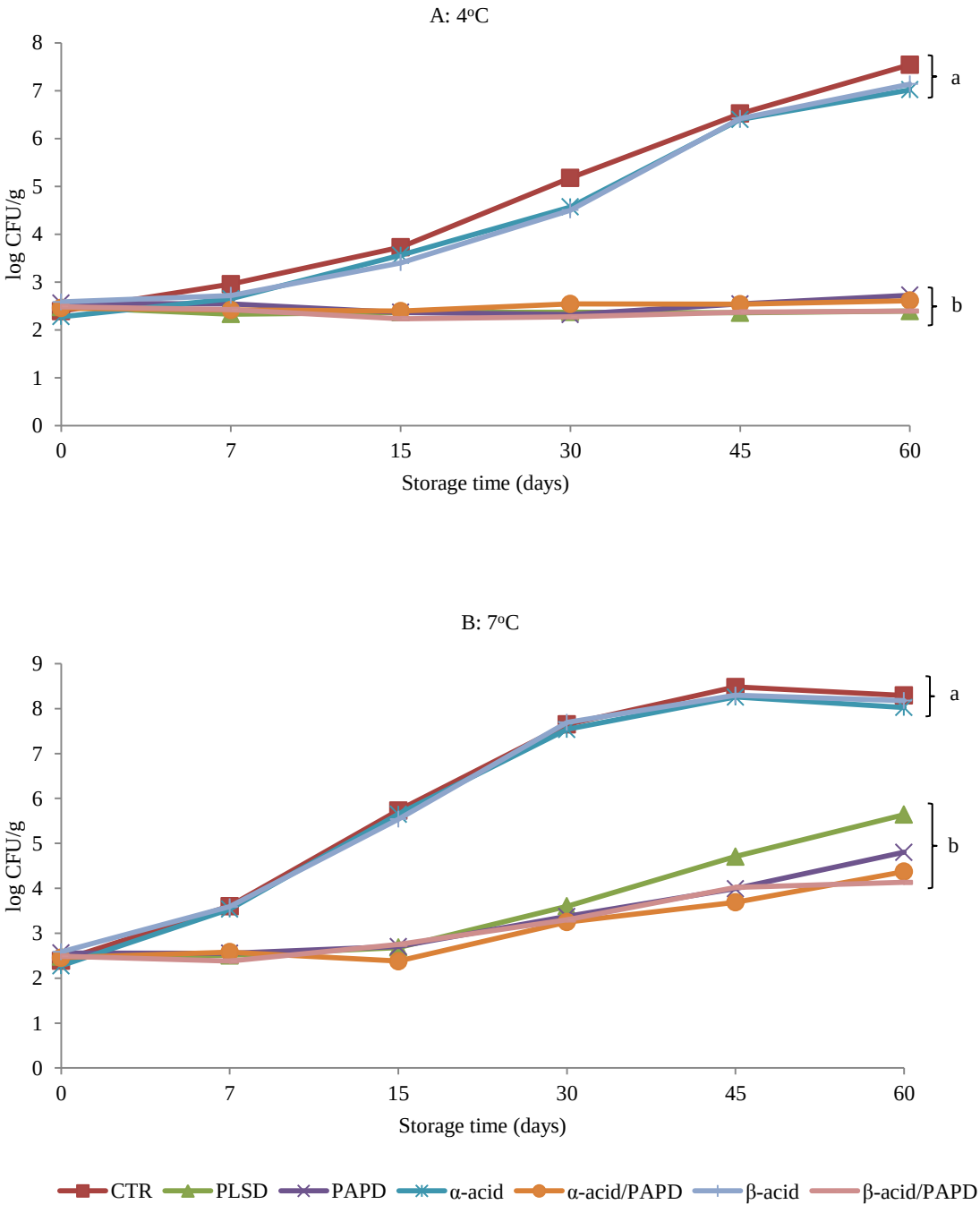

Figure 4.2 *L. monocytogenes* populations in vacuum-packaged deli-style turkey meat with various inhibitors during 60 days of storage at 4 (A) and 7°C (B).
[a-b] Slope of graphs with same letters in the same figure were not significantly different ($P \geq 0.05$).

Surface inoculation of deli-style turkey, which was sliced after 30 and 60 days of storage at 4^{o}C, yielded average *L. monocytogenes* populations of 2.18 to 2.45 log CFU/g and 2.66 to 2.78 log CFU/g, respectively (Fig. 4.3, 4.4 and Table A.9, A.10). During 10 days of storage at 4^{o}C, *Listeria* populations decreased < 0.6 log CFU/g using PLSD, PAPD, α-acid/PAPD and β-acid/PAPD, whereas the pathogen increased by 0.5 to 0.9 log CFU/g in CTR, α-acid and β-acid although no significant difference ($P \geq 0.05$) was seen, regardless of treatment (Fig. 4.3 and Table A.9). During storage at 7^{o}C, PLSD, PAPD, α-acid/PAPD, and β-acid/PAPD were listeristatic with similar trend of *Listeria* growth ($P \geq 0.05$) while both CTR and β-acid allowed *Listeria* to grow by 1.2 to 2.0 log CFU/g, resulting in significantly higher populations than the other treatments (Fig. 4.3, 4.4, and Table A.9, A.10).

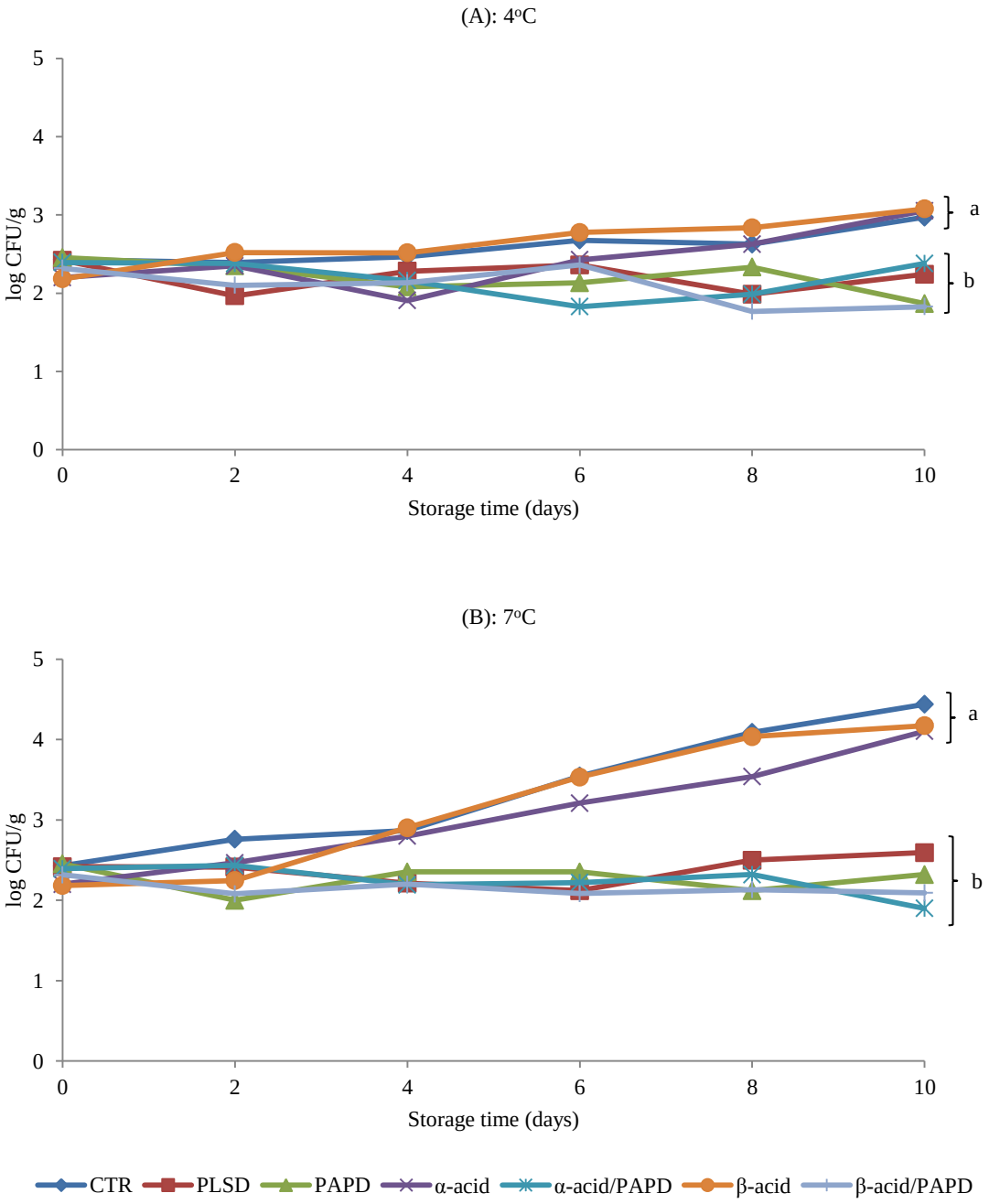

Figure 4.3 *L. monocytogenes* populations during 10 days of storage at 4 (A) and 7°C (B) in aerobic-packaged deli-style turkey meat with various inhibitors and sliced after 30 days of storage.

[a-b] Slope of graphs with same letters in the same figure were not significantly different ($P \geq 0.05$).

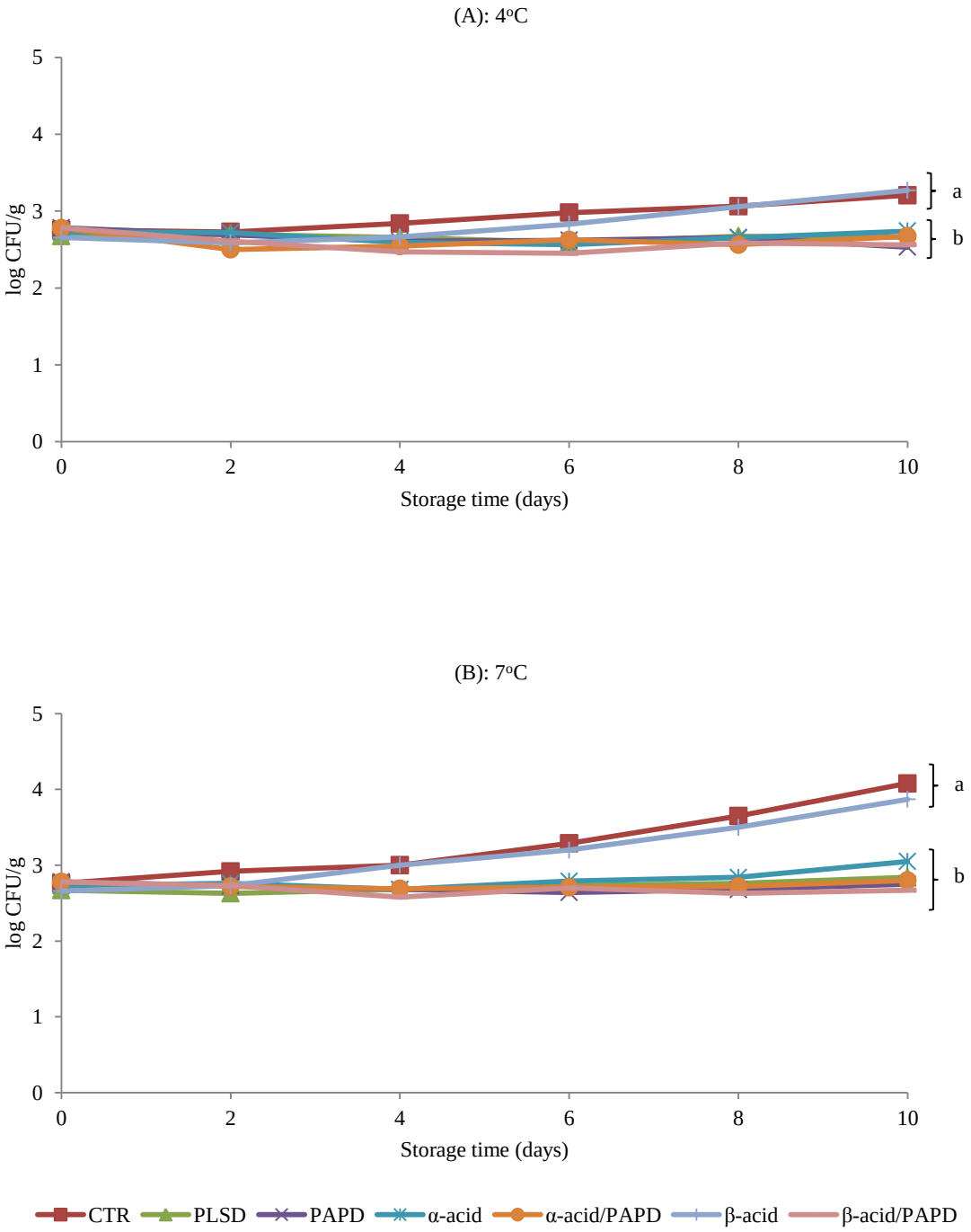

Figure 4.4 *L. monocytogenes* populations during 10 days of storage at 4 (A) and 7°C (B) in aerobic-packaged deli-style turkey meat with various inhibitors and sliced after 60 days of storage.

[a-b] Slope of graphs with same letters in the same figure were not significantly different ($P \geq 0.05$).

The antilisterial activity of hop α-acid and β-acid at 5 ppm in deli-turkey meat was different from the results in liquid media (Chapter 3). In liquid media, 5 ppm β-acid, 5 ppm α-acid/0.5% PAPD and 5 ppm β-acid/0.5% PAPD mixtures was listeristatic, while 5 ppm α-acid allowed *Listeria* to increase 2.4 logs after 6 days of storage at 7^{o}C (Fig. 3.3). In deli-style turkey, however, *Listeria* populations increased by 0.06 to 1.35 log CFU/g for both α- and β-acids at 5 ppm after 6 days of storage at 7^{o}C, whereas listeristatic effects were seen for both 5 ppm α-acid/0.5% PAPD and 5 ppm β-acid/0.5% PAPD mixtures (Fig. 4.3 and 4.4). These results indicate hop acid at 5 ppm alone is not sufficient to inhibit *Listeria* in deli-meat while the combination of hop acid/PAPD is more effective. These results also support the previous findings that *L. monocytogenes* was completely inhibited using the combination of 3.0 ppm hop β-acid, 1.0% potassium lactate, and 0.25% sodium diacetate in broth (Shen and Sofos, 2008). However, when used alone, a very high concentration of hop β-acid (20,000 ppm) was required to reduce the pathogen by 2.1 log CFU/package (Seman et al., 2004). Kramer et al. (2014) also found that the MIC of β-acid extract was 12.5 ppm in broth media and 1000 ppm in the model meat marinate.

The discrepancy in hop's antilisterial activity between liquid media and deli-meat is expected from two reasons: Firstly, hop acid is less mobile in meat batter compared to liquid media. Secondly, hop acid is sequestrated by fat and protein components in meat batter and become less available to react with *Listeria* membranes. Being hydrophobic, hop acids can react with both microbial cell membranes (Schmalreck and Teuber, 1975) and food lipids (Larson et al., 1996). Hence, the antilisterial activity of five hop acids (α-acid, β-acid, acid-tetra, K-tetra, and K-hexa) was assessed with/without PAPD in skim milk and 2% milk (similar fat as deli-

turkey) in order to investigate if the discrepancy between liquid media and deli-meat is due to the sequestration of hop acid by fat in meat batter.

The average *L. monocytogenes* inoculum was 4.2 log CFU/mL for skim milk and 2% milk (Fig. 4.5). Regardless of hop acid type, 0.5% PAPD and 0.5%PAPD/5 ppm hop acid showed better inhibition in skim milk and TSBYE than the inhibitor-free controls, with intermediate inhibition seen when the five hop acid extracts were used individually (Fig. 4.5). Similarly in 2% milk, 0.5% PAPD and 0.5% PAPD/5 ppm hop acid showed better inhibition than the control in TSBYE, while intermediate inhibition was observed for the remaining treatments including the control in 2% milk (Fig. 4.5). These results clearly indicate that the addition of hop acids at 5 ppm is not sufficient to inhibit *Listeria*, especially in 2% fat milk. Larson et al. (1996) observed no inhibition differences between skim milk and skim milk containing 1 and 10 ppm hop β-acid during 30 days of storage at 4^{o}C, while a listericidal effect was seen for hop β-acid at 100 and 1,000 ppm.

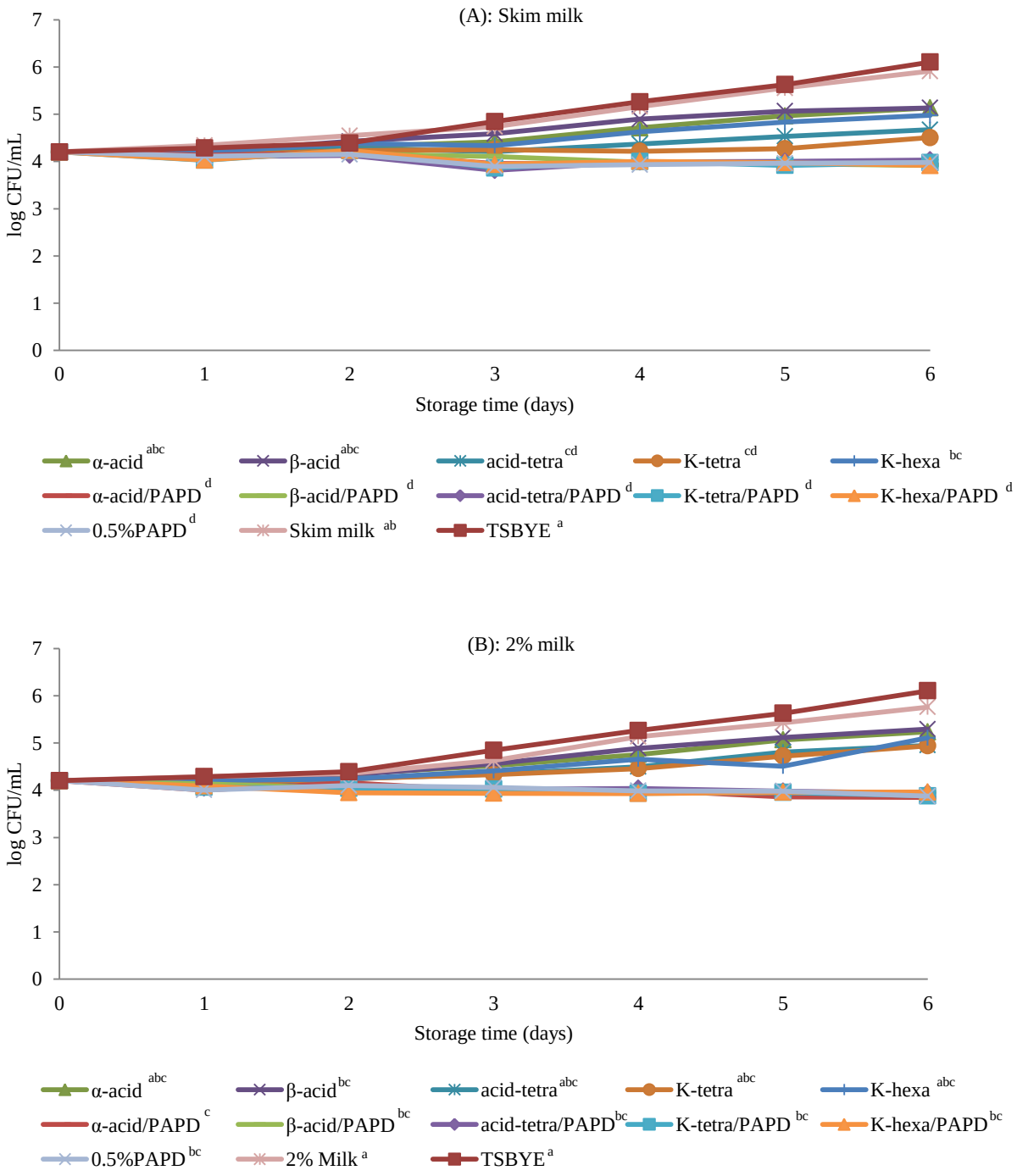

Figure 4.5 *L. monocytogenes* populations in skim milk (A) and 2% milk (B) with or without different hop acid extracts at 5 ppm or 0.5% PAPD during 6 days of storage at 7°C. [a-d] Slope of graphs with same letters in the same figure were not significantly different ($P \geq 0.05$).

4.4 Conclusion

Addition of organic acids in processed meats is one common intervention strategy to minimize *Listeria* growth. Although antilisterial activity of hop acids has been assessed and well documented using liquid media, their use in processed meats has not been extensively studied, except a few trials using hops solution sprays or dips for processed meats. This study indicated that hop acids at 5 ppm failed to inhibit *Listeria* growth or induce any synergistic effect with 0.5% PAPD in deli turkey meat and milk during storage of 6 – 60 days at 4 or 7^{o}C. Based on these findings, the addition of hop β-acid in the amount of 4.4 mg/kg (ppm) in cooked meat and 5.5 mg/kg (ppm) in casings for meat products (US-FDA GRAS Notice Nr 000063) (FDA, 2001) appears to be insufficient to inhibit *Listeria* when the hop acid is formulated.

CHAPTER 5: ANTILISTERIAL EFFECT OF HOP ALPHA AND BETA ACIDS IN TURKEY SLURRY AT 7 AND 37ºC

5.1 Introduction

In our previous study (chapters 4), single addition of 5 ppm hop α- or β-acid to deli-turkey meat was not sufficient to inhibit *Listeria* growth with no synergistic effect seen with the mixture of potassium acetate/potassium diacetate (PAPD). Based on these results, the next question to be answered is what minimum concentration of hop acid is for inhibiting *L. monocytogenes* in meat products. To answer the question, turkey slurry was prepared and used as a meat model system. The purpose of this study, therefore, was to determine the concentrations of hop α- and β-acids required for inhibiting *Listeria* in turkey slurry during storage for one day at 37^{o}C and 12 days at 7^{o}C. The hypothesis of this research is that both hop α- and β-acids can inhibit *L. monocytogenes* at certain concentrations higher than 5 ppm in turkey slurry during storage at 7 and 37^{o}C.

5.2 Material and methods

5.2.1 Preparation of hop acids (alpha and beta) and *L. monocytogenes* strains

Both hop α- and β-acids were obtained from Kalsec Inc. (Kalamazoo, WI) with the concentration as shown in Table 3.1. For the assessment of antilisterial activity, the hop acid extracts were dissolved in 100% ethanol and added to turkey slurries at 0 – 1000 ppm.

The cocktail of six *L. monocytogenes* strains was prepared to contain ~1 x 10^{8} CFU/mL and confirmed the *L. monocytogenes* population in the inoculum as explained in Chapter 3.

5.2.2 Turkey slurries preparation with hop acids

Turkey slurry was prepared at the Michigan State University (MSU) Meat laboratory (East Lansing, MI) by grinding turkey breast through a 0.95-cm plate followed by mixing the ground turkey (25%) with brine solution (75%), containing 70% water, 2.28% salt, 2.00% sugar, 0.48% phosphate, and 0.24% nitrite for 1 min in a small food chopper (Model KFC3511, KitchenAid, St. Joseph, MI). The slurry (100 g) was then pasteurized in each of 250 mL flasks by submerging in a water bath (85^{o}C) until the internal temperature of turkey slurry reached to 72^{o}C. To the flask, hop α- or β-acid was added, mixed thoroughly for 2 min with a stir bar, and immersed again to the water bath for 3 min to simulate the heat exposure during cooking, prior to cooling to 37^{o}C in icy slurry.

5.2.3 Antilisterial activity of hop extracts at 7 and 37^{o}C

Hop α-and β-acids were individually dissolved in 1 mL of 100% ethanol and added to the turkey slurries to achieve the concentrations of 0, 250, 500, 750, and 1000 ppm (w/w) prior to incubation at 37^{o}C or 0, 5, 25, 50, 100, 500, and 1000 ppm (w/w) for incubation at 7^{o}C. Turkey slurry without hop acid was also prepared to serve as a control with the same amount of ethanol used for dissolving the hop acids. The prepared six-strain *L. monocytogenes* cocktail was then added to each flask to achieve approximately 2.0 – 3.0 log CFU/g, mixed thoroughly, and incubated at 37^{o}C for 24 h or 7^{o}C for 12 days. Samples were taken initially and after 24 h incubation at 37^{o}C, or initially and every 3 days at 7^{o}C. Appropriate serial dilutions in sterile phosphate buffered saline (PBS) were plated on modified Oxford agar (MOX) (Difco, BD) and incubated at 37^{o}C for 48 h to enumerate *L. monocytogenes* populations.

5.2.4 Statistical analysis

The microbiological data from triplicate experiments were converted to log CFU/g. An analysis of variance (ANOVA) was performed using the mixed procedure of SAS software (SAS Institute, 2002). The slope of graph of *Listeria* population during storage for each treatment (growth rate) was calculated to better assess the effect of treatment on *Listeria* inhibition. Statistically significant differences between the treatments were determined using Tukey's Test at $\alpha = 0.05$.

5.3 Results and discussion

5.3.1 Antilisterial activity of hop acids at 37°C

Antilisterial activity of 0 (Control), 250, 500, 750, and 1,000 ppm hop α- and β-acid were evaluated in turkey slurries after incubation at 37°C for 24 h (Table 5.1). The initial *L. monocytogenes* inoculum level ranged from 2.22 to 2.40 log CFU/g for all treatments with no significant diffrence at 0 h at 37°C ($P \geq 0.05$). Considering no immediate lethal effect upon the exposure to hop β-acids in liquid media, this result agrees with the report of Shen and Sofos (2008). After incubating for 24 h, *Listeria* populations were less than the detection limit (< 10 cells/g) at 750 ppm α-acid and 1,000 ppm β-acids, whereas *Listeria* growth using 500 ppm α- and β-acids was half that of the control (8.02 log CFU/g) (Table 5.1).

In chapter 3, *Listeria* populations were less than detection limit (< 10 cells/g) for 50 ppm α-acid. Using 25 ppm α-acid and β-acid, populations were reduced to 1/8 and half of control, respectively, in trypticase soy broth with yeast extract (TSBYE) at 37°C for 24 h. The concentration of 25 ppm was about 30 times lower than the requirement for listericidal activity in turkey slurry. Larson et al. (1996) also reported that *Listeria* growth was completely inhibited in

trypticase soy broth containing 10 ppm of hop extract II (41% β- and 12% α-acids) and hop extract III (30% colupulone and 65% β-acids) after 24 h of incubation at 37°C. In whole milk at 4°C, however, *Listeria* inhibition was seen when hop extract III was increased to 1000 ppm. Again, these findings indicate that the required concentration of hop acid for a listericidal effect in food is 30 times higher than in liquid media at 37°C.

Table 5.1 Population of *L. monocytogenes*[1] (log CFU/g) in turkey slurries containing 0 to 1000 ppm α-acid or β-acid after incubating at 37°C for 24 h.

Treatment	Number of *L. monocytogenes* (log CFU/g)*	
	Time	
	0 h	24 h
α-acid 250 ppm	2.39 ± 0.33 [a]	4.38 ± 0.42 [cd]
α-acid 500 ppm	2.38 ± 0.34 [a]	3.96 ± 0.04 [c]
α-acid 750 ppm	2.30 ± 0.30 [a]	< 1.00 [a]
α-acid 1000 ppm	2.22 ± 0.19 [a]	< 1.00 [a]
β-acid 250 ppm	2.29 ± 0.47 [a]	4.60 ± 0.18 [d]
β-acid 500 ppm	2.39 ± 0.32 [a]	4.01 ± 0.06 [c]
β-acid 750 ppm	2.35 ± 0.33 [a]	1.73 ± 0.24 [b]
β-acid 1000 ppm	2.35 ± 0.34 [a]	< 1.00 [a]
0 ppm (Control)	2.40 ± 0.42 [a]	8.02 ± 0.31 [e]

[1]Means ± standard deviation of $n = 6$ observations for each reading.
[a-e] Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
* No viable *L. monocytogenes* detection or the counts below minimum detectability of the methodology (10 cells per gram) was marked as < 1.00.

5.3.2 Antilisterial activity of hop acids at 7°C

Based on our previous results in liquid media and deli-style turkey, antilisterial activities of α- and β-acids at 0, 25, 50, 100, 500, and 1,000 ppm were evaluated in turkey slurries during 12 days of storage at 7°C (Fig. 5.1). Both α- and β-acids were listericidal at > 500 ppm, indicating that the listericidal concentration is ~100 times higher in turkey slurry than in liquid media at 7°C.

Although many investigators reported that hop acids at ≤ 10 ppm effectively inhibited the growth of *L. monocytogenes* in liquid media (Millis and Schendel, 1994; Barney et. al., 1995; Larson et. al., 1996; Shen and Sofos, 2008), our work shows that hop acids at < 100 ppm were not effective in turkey slurries at 7°C. In accordance with our results, Larson et al. (1996) reported that a hop acid extract containing 30% colupulone and 65% β-acids was listericidal at 1,000 ppm in skim milk and 2% milk during 35 days of storage at 4°C, with moderate inhibition and almost no inhibition at 100 and ≤ 10 ppm, respectively. Again, these findings indicate that the required concentration of hop acids for inhibition of *Listeria* in actual foods is not as same as the concentration observed in liquid media.

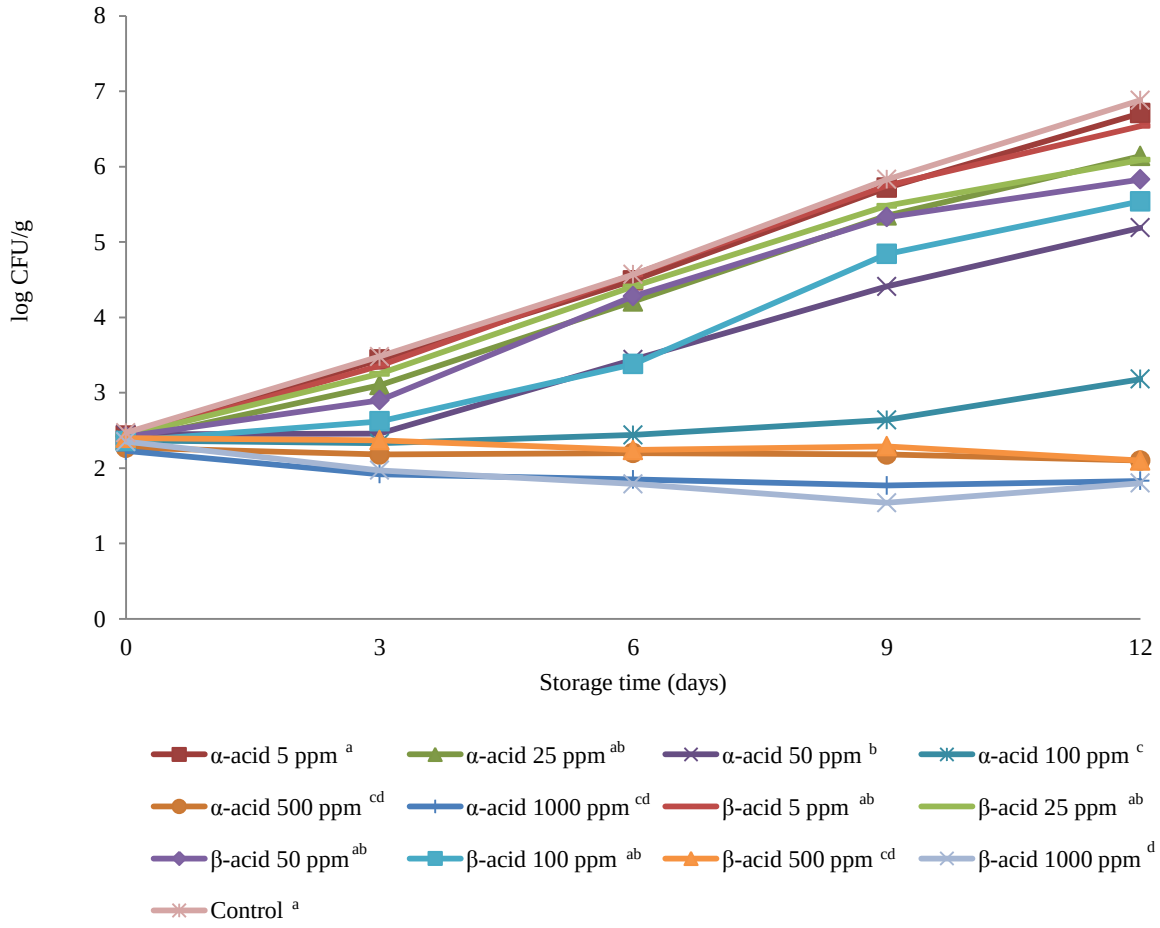

Figure 5.1 Population of *L. monocytogenes* in turkey slurries containing 0 to 1000 ppm α-acid or β-acid during 12 days of storage at 7°C.

[a-d] Slope of graphs with same letters were not significantly different ($P \geq 0.05$).

In foods, hop acid is not a popular food ingredient due to the undesirable bitter taste. Although our results indicated that hop α- and β-acids at > 500 ppm are unlikely added to foods, hop acids could be used in combination with organic acids to reduce both the sour taste from organic acids and the bitter flavor from hop acids. In sensory evaluations, negative odor was detected in ham containing 0.2% sodium diacetate (Stekelenburg and Kant-Muermans, 2001) and bitter taste at > 50 ppm for purified hop β-acid (Millis et al., 1994).

In our previous study (chapter 3), addition of 1% PAPD (80% potassium acetate/20% potassium diacetate) containing 0.2% potassium diacetate resulted in lower flavor and overall acceptability scores in low-sodium frankfurters compared to the inhibitor-free control (Sansawat et al., 2013). As a result, it will be interesting if the combination of PAPD at < 1% and hop acid at < 50 ppm could minimize both sour and bitter flavor while maintaining the *Listeria* inhibition.

Based on these findings, the USDA allowance for hop acids used meat products (4.4 mg/kg in cooked meats and 5.5 mg/kg in meat product in casings) (US/FDA GRAS Notice Nr 000063) (FDA, 2001) needs to be updated for practical application in case of formulation to meat batter.

In liquid media, hop acid can directly contact *L. monocytogenes* and result in effective inhibition, which is not true in meat batters. In our study, the minimal inhibitory concentration of hop α- and β-acid was between 6.3 and 3.1 ppm in liquid media (Table 3.4, Chapter 3), but no inhibition was observed in deli-turkey, skim milk, and 2% milk at 5 ppm regardless of hop α- or β-acid (Fig. 4.2, 4.5, 4.6 Chapter 4). It is known that increased hydrophobicity of hop acid leads to greater antimicrobial activity due to increased interaction with the bacterial cell membrane (Etoh et al., 1994; Schmalreck et al., 1975). Reduced activity of hop extract in food is expected from the sequestration of hydrophobic groups by food lipids (Larson et al., 1996). As a result,

encapsulation of hop acids would be additional solution so that the encapsulated hop acids can be less sequestrated during batter mixing and effectively released after emulsifying fats and coagulating proteins during cooking.

5.4 Conclusion

Hop α- and β-acids exhibited antilisterial activity in turkey slurries at the concentrations ≥ 750 ppm during storage at 37°C for 24 h or at the concentrations ≥ 500 ppm at 7°C for 12 days. However, the high concentration of hop acids might be not practical due to the prediction of negative sensory impacts. Two potential solutions for the implementation of hop acids could be: (1) combination with an organic acid at the levels below the threshold for bitter and sour taste, and (2) encapsulation of the hop acids to avoid any sequestration by food components during batter mixing and maintain antilisterial activity with no sensory issue.

SUMMARY

Continuous outbreaks of listeriosis urged food processors to find a better way to control *L. monocytogenes* especially in ready-to-eat (RTE) meat products. Formulation of antimicrobial agents in foods is one of common methods to control bacteria including *L. monocytogenes.* Regardless of surface application or formulation, development of a new antilisterial agent and innovative combinations of pre-existing additives are always desirable for pathogen inhibition and improved product quality. Results from this research indicated that the mixtures of hop acids and organic acid salts were more effective than any single application on *Listeria* inhibition in liquid media. Concerning *Listeria* inhibition and product sensory quality, the mixture of potassium acetate and potassium diacetate (PAPD) out of 9 organic acids/mixtures was the most effective in *Listeria* inhibition but not for eating quality of frankfurter in low-sodium. In case of hop acids, addition of α- or β-acid out of 8 hop acids was most effective in *Listeria* inhibition in liquid media, but the effectiveness was not the same in meat paste. Upon mixing the best organic acid and one of the two best hop acids, they induced synergistic effects on *Listeria* inhibition in liquid media, but not in meat paste. These results are expected from the sequestration of hop acids by lipids and proteins in meat batter. Therefore, it will be interesting to conduct an additional study to find the right levels of hop acid and PAPD in mixture that can provide effective *Listeria* inhibition with no sensory quality loss. More interestingly, encapsulaiton of hop acids will be desirable if it can prevent any sequestration of hop acids during meat batter preparation.

FUTURE RECOMMENDATIONS

Results of this dissertation indicated that the combination of organic acids and hop acids possess the potential to improve antilisterial activity and sensory attributes. When incubating *Listeria* with 0.5% PAPD and hop α- or β-acid at 25 ppm, the pathogen populations decreased from 5.7 log CFU/mL to non-detectable level in liquid media. In single addition, the required hop acid for *Listeria* inhibition was 500 ppm in meat batter, whereas the minimum inhibitory activity of hop acid was between 3.1 – 6.3 ppm in liquid media. Our results also indicate that when formulated hop acids to deli-style turkey meats at the allowance of USDA-FSIS, the hop acids did not inhibit the growth of *L. monocytogenes* nor generate further inhibition upon combining with PAPD. Therefore, these results suggest that USDA-FSIS should reconsider the allowance level in case of formulation. In future studies, it will be interesting to find that if formulation of encapsulated hop acids can provide better inhibition on *L. monocytogenes* in meat paste with no sensory issue. It will be also interesting to know that if mixture of encapsulated hop acids with PAPD or other antimicrobial agents can generate synergistic effects in deli-style turkey. Given no inhibition in the range of sensory issue (< 25 ppm hop acids), we could suggest that hop acids may not be an adequate inhibitor in processed meat products. Providing better inhibition and synergistic effects with other inhibitors upon encapsulation, the hop acids could be useful as a natural food preservative.

APPENDICES

APPENDIX A

Tables of supplemental data

Table A.1 Population of *L. monocytogenes*[1,2,3] on vacuum-packaged full-sodium frankfurters with powdered or liquid inhibitors[4] during 90 days of storage at 4, 7 and 10ºC.

Treatments	0	15	30	45	60	75	90
Storage at 4ºC							
CTR (0%)	4.57 ± 0.17^{a}	5.88 ± 0.18^{a}	7.10 ± 0.32^{a}	7.12 ± 1.49^{a}	7.75 ± 0.31^{a}	7.70 ± 0.19^{a}	7.56 ± 0.41^{a}
PI-I (0.5%)	4.56 ± 0.25^{a}	4.58 ± 0.15^{b}	4.77 ± 0.39^{b}	5.31 ± 1.15^{b}	5.76 ± 1.27^{b}	6.23 ± 0.78^{b}	6.90 ± 0.44^{ab}
PI-2 (1.00%)	4.52 ± 0.17^{a}	4.29 ± 0.26^{b}	4.26 ± 0.01^{bc}	4.30 ± 0.33^{b}	4.54 ± 0.46^{bc}	4.35 ± 0.40^{cd}	4.70 ± 0.80^{cd}
PI-3 (0.65%)	4.58 ± 0.54^{a}	4.28 ± 0.17^{b}	4.21 ± 0.02^{bc}	4.18 ± 0.15^{b}	4.30 ± 0.16^{bc}	4.13 ± 0.11^{cd}	4.13 ± 0.10^{d}
PI-4 (0.75%)	4.57 ± 0.26^{a}	4.26 ± 0.22^{b}	4.22 ± 0.12^{bc}	4.20 ± 0.27^{b}	4.11 ± 0.13^{c}	4.05 ± 0.15^{d}	4.02 ± 0.22^{d}
PI-5 (1.00%)	4.60 ± 0.34^{a}	4.19 ± 0.23^{b}	4.10 ± 0.22^{c}	4.11 ± 0.36^{b}	4.09 ± 0.18^{c}	3.94 ± 0.24^{d}	4.06 ± 0.31^{d}
LI-1 (2.5%)	4.52 ± 0.39^{a}	4.26 ± 0.27^{b}	4.32 ± 0.17^{bc}	4.60 ± 0.58^{b}	5.31 ± 0.98^{bc}	5.39 ± 1.19^{bc}	5.88 ± 1.08^{bc}
LI-2 (2.5%)	4.56 ± 0.47^{a}	4.18 ± 0.23^{b}	4.12 ± 0.16^{c}	4.11 ± 0.24^{b}	4.18 ± 0.41^{bc}	4.18 ± 0.32^{cd}	4.23 ± 0.52^{d}
LI-3 (2.5%)	4.57 ± 0.53^{a}	4.26 ± 0.31^{b}	4.23 ± 0.05^{bc}	4.68 ± 0.83^{b}	5.25 ± 1.35^{bc}	5.05 ± 0.78^{bcd}	5.77 ± 0.94^{bc}
LI-4 (2.5%)	4.59 ± 0.24^{a}	4.31 ± 0.21^{b}	4.17 ± 0.10^{c}	4.37 ± 0.28^{b}	4.69 ± 0.56^{bc}	4.55 ± 0.74^{cd}	4.54 ± 0.69^{cd}
Storage at 7ºC							
CTR (0%)	4.57 ± 0.17^{a}	6.99 ± 0.44^{a}	7.56 ± 0.25^{a}	7.73 ± 0.51^{a}	7.49 ± 0.38^{a}	7.32 ± 0.44^{a}	7.15 ± 0.29^{ab}
PI-I (0.5%)	4.56 ± 0.25^{a}	5.77 ± 0.64^{b}	6.92 ± 0.25^{b}	7.10 ± 0.54^{a}	7.07 ± 0.66^{ab}	7.62 ± 0.09^{a}	7.78 ± 0.05^{a}
PI-2 (1.00%)	4.52 ± 0.17^{a}	4.26 ± 0.25^{d}	4.63 ± 0.44^{cd}	5.39 ± 0.77^{cd}	5.26 ± 0.97^{cde}	6.19 ± 0.56^{abc}	6.79 ± 0.57^{abc}
PI-3 (0.65%)	4.58 ± 0.54^{a}	4.24 ± 0.17^{d}	4.19 ± 0.18^{d}	4.55 ± 0.54^{de}	4.61 ± 1.04^{de}	4.77 ± 0.89^{cd}	6.13 ± 0.62^{bc}
PI-4 (0.75%)	4.57 ± 0.26^{a}	4.28 ± 0.14^{d}	4.32 ± 0.34^{d}	4.43 ± 0.39^{de}	3.98 ± 0.07^{e}	4.10 ± 0.32^{d}	4.10 ± 0.46^{d}
PI-5 (1.00%)	4.60 ± 0.34^{a}	4.22 ± 0.17^{d}	4.17 ± 0.28^{d}	4.23 ± 0.32^{e}	4.09 ± 0.10^{e}	4.05 ± 0.38^{d}	4.41 ± 0.74^{d}
LI-1 (2.5%)	4.52 ± 0.39^{a}	5.12 ± 0.24^{bc}	5.20 ± 0.74^{c}	6.74 ± 0.65^{ab}	6.56 ± 0.63^{abc}	7.12 ± 0.38^{ab}	7.28 ± 0.09^{ab}
LI-2 (2.5%)	4.56 ± 0.47^{a}	4.18 ± 0.29^{d}	4.53 ± 0.56^{cd}	4.94 ± 1.24^{cde}	4.78 ± 0.98^{de}	5.31 ± 1.41^{bcd}	5.73 ± 0.58^{c}
LI-3 (2.5%)	4.57 ± 0.53^{a}	4.76 ± 0.40^{cd}	5.23 ± 0.74^{c}	5.96 ± 0.95^{bc}	5.83 ± 0.78^{bcd}	6.28 ± 0.96^{abc}	7.18 ± 0.45^{ab}
LI-4 (2.5%)	4.59 ± 0.24^{a}	4.50 ± 0.22^{cd}	4.58 ± 0.27^{cd}	5.22 ± 0.34^{cde}	5.47 ± 0.98^{bcde}	5.80 ± 0.52^{abcd}	6.46 ± 0.33^{bc}
Storage at 10ºC							
CTR (0%)	4.57 ± 0.17^{a}	7.24 ± 0.20^{a}	7.48 ± 0. 35^{a}	7.30 ± 0.45^{a}	6.62 ± 0.64^{abc}	6.15 ± 0.79^{ab}	5.34 ± 1.43^{abc}
PI-I (0.5%)	4.56 ± 0.25^{a}	7.35 ± 0.25^{ab}	7.56 ± 0.22^{a}	7.33 ± 0.23^{a}	7.08 ± 0.20^{ab}	6.91 ± 0.66^{ab}	6.81 ± 0.49^{a}
PI-2 (1.00%)	4.52 ± 0.17^{a}	6.01 ± 0.23^{cd}	7.19 ± 0.25^{ab}	7.51 ± 0.06^{a}	7.50 ± 0.13^{a}	7.18 ± 0.23^{a}	7.86 ± 0.29^{a}
PI-3 (0.65%)	4.58 ± 0.54^{a}	5.49 ± 0.30^{de}	6.59 ± 0.71^{abc}	6.90 ± 0.11^{a}	6.62 ±0.08^{abc}	6.58± 0.22^{ab}	6.59 ± 0.39^{ab}
PI-4 (0.75%)	4.57 ± 0.26^{a}	4.64 ± 0.46^{e}	5.44 ± 0.95^{cd}	5.33 ± 0.67^{b}	5.10 ± 0.24^{bc}	4.65 ± 0.31^{b}	4.64 ± 0.02^{bc}
PI-5 (1.00%)	4.60 ± 0.34^{a}	4.76 ± 0.14^{e}	4.89 ± 1.03^{d}	5.08 ± 0.48^{b}	5.01 ± 0.32^{c}	4.74 ± 0.41^{b}	4.03 ± 0.27^{c}
LI-1 (2.5%)	4.52 ± 0.39^{a}	6.91 ± 0.20^{abc}	7.60 ± 0.23^{a}	7.48 ± 0.09^{a}	7.42 ± 0.14^{a}	7.17 ± 0.25^{a}	7.23 ± 0.65^{a}
LI-2 (2.5%)	4.56 ± 0.47^{a}	5.12 ± 0.69^{de}	6.16 ± 0.2^{abcd}	6.31 ± 0.27^{ab}	6.39 ± 0.33^{abc}	6.20 ± 0.56^{ab}	6.20 ± 0.83^{ab}
LI-3 (2.5%)	4.57 ± 0.53^{a}	6.80 ± 0.25^{abc}	6.88 ± 0.40^{abc}	6.98 ± 0.03^{a}	6.79 ± 0.25^{abc}	6.81 ± 0.20^{ab}	6.69 ± 0.22^{ab}
LI-4 (2.5%)	4.59 ± 0.24^{a}	6.26 ± 0.13^{bcd}	5.90 ± 0.17^{bcd}	7.03 ± 0.06^{a}	6.16 ± 0.11^{abc}	5.12 ± 0.29^{ab}	5.17 ± 0.19^{abc}

[1]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
[2]Means ± standard deviation of $n = 6$ observations for each reading, except for control.
[3]The minimum detectability of the methodology was > 10 cells per gram.
[4]Inhibitors in the formulation as in Table 2.3

Table A.2 Area[1] under graph of *L. monocytogenes* population on vacuum-packaged full-sodium frankfurters with powdered or liquid inhibitors[2] during 90 days of storage at 4, 7 and 10ºC.

Treatments	Area under graph		
	4ºC	7ºC	10ºC
CTR (0%)	623.37 [a]	649.77 [a]	606.82 [a]
PI-I (0.5%)	484.94 [b]	609.01 [ab]	630.24 [a]
PI-2 (1.00%)	394.88 [bc]	470.44 [cde]	618.35 [a]
PI-3 (0.65%)	380.91[c]	414.92 [de]	568.25 [a]
PI-4 (0.75%)	376.30 [c]	380.87 [e]	448.09 [b]
PI-5 (1.00%)	370.27 [c]	377.82 [e]	433.90 [b]
LI-1 (2.5%)	435.78 [bc]	549.16 [abc]	641.97 [a]
LI-2 (2.5%)	376.76 [c]	432.48 [de]	535.09 [ab]
LI-3 (2.5%)	428.92 [bc]	508.36 [bcd]	599.99 [a]
LI-4 (2.5%)	398.79 [bc]	465.57 [cde]	532.26 [ab]

[1]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
[2]Inhibitors in the formulation as in Table 2.3

Table A.3 Population of *L. monocytogenes*[1,2,3] on vacuum-packaged low-sodium frankfurters with powdered or liquid inhibitors[4] during 90 days of storage at 4, 7 and 10ºC.

Treatments	0	15	30	45	60	75	90
Storage at 4ºC							
CTR (0%)	4.74 ± 0.12^{a}	6.28 ± 0.87^{a}	6.65 ± 1.45^{a}	7.26 ± 0.93^{a}	7.37 ± 0.44^{a}	7.25 ± 0.88^{a}	6.96 ± 0.84^{a}
PI-2 (1.00%)	4.69 ± 0.19^{a}	4.69 ± 0.25^{b}	5.16 ± 0.59^{ab}	5.34 ± 0.98^{bc}	5.95 ± 0.88^{b}	6.15 ± 1.82^{ab}	6.51 ± 0.95^{a}
PI-3 (0.65%)	4.79 ± 0.04^{a}	4.52 ± 0.21^{b}	4.69 ± 0.24^{b}	4.91 ± 0.61^{bc}	4.94 ± 0.30^{bc}	5.29 ± 0.90^{b}	5.36 ± 1.14^{ab}
PI-5 (1.00%)	4.70 ± 0.07^{a}	4.35 ± 0.17^{b}	4.34 ± 0.05^{b}	4.45 ± 0.19^{c}	4.21 ± 0.09^{c}	4.24 ± 0.08^{b}	4.15 ± 0.46^{b}
LI-4 (2.5%)	4.71 ± 0.08^{a}	4.81 ± 0.36^{b}	5.17 ± 0.82^{ab}	5.81 ± 0.93^{b}	6.11 ± 0.93^{b}	5.77 ± 1.46^{ab}	5.96 ± 1.75^{a}
Storage at 7ºC							
CTR (0%)	4.74 ± 0.12^{a}	7.39 ± 0.56^{a}	7.38 ± 0.46^{a}	7.60 ± 0.30^{a}	7.54 ± 0.05^{a}	7.29 ± 0.10^{a}	7.16 ± 0.63^{a}
PI-2 (1.00%)	4.69 ± 0.19^{a}	5.32 ± 0.55^{b}	6.27 ± 0.87^{ab}	7.10 ± 0.67^{a}	7.15 ± 0.64^{a}	7.53 ± 0.39^{a}	7.36 ± 0.57^{a}
PI-3 (0.65%)	4.79 ± 0.04^{a}	4.73 ± 0.37^{bc}	5.28 ± 0.59^{bc}	5.92 ± 0.76^{ab}	6.38 ± 0.68^{a}	6.47 ± 0.58^{a}	6.13 ± 0.49^{b}
PI-5 (1.00%)	4.70 ± 0.07^{a}	4.34 ± 0.20^{c}	4.30 ± 0.14^{c}	4.30 ± 0.13^{b}	4.25 ± 0.12^{b}	4.53 ± 0.46^{b}	4.46 ± 0.63^{c}
LI-4 (2.5%)	4.71 ± 0.08^{a}	5.31 ± 0.79^{b}	6.01 ± 1.33^{ab}	6.37 ± 1.43^{a}	6.50 ± 1.00^{a}	6.83 ± 1.00^{a}	6.91 ± 0.44^{a}
Storage at 10ºC							
CTR (0%)	4.74 ± 0.12^{a}	7.60 ± 0.31^{a}	7.55 ± 0.09^{a}	7.02 ± 0.38^{ab}	6.56 ± 0.46^{a}	5.69 ± 0.72^{ab}	5.77 ± 0.58^{ab}
PI-2 (1.00%)	4.69 ± 0.19^{a}	6.86 ± 0.24^{ab}	7.62 ± 0.47^{a}	7.70 ± 0.12^{a}	7.21 ± 0.52^{a}	7.01 ± 0.30^{a}	6.98 ± 0.37^{a}
PI-3 (0.65%)	4.79 ± 0.04^{a}	5.55 ± 0.75^{cd}	7.11 ± 0.17^{ab}	7.03 ± 0.62^{ab}	6.69 ± 0.64^{a}	6.12 ± 1.50^{ab}	5.89 ±1.07^{ab}
PI-5 (1.00%)	4.70 ± 0.07^{a}	4.48 ± 0.13^{d}	4.49 ± 0.25^{c}	4.67 ± 0.50^{c}	4.81 ± 1.25^{b}	4.61 ± 1.13^{b}	4.72 ± 0.12^{b}
LI-4 (2.5%)	4.71 ± 0.08^{a}	6.32 ± 1.04^{bc}	6.82 ± 0.53^{b}	6.50 ± 0.95^{b}	6.57 ± 0.67^{a}	6.06 ± 1.29^{ab}	6.16 ± 1.38^{ab}

[1]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
[2]Means ± standard deviation of $n = 6$ observations for each reading, except for control.
[3]The minimum detectability of the methodology was > 10 cells per gram.
[4]Inhibitors in the formulation as in Table 2.3

Table A.4 Area[1] under graph of *L. monocytogenes* population on vacuum-packaged low-sodium frankfurters with powdered or liquid inhibitors[2] during 90 days of storage at 4, 7 and 10ºC.

Treatments	Area under graph		
	4ºC	7ºC	10ºC
CTR (0%)	609.77 ^{a}	647.35 ^{a}	595.08 ^{a}
PI-2 (1.00%)	493.36 ^{ab}	590.99 ^{a}	633.57 ^{a}
PI-3 (0.65%)	441.32 ^{b}	513.50 ^{ab}	567.72 ^{a}
PI-5 (1.00%)	390.18 ^{b}	394.36 ^{b}	416.55 ^{b}
LI-4 (2.5%)	495.14 ^{ab}	552.65 ^{a}	565.67 ^{a}

[1]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
[2]Inhibitors in the formulation as in Table 2.3

Table A.5 Population of mesophilic aerobic bacteria[1,2,3] on vacuum-packaged frankfurters with powdered or liquid inhibitors[4] during 90 days of storage at 4, 7 and 10ºC.

Treatments	0	15	30	45	60	75	90
Storage at 4°C							
CTR (0%)	1.40 ± 0.96^{a}	3.04 ± 2.33^{a}	6.57 ± 0.29^{a}	7.50 ± 0.11^{a}	6.98 ± 0.83^{a}	6.74 ± 1.50^{a}	7.07 ± 1.26^{a}
PI-I (0.5%)	1.56 ± 0.50^{a}	1.83 ± 1.69^{a}	3.00 ± 1.60^{b}	5.71 ± 0.66^{b}	6.00 ± 0.78^{ab}	5.15 ± 1.91^{ab}	6.26 ± 2.48^{ab}
PI-2 (1.00%)	1.64 ± 0.18^{a}	1.54 ± 0.99^{a}	2.54 ± 1.25^{b}	5.55 ± 0.74^{b}	5.32 ± 0.29^{b}	4.82 ± 0.49^{ab}	5.05 ± 1.66^{ab}
PI-3 (0.65%)	1.91 ± 0.55^{a}	1.69 ± 1.03^{a}	2.98 ± 0.85^{b}	5.60 ± 0.72^{b}	5.27 ± 0.28^{b}	5.47 ± 0.92^{ab}	4.74 ± 1.65^{b}
PI-4 (0.75%)	1.43 ± 0.96^{a}	1.48 ± 0.56^{a}	2.88 ± 0.87^{b}	5.36 ± 0.89^{b}	5.35 ± 0.35^{b}	4.91 ± 1.07^{ab}	4.49 ± 1.44^{b}
PI-5 (1.00%)	1.25 ± 0.36^{a}	1.61 ± 0.99^{a}	2.05 ± 0.91^{b}	5.27 ± 0.77^{b}	4.69 ± 0.70^{b}	4.06 ± 0.51^{b}	5.14 ± 1.19^{ab}
LI-1 (2.5%)	1.42 ± 0.39^{a}	2.05 ± 1.19^{a}	2.76 ± 0.75^{b}	5.69 ± 0.67^{b}	6.03 ± 0.82^{ab}	5.86 ± 1.47^{ab}	6.12 ± 1.77^{ab}
LI-2 (2.5%)	1.77 ± 0.47^{a}	1.39 ± 1.33^{a}	2.17 ± 0.83^{b}	4.74 ± 0.58^{b}	5.12 ± 0.38^{b}	4.90 ± 1.58^{ab}	5.39 ± 1.60^{ab}
LI-3 (2.5%)	1.77 ± 0.53^{a}	1.36 ± 0.58^{a}	3.12 ± 1.84^{b}	5.82 ± 0.58^{b}	5.71 ± 0.43^{ab}	5.14 ± 1.19^{ab}	6.53 ± 0.79^{ab}
LI-4 (2.5%)	1.66 ± 0.25^{a}	1.73 ± 0.25^{a}	2.74 ± 0.76^{b}	5.51 ± 0.73^{b}	5.74 ± 0.55^{ab}	5.35 ± 1.05^{ab}	5.79 ± 0.86^{ab}
Storage at 7°C							
CTR (0%)	1.40 ± 0.96^{a}	4.17 ± 3.40^{a}	6.74 ± 0.29^{a}	7.60 ± 0.08^{a}	6.78 ± 0.84^{a}	6.64 ± 0.95^{a}	6.36 ± 1.85^{ab}
PI-I (0.5%)	1.56 ± 0.50^{a}	1.73 ± 2.16^{ab}	3.47 ± 1.19^{b}	6.13 ± 0.40^{ab}	6.65 ± 0.61^{a}	5.64 ± 1.13^{ab}	6.19 ± 1.82^{ab}
PI-2 (1.00%)	1.64 ± 0.18^{a}	1.36 ± 1.11^{b}	3.53 ± 1.06^{b}	5.81 ± 0.25^{b}	5.25 ± 0.41^{b}	4.92 ± 0.57^{ab}	5.12 ± 0.94^{b}
PI-3 (0.65%)	1.91 ± 0.55^{a}	2.31 ± 1.70^{ab}	3.40 ± 1.06^{b}	5.62 ± 0.49^{b}	5.91 ± 0.69^{ab}	5.17 ± 0.71^{ab}	5.83 ± 1.27^{ab}
PI-4 (0.75%)	1.43 ± 0.96^{a}	2.09 ± 1.88^{ab}	3.75 ± 0.75^{b}	5.53 ± 0.61^{b}	5.72 ± 0.58^{ab}	5.23 ± 1.44^{ab}	5.18 ± 1.02^{b}
PI-5 (1.00%)	1.25 ± 0.36^{a}	1.43 ± 1.79^{b}	3.62 ± 0.85^{b}	5.50 ± 0.62^{b}	5.41 ± 0.37^{b}	4.57 ± 0.54^{b}	5.20 ± 0.87^{ab}
LI-1 (2.5%)	1.42 ± 0.39^{a}	2.21 ± 1.65^{ab}	4.09 ± 0.85^{b}	6.21 ± 0.51^{ab}	6.36 ± 0.47^{ab}	5.95 ± 1.51^{ab}	6.50 ± 1.40^{ab}
LI-2 (2.5%)	1.77 ± 0.47^{a}	1.98 ± 1.53^{ab}	4.19 ± 0.59^{b}	5.65 ± 0.87^{b}	5.93 ± 0.77^{ab}	5.89 ± 1.19^{ab}	5.85 ± 1.47^{ab}
LI-3 (2.5%)	1.77 ± 0.53^{a}	2.52 ± 1.85^{ab}	4.61 ± 1.21^{ab}	6.48 ± 0.63^{ab}	6.30 ± 0.81^{ab}	6.22 ± 1.23^{ab}	6.58 ± 1.17^{a}
LI-4 (2.5%)	1.66 ± 0.25^{a}	2.12 ± 1.52^{ab}	4.41 ± 1.14^{b}	6.34 ± 0.46^{ab}	6.27 ± 0.67^{ab}	6.50 ± 0.45^{a}	6.27 ± 1.25^{ab}
Storage at 10°C							
CTR (0%)	1.40 ± 0.96^{a}	4.22 ± 3.42^{a}	7.63 ± 0.58^{a}	7.50 ± 0.30^{abc}	7.06 ± 1.03^{a}	7.59 ± 0.36^{a}	7.20 ± 0.78^{a}
PI-I (0.5%)	1.56 ± 0.50^{a}	3.58 ± 3.13^{a}	7.07 ± 0.19^{abc}	7.64 ± 0.33^{ab}	7.07 ± 0.94^{a}	6.92 ± 1.07^{a}	7.44 ± 0.67^{a}
PI-2 (1.00%)	1.64 ± 0.18^{a}	3.38 ± 3.25^{a}	6.41 ± 0.78^{bcd}	7.54 ± 0.07^{abc}	7.07 ± 0.81^{a}	6.64 ± 1.60^{a}	7.06 ± 0.92^{a}
PI-3 (0.65%)	1.91 ± 0.55^{a}	3.50 ± 2.77^{a}	7.17 ± 0.14^{ab}	7.37 ± 0.26^{abc}	6.86 ± 1.10^{a}	6.63 ± 2.18^{a}	7.17 ± 0.48^{a}
PI-4 (0.75%)	1.43 ± 0.96^{a}	3.61 ± 3.12^{a}	6.09 ± 0.44^{bcd}	6.78 ± 0.39^{bcd}	6.27 ± 0.60^{a}	7.14 ± 0.92^{a}	7.10 ± 0.25^{a}
PI-5 (1.00%)	1.25 ± 0.36^{a}	2.75 ± 2.07^{a}	5.66 ± 0.52^{d}	6.31 ± 0.24^{d}	6.09 ± 1.04^{a}	6.34 ± 1.52^{a}	7.03 ± 0.27^{a}
LI-1 (2.5%)	1.42 ± 0.39^{a}	3.95 ± 3.00^{a}	7.00 ± 0.34^{abc}	7.61 ± 0.37^{abc}	6.78 ± 1.13^{a}	7.35 ± 0.20^{a}	7.21 ± 0.79^{a}
LI-2 (2.5%)	1.77 ± 0.47^{a}	3.39 ± 2.77^{a}	5.99 ± 0.57^{cd}	6.69 ± 0.05^{cd}	6.21 ± 0.94^{a}	6.25 ± 0.77^{a}	7.01 ± 0.11^{a}
LI-3 (2.5%)	1.77 ± 0.53^{a}	3.76 ± 3.15^{a}	6.91 ± 0.34^{abc}	7.72 ± 0.40^{a}	7.17 ± 0.69^{a}	7.30 ± 0.18^{a}	7.23 ± 0.27^{a}
LI-4 (2.5%)	1.66 ± 0.25^{a}	3.90 ± 3.18^{a}	6.73 ± 0.34^{abcd}	7.51 ± 0.50^{abc}	6.75 ± 1.02^{a}	6.70 ± 1.09^{a}	7.07 ± 0.30^{a}

[1]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
[2]Means ± standard deviation of $n = 6$ observations for each reading, except for control.
[3]The minimum detectability of the methodology was > 10 cells per gram.
[4]Inhibitors in the formulation as in Table 2.3

Table A.6 Population of mesophilic aerobic bacteria[1,2,3] on vacuum-packaged low-sodium frankfurters with powdered or liquid *Listeria* growth inhibitors[4] during 90 days of storage at 4, 7 and 10ºC.

Treatments	0	15	30	45	60	75	90
Storage at 4ºC							
CTR (0%)	2.27 ± 0.36^{a}	6.41 ± 0.53^{a}	6.99 ± 0.18^{a}	7.45 ± 0.45^{a}	7.35 ± 0.57^{a}	7.52 ± 0.46^{a}	7.49 ± 0.56^{a}
PI-2 (1.00%)	2.36 ± 0.72^{a}	3.81 ± 0.19^{b}	5.10 ± 1.01^{b}	5.95 ± 1.48^{ab}	6.27 ± 1.03^{ab}	6.95 ± 0.63^{abc}	7.13 ± 0.52^{a}
PI-3 (0.65%)	2.50 ± 0.42^{a}	3.34 ± 0.75^{b}	4.28 ± 0.96^{bc}	5.77 ± 0.81^{ab}	6.38 ± 0.82^{ab}	7.14 ± 0.56^{ab}	7.49 ± 0.22^{a}
PI-5 (1.00%)	2.57 ± 0.20^{a}	3.03 ± 0.43^{b}	3.53 ± 0.37^{c}	4.06 ± 0.65^{b}	4.88 ± 1.11^{b}	6.08 ± 0.58^{c}	7.33 ± 0.72^{a}
LI-4 (2.5%)	2.30 ± 0.52^{a}	3.17 ± 0.24^{b}	5.28 ± 0.38^{b}	5.81 ± 0.58^{ab}	6.17 ± 0.80^{ab}	6.26 ± 0.55^{bc}	6.81 ± 0.21^{a}
Storage at 7ºC							
CTR (0%)	2.27 ± 0.36^{a}	6.72 ± 0.38^{a}	6.96 ± 0.39^{a}	7.43 ± 0.33^{a}	7.58 ± 0.25^{a}	7.71 ± 0.05^{a}	7.90 ± 0.30^{a}
PI-2 (1.00%)	2.36 ± 0.72^{a}	4.69 ± 0.95^{b}	5.94 ± 0.86^{ab}	6.72 ± 0.54^{a}	6.97 ± 1.19^{a}	7.42 ± 0.37^{a}	7.27 ± 0.40^{a}
PI-3 (0.65%)	2.50 ± 0.42^{a}	4.30 ± 1.21^{bc}	5.57 ± 0.98^{ab}	6.62 ± 0.49^{ab}	7.15 ± 0.77^{a}	7.22 ± 0.61^{ab}	7.40 ± 0.50^{a}
PI-5 (1.00%)	2.57 ± 0.20^{a}	3.07 ± 0.62^{c}	4.22 ± 0.44^{b}	4.75 ± 1.19^{b}	5.06 ± 0.84^{b}	6.41 ± 0.15^{b}	7.18 ± 0.73^{a}
LI-4 (2.5%)	2.30 ± 0.52^{a}	4.66 ± 0.48^{b}	5.78 ± 1.06^{ab}	5.91 ± 0.58^{ab}	6.67 ± 0.35^{a}	6.95 ± 0.12^{ab}	7.16 ± 0.21^{a}
Storage at 10ºC							
CTR (0%)	2.27 ± 0.36^{a}	7.32 ± 0.31^{a}	7.82 ± 0.09^{a}	7.41 ± 0.02^{a}	7.60 ± 0.50^{a}	7.55 ± 0.30^{a}	7.54 ± 0.22^{a}
PI-2 (1.00%)	2.36 ± 0.72^{a}	6.04 ± 0.31^{b}	6.95 ± 0.41^{a}	7.19 ± 0.49^{ab}	7.45 ± 0.19^{a}	7.46 ± 0.48^{a}	7.72 ± 0.09^{a}
PI-3 (0.65%)	2.50 ± 0.42^{a}	5.41 ± 0.68^{b}	6.59 ± 0.65^{a}	6.43 ± 1.23^{ab}	6.94 ± 0.72^{ab}	7.17 ± 0.38^{ab}	7.70 ± 0.49^{a}
PI-5 (1.00%)	2.57 ± 0.20^{a}	3.91 ± 0.13^{c}	4.88 ± 0.65^{b}	5.53 ± 1.02^{b}	6.06 ± 0.67^{b}	6.57 ± 0.64^{b}	7.64 ± 0.49^{a}
LI-4 (2.5%)	2.30 ± 0.52^{a}	6.11 ± 0.12^{b}	6.87 ± 0.38^{a}	6.96 ± 0.26^{ab}	7.04 ± 0.21^{ab}	6.91 ± 0.52^{ab}	6.96 ± 0.77^{a}

[1]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
[2]Means ± standard deviation of $n = 6$ observations for each reading, except for control.
[3]The minimum detectability of the methodology was > 10 cells per gram.
[4]Inhibitors in the formulation as in Table 2.3

Table A.7 Population of *L. monocytogenes*[1] in TSBYE with or without different hop acid extracts at 5 ppm, 0.5 or 1% PAPD, and mixtures of 5 ppm hop acid extracts/0.5% PAPD during 6 days of storage at 7°C.

Treatment	Populations of *L. monocytogenes*[2] (log CFU/mL)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
α-acid	3.68 ± 0.18[a]	4.30 ± 0.35[ab]	4.79 ± 0.63[b]	5.26 ± 0.54[c]	5.69 ± 0.64[b]	6.12 ± 0.64[bc]
β-acid	3.80 ± 0.73[a]	3.55 ± 0.55[ab]	3.51 ± 0.61[a]	3.52 ± 0.50[ab]	3.63 ± 0.51[a]	3.76 ± 1.02[a]
acid-tetra	3.80 ± 0.45[a]	4.14 ± 0.47[ab]	4.28 ± 0.22[ab]	4.96 ± 0.84[bc]	5.33 ± 0.71[b]	5.55 ± 0.66[b]
K-tetra	4.04 ± 0.41[a]	4.48 ± 0.36[ab]	5.15 ± 0.21[b]	5.80 ± 0.31[c]	6.32 ± 0.48[b]	7.02 ± 0.67[bc]
K-hexa	4.01 ± 0.45[a]	4.53 ± 0.36[ab]	5.37 ± 0.32[b]	5.96 ± 0.41[c]	6.55 ± 0.61[b]	7.02 ± 0.75[bc]
α-acid/PAPD	3.45 ± 0.41[a]	3.27 ± 0.46[a]	3.32 ± 0.31[a]	3.40 ± 0.53[a]	3.09 ± 0.44[a]	3.06 ± 0.30[a]
β-acid/PAPD	3.84 ± 0.42[a]	3.40 ± 0.40[ab]	3.36 ± 0.39[a]	3.40 ± 0.46[a]	3.36 ± 0.44[a]	3.35 ± 0.49[a]
acid-tetra/PAPD	3.71 ± 0.60[a]	3.40 ± 0.78[ab]	3.41 ± 0.45[a]	3.50 ± 0.56[ab]	3.39 ± 0.58[a]	3.49 ± 0.46[a]
K-tetra/PAPD	3.78 ± 0.84[a]	3.56 ± 0.55[ab]	3.60 ± 0.52[a]	3.50 ± 0.50[ab]	3.49 ± 0.56[a]	3.43 ± 0.47[a]
K-hexa/PAPD	3.79 ± 0.68[a]	3.62 ± 0.49[ab]	3.48 ± 0.50[a]	3.51 ± 0.58[ab]	3.44 ± 0.45[a]	3.41 ± 0.35[a]
0.5%PAPD	3.86 ± 0.76[a]	3.59 ± 0.51[ab]	3.55 ± 0.60[a]	3.55 ± 0.61[ab]	3.61 ± 0.61[a]	3.64 ± 0.57[a]
1%PAPD	3.69 ± 0.68[a]	3.48 ± 0.47[ab]	3.44 ± 0.51[a]	3.27 ± 0.49[a]	3.36 ± 0.47[a]	3.33 ± 0.39[a]
TSBYE	4.25 ± 0.64[a]	4.74 ± 0.41[b]	5.36 ± 0.30[b]	6.14 ± 0.46[c]	6.67 ± 0.75[b]	7.26 ± 0.99[c]

[1]*Listeria* inoculated with 3.84 ± 0.28 log CFU/mL.
[2]Means ± standard deviation of $n = 6$ observations for each reading.
[a-c]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).

Table A.8 Population of *L. monocytogenes*[1,2] on vacuum-packaged deli-style turkey meat with various inhibitors during 60 days of storage at 4 and 7°C.

Treatment	Population of *Listeria monocytogenes* (log CFU/g) on storage day					
	Day 0	Day 7	Day 15	Day 30	Day 45	Day 60
Storage at 4 °C						
CTR	2.39 ± 0.36 [a]	2.96 ± 0.11 [a]	3.73 ± 0.37 [b]	5.18 ± 0.13 [b]	6.52 ± 0.38 [b]	7.54 ± 0.13 [b]
PLSD	2.48 ± 0.52 [a]	2.33 ± 0.06 [a]	2.37 ± 0.31 [a]	2.37 ± 0.34 [a]	2.36 ± 0.37 [a]	2.39 ± 0.36 [a]
PAPD	2.56 ± 0.37 [a]	2.55 ± 0.29 [a]	2.37 ± 0.15 [a]	2.32 ± 0.32 [a]	2.54 ± 0.60 [a]	2.73 ± 0.51 [a]
α-acid	2.28 ± 0.55 [a]	2.65 ± 0.18 [a]	3.56 ± 0.43 [b]	4.57 ± 0.14 [b]	6.40 ± 0.42 [b]	7.02 ± 0.48 [b]
α-acid/PAPD	2.46 ± 0.17 [a]	2.42 ± 0.31 [a]	2.39 ± 0.26 [a]	2.54 ± 0.47 [a]	2.54 ± 0.34 [a]	2.61 ± 0.60 [a]
β-acid	2.59 ± 0.27 [a]	2.72 ± 0.12 [a]	3.40 ± 0.22 [b]	4.50 ± 0.43 [b]	6.42 ± 0.43 [b]	7.14 ± 0.18 [b]
β-acid/PAPD	2.49 ± 0.29 [a]	2.43 ± 0.38 [a]	2.24 ± 0.14 [a]	2.27 ± 0.47 [a]	2.37 ± 0.38 [a]	2.39 ± 0.44 [a]
Storage at 7 °C						
CTR	2.39 ± 0.36 [x]	3.60 ± 0.51 [y]	5.73 ± 0.33 [y]	7.65 ± 0.24 [y]	8.48 ± 0.19 [y]	8.29 ± 0.10 [z]
PLSD	2.48 ± 0.52 [x]	2.51 ± 0.36 [xy]	2.68 ± 0.50 [x]	3.60 ± 0.63 [x]	4.71 ± 0.99 [x]	5.64 ± 1.04 [y]
PAPD	2.56 ± 0.37 [x]	2.55 ± 0.32 [xy]	2.70 ± 0.25 [x]	3.37 ± 0.50 [x]	3.99 ± 0.77 [x]	4.80 ± 0.39 [xy]
α-acid	2.28 ± 0.55 [x]	3.54 ± 0.49 [y]	5.65 ± 0.37 [y]	7.54 ± 0.25 [y]	8.26 ± 0.16 [y]	8.03 ± 0.11 [z]
α-acid/PAPD	2.46 ± 0.17 [x]	2.58 ± 0.36 [xy]	2.38 ± 0.28 [x]	3.25 ± 0.54 [x]	3.69 ± 0.77 [x]	4.37 ± 0.59 [xy]
β-acid	2.59 ± 0.27 [x]	3.59 ± 0.55 [y]	5.54 ± 0.48 [y]	7.69 ± 0.01 [y]	8.30 ± 0.33 [y]	8.18 ± 0.35 [z]
β-acid/PAPD	2.49 ± 0.29 [x]	2.38 ± 0.11 [x]	2.75 ± 0.02 [x]	3.30 ± 0.19 [x]	4.02 ± 0.41 [x]	4.13 ± 0.78 [x]

[1]Means ± standard deviation of $n = 6$ observations for each reading.
[2]The minimum detectability of the methodology was > 10 cells per gram.
[a-c, x-z]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).

Table A.9 Population of *L. monocytogenes*[1,2] at 4 and 7°C on aerobic-packaged deli-style turkey meat with various inhibitors and sliced after 30 days of storage whole sticks.

Treatment	Population of *Listeria monocytogenes* (log CFU/g) on storage day					
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Storage at 4 °C						
CTR	2.43 ± 0.41 [a]	2.39 ± 0.36 [a]	2.47 ± 0.21 [a]	2.68 ± 0.24 [a]	2.63 ± 0.57 [a]	2.97 ± 0.43 [a]
PLSD	2.42 ± 0.43 [a]	1.97 ± 0.85 [a]	2.28 ± 0.50 [a]	2.36 ± 0.10 [a]	1.99 ± 0.85 [a]	2.24 ± 0.47 [a]
PAPD	2.45 ± 0.30 [a]	2.35 ± 0.37 [a]	2.08 ± 0.43 [a]	2.13 ± 0.38 [a]	2.33 ± 0.31 [a]	1.87 ± 0.81 [a]
α-acid	2.20 ± 0.35 [a]	2.35 ± 0.56 [a]	1.91 ± 0.81 [a]	2.43 ± 0.10 [a]	2.63 ± 0.54 [a]	3.05 ± 0.16 [a]
α-acid/PAPD	2.39 ± 0.45 [a]	2.38 ± 0.33 [a]	2.16 ± 0.41 [a]	1.83 ± 0.76 [a]	1.99 ± 0.85 [a]	2.38 ± 0.17 [a]
β-acid	2.18 ± 0.60 [a]	2.52 ± 0.31 [a]	2.52 ± 0.45 [a]	2.78 ± 0.42 [a]	2.84 ± 0.35 [a]	3.08 ± 0.07 [a]
β-acid/PAPD	2.32 ± 0.58 [a]	2.10 ± 0.35 [a]	2.13 ± 0.38 [a]	2.36 ± 0.10 [a]	1.77 ± 0.68 [a]	1.83 ± 0.72 [a]
Storage at 7 °C						
CTR	2.43 ± 0.41 [x]	2.76 ± 0.27 [x]	2.87 ± 0.51 [x]	3.55 ± 0.41 [y]	4.09 ± 0.12 [y]	4.44 ± 0.21 [z]
PLSD	2.42 ± 0.43 [x]	2.41 ± 0.37 [x]	2.21 ± 0.45 [x]	2.12 ± 0.39 [x]	2.50 ± 0.44 [x]	2.59 ± 0.36 [xy]
PAPD	2.45 ± 0.30 [x]	2.00 ± 0.87 [x]	2.35 ± 0.16 [x]	2.35 ± 0.15 [xy]	2.12 ± 0.39 [x]	2.32 ± 0.28 [x]
α-acid	2.20 ± 0.35 [x]	2.47 ± 0.66 [x]	2.80 ± 0.54 [x]	3.21 ± 0.37 [xy]	3.54 ± 0.16 [y]	4.10 ± 0.57 [yz]
α-acid/PAPD	2.39 ± 0.45 [x]	2.43 ± 0.15 [x]	2.19 ± 0.43 [x]	2.22 ± 0.24 [x]	2.32 ± 0.28 [x]	1.90 ± 0.78 [x]
β-acid	2.18 ± 0.60 [x]	2.25 ± 0.99 [x]	2.90 ± 0.34 [x]	3.53 ± 0.20 [y]	4.04 ± 0.16 [y]	4.17 ± 0.08 [z]
β-acid/PAPD	2.32 ± 0.58 [x]	2.08 ± 0.43 [x]	2.20 ± 0.46 [x]	2.08 ± 0.94 [x]	2.13 ± 0.23 [x]	2.09 ± 0.95 [x]

[1]Means ± standard deviation of $n = 6$ observations for each reading.
[2]The minimum detectability of the methodology was > 10 cells per gram.
[a-c, x-z]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).

Table A.10 Population of *L. monocytogenes*[1,2] at 4 and 7°C on aerobic-packaged deli-style turkey meat with various inhibitors and sliced after 60 days of storage whole sticks.

Treatment	Population of *Listeria monocytogenes* (log CFU/g) on storage day					
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Storage at 4 °C						
CTR	2.76 ± 0.23 [a]	2.73 ± 0.24 [a]	2.84 ± 0.39 [a]	2.98 ± 0.05 [a]	3.06 ± 0.04 [a]	3.21 ± 0.06 [a]
PLSD	2.67 ± 0.45 [a]	2.70 ± 0.33 [a]	2.66 ± 0.26 [a]	2.59 ± 0.27 [a]	2.67 ± 0.28 [a]	2.66 ± 0.22 [a]
PAPD	2.78 ± 0.35 [a]	2.69 ± 0.31 [a]	2.61 ± 0.34 [a]	2.62 ± 0.31 [a]	2.66 ± 0.26 [a]	2.53 ± 0.13 [a]
α-acid	2.73 ± 0.34 [a]	2.72 ± 0.26 [a]	2.59 ± 0.36 [a]	2.56 ± 0.28 [a]	2.65 ± 0.37 [a]	2.74 ± 0.24 [a]
α-acid/PAPD	2.78 ± 0.11 [a]	2.50 ± 0.35 [a]	2.54 ± 0.34 [a]	2.63 ± 0.38 [a]	2.56 ± 0.28 [a]	2.67 ± 0.36 [a]
β-acid	2.66 ± 0.26 [a]	2.59 ± 0.41 [a]	2.66 ± 0.55 [a]	2.83 ± 0.63 [a]	3.06 ± 0.65 [a]	3.27 ± 0.86 [a]
β-acid/PAPD	2.78 ± 0.19 [a]	2.61 ± 0.32 [a]	2.47 ± 0.29 [a]	2.45 ± 0.18 [a]	2.59 ± 0.26 [a]	2.56 ± 0.17 [a]
Storage at 7 °C						
CTR	2.76 ± 0.23 [x]	2.92 ± 0.17 [x]	3.00 ± 0.03 [x]	3.29 ± 0.13 [x]	3.65 ± 0.01 [y]	4.08 ± 0.16 [y]
PLSD	2.67 ± 0.45 [x]	2.63 ± 0.16 [x]	2.68 ± 0.19 [x]	2.73 ± 0.21 [x]	2.76 ± 0.23 [x]	2.84 ± 0.28 [x]
PAPD	2.78 ± 0.35 [x]	2.73 ± 0.33 [x]	2.68 ± 0.24 [x]	2.64 ± 0.19 [x]	2.68 ± 0.24 [x]	2.75 ± 0.25 [x]
α-acid	2.73 ± 0.34 [x]	2.76 ± 0.26 [x]	2.68 ± 0.07 [x]	2.79 ± 0.20 [x]	2.84 ± 0.10 [x]	3.05 ± 0.09 [x]
α-acid/PAPD	2.78 ± 0.11 [x]	2.72 ± 0.32 [x]	2.69 ± 0.24 [x]	2.71 ± 0.18 [x]	2.72 ± 0.23 [x]	2.80 ± 0.33 [x]
β-acid	2.66 ± 0.26 [x]	2.73 ± 0.38 [x]	3.00 ± 0.80 [x]	3.20 ± 1.04 [x]	3.50 ± 1.23 [y]	3.87 ± 1.32 [y]
β-acid/PAPD	2.78 ± 0.19 [x]	2.73 ± 0.26 [x]	2.58 ± 0.09 [x]	2.70 ± 0.28 [x]	2.63 ± 0.08 [x]	2.67 ± 0.18 [x]

[1]Means ± standard deviation of $n = 6$ observations for each reading.
[2]The minimum detectability of the methodology was > 10 cells per gram.
[a-b, x-y]Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).

Table A.11 Population of *L. monocytogenes*[1,2] (log CFU/mL) in skim milk with or without different hop acid extracts at 5 ppm or 0.5% PAPD during 6 days of storage at 7°C.

Treatment	Storage time (day)					
	1	2	3	4	5	6
α-acid	4.19 ± 0.27[a]	4.31 ± 0.23[a]	4.41 ± 0.50[a]	4.71 ± 0.53[ab]	4.97 ± 0.76[ab]	5.13 ± 0.80[abc]
β-acid	4.20 ± 0.21[a]	4.43 ± 0.38[a]	4.59 ± 0.44[a]	4.90 ± 0.40[ab]	5.06 ± 0.71[ab]	5.13 ± 0.73[abc]
acid-tetra	4.20 ± 0.23[a]	4.28 ± 0.43[a]	4.21 ± 0.39[a]	4.37 ± 0.33[ab]	4.53 ± 0.49[ab]	4.67 ± 0.60[ab]
K-tetra	4.13 ± 0.26[a]	4.23 ± 0.56[a]	4.25 ± 0.21[a]	4.22 ± 0.30[ab]	4.27 ± 0.45[ab]	4.51 ± 0.43[ab]
K-hexa	4.20 ± 0.22[a]	4.38 ± 0.44[a]	4.34 ± 0.53[a]	4.63 ± 0.51[ab]	4.84 ± 0.62[ab]	4.98 ± 0.63[abc]
α-acid/PAPD	4.14 ± 0.20[a]	4.18 ± 0.66[a]	3.96 ± 0.43[a]	3.95 ± 0.32[a]	3.98 ± 0.42[a]	3.93 ± 0.37[a]
β-acid/PAPD	4.08 ± 0.29[a]	4.16 ± 0.53[a]	4.11 ± 0.33[a]	3.98 ± 0.35[a]	3.95 ± 0.31[a]	3.92 ± 0.24[a]
acid-tetra/PAPD	4.10 ± 0.29[a]	4.13 ± 0.62[a]	3.81 ± 0.51[a]	3.99 ± 0.29[a]	4.00 ± 0.36[a]	4.03 ± 0.35[a]
K-tetra/PAPD	4.03 ± 0.38[a]	4.23 ± 0.53[a]	3.86 ± 0.37[a]	4.00 ± 0.35[a]	3.92 ± 0.48[a]	3.97 ± 0.33[a]
K-hexa/PAPD	4.03 ± 0.37[a]	4.23 ± 0.57[a]	3.94 ± 0.25[a]	4.00 ± 0.40[a]	3.98 ± 0.34[a]	3.92 ± 0.30[a]
0.5%PAPD	4.11 ± 0.20[a]	4.15 ± 0.53[a]	3.89 ± 0.38[a]	3.93 ± 0.33[a]	3.96 ± 0.35[a]	3.98 ± 0.30[a]
Skim milk	4.34 ± 0.26[a]	4.55 ± 0.45[a]	4.74 ± 0.42[a]	5.16 ± 0.65[ab]	5.56 ± 0.66[b]	5.91 ± 0.52[bc]
TSBYE	4.29 ± 0.24[a]	4.39 ± 0.34[a]	4.84 ± 0.62[a]	5.26 ± 0.65[b]	5.63 ± 0.49[b]	6.10 ± 0.32[c]

[1]Means ± standard deviation of $n = 6$ observations for each reading.
[2]The minimum detectability of the methodology was > 10 cells per gram.
[a-c] Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).

Table A.12 Population of *L. monocytogenes*[1,2] (log CFU/mL) in 2% milk with or without different hop acid extracts at 5 ppm or 0.5% PAPD during 6 days of storage at 7°C.

Treatment	Storage time (day)					
	1	2	3	4	5	6
α-acid	4.16 ± 0.22[a]	4.35 ± 0.30[a]	4.52 ± 0.47[a]	4.76 ± 0.61[a]	5.07 ± 0.86[a]	5.24 ± 0.90[ab]
β-acid	4.17 ± 0.21[a]	4.33 ± 0.36[a]	4.56 ± 0.48[a]	4.89 ± 0.74[a]	5.12 ± 0.86[a]	5.29 ± 0.93[ab]
acid-tetra	4.11 ± 0.34[a]	4.25 ± 0.27[a]	4.34 ± 0.40[a]	4.50 ± 0.45[a]	4.80 ± 0.81[a]	4.95 ± 1.03[ab]
K-tetra	4.14 ± 0.27[a]	4.24 ± 0.35[a]	4.33 ± 0.31[a]	4.46 ± 0.45[a]	4.72 ± 0.82[a]	4.94 ± 0.85[ab]
K-hexa	4.20 ± 0.22[a]	4.24 ± 0.42[a]	4.41 ± 0.43[a]	4.66 ± 0.63[a]	4.51 ± 1.17[a]	5.11 ± 0.81[ab]
α-acid/PAPD	4.00 ± 0.34[a]	4.16 ± 0.30[a]	3.98 ± 0.42[a]	4.01 ± 0.30[a]	3.86 ± 0.41[a]	3.85 ± 0.42[a]
β-acid/PAPD	4.14 ± 0.21[a]	4.04 ± 0.36[a]	4.04 ± 0.39[a]	3.96 ± 0.31[a]	3.95 ± 0.37[a]	3.95 ± 0.36[a]
acid-tetra/PAPD	4.04 ± 0.36[a]	4.06 ± 0.39[a]	4.02 ± 0.42[a]	4.04 ± 0.37[a]	3.98 ± 0.29[a]	3.94 ± 0.30[a]
K-tetra/PAPD	4.06 ± 0.31[a]	4.04 ± 0.42[a]	4.02 ± 0.37[a]	3.94 ± 0.36[a]	3.95 ± 0.38[a]	3.88 ± 0.38[a]
K-hexa/PAPD	4.09 ± 0.23[a]	3.95 ± 0.41[a]	3.94 ± 0.49[a]	3.93 ± 0.30[a]	3.96 ± 0.40[a]	3.96 ± 0.40[a]
0.5%PAPD	4.00 ± 0.31[a]	4.10 ± 0.23[a]	4.06 ± 0.30[a]	3.99 ± 0.42[a]	3.98 ± 0.50[a]	3.88 ± 0.29[a]
2% Milk	4.29 ± 0.23[a]	4.35 ± 0.29[a]	4.63 ± 0.39[a]	5.13 ± 0.59[a]	5.63 ± 0.49[a]	5.76 ± 0.68[ab]
TSBYE	4.29 ± 0.24[a]	4.39 ± 0.34[a]	4.84 ± 0.62[a]	5.26 ± 0.65[a]	5.63 ± 0.49[a]	6.10 ± 0.32[b]

[1]Means ± standard deviation of $n = 6$ observations for each reading.
[2]The minimum detectability of the methodology was > 10 cells per gram.
[a-c] Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).

Table A.13 Population of *L. monocytogenes*[1,2] (log CFU/g) in turkey slurries containing α-acid or β-acid at 0 to 1000 ppm during 12 days of storage at 7°C.

Treatment	Number of *L. monocytogenes* (log CFU/g)				
	Day 0	Day 3	Day 6	Day 9	Day 12
α-acid 5 ppm	2.43 ± 0.43 [a, A]	3.44 ± 0.26 [d, AB]	4.49 ± 0.34 [d, B]	5.72 ± 0.49 [b, C]	6.71 ± 0.52 [b, C]
α-acid 25 ppm	2.37 ± 0.45 [a, A]	3.10 ± 0.58 [bcd, AB]	4.21 ± 0.34 [d, BC]	5.35 ± 0.63 [b, CD]	6.14 ± 0.62 [b, D]
α-acid 50 ppm	2.45 ± 0.43 [a, A]	2.46 ± 0.45 [abcd, A]	3.44 ± 0.28 [cd, AB]	4.41 ± 0.48 [b, BC]	5.19 ± 0.63 [b, C]
α-acid 100 ppm	2.37 ± 0.33 [a, A]	2.33 ± 0.43 [abcd, A]	2.44 ± 0.52 [abc, A]	2.64 ± 0.48 [a, A]	3.18 ± 0.70 [a, A]
α-acid 500 ppm	2.27 ± 0.30 [a, A]	2.18 ± 0.37 [abc, A]	2.20 ± 0.48 [ab, A]	2.18 ± 0.58 [a, A]	2.10 ± 0.47 [a, A]
α-acid 1000 ppm	2.23 ± 0.44 [a, A]	1.92 ± 0.42 [a, A]	1.85 ± 0.39 [a, A]	1.77 ± 0.46 [a, A]	1.83 ± 0.50 [a, A]
β-acid 5 ppm	2.41 ± 0.44 [a, A]	3.36 ± 0.31 [d, A]	4.57 ± 0.38 [d, B]	5.75 ± 0.43 [b, C]	6.54 ± 0.52 [b, C]
β-acid 25 ppm	2.43 ± 0.46 [a, A]	3.25 ± 0.32 [cd, AB]	4.41 ± 0.32 [d, BC]	5.48 ± 0.59 [b, CD]	6.09 ± 0.60 [b, D]
β-acid 50 ppm	2.42 ± 0.44 [a, A]	2.90 ± 0.37 [abcd, A]	4.28 ± 0.29 [d, B]	5.33 ± 0.45 [b, BC]	5.83 ± 0.70 [b, C]
β-acid 100 ppm	2.36 ± 0.46 [a, A]	2.62 ± 0.36 [abcd, A]	3.38 ± 0.27 [bcd, A]	4.84 ± 0.49 [b, B]	5.54 ± 0.73 [b, B]
β-acid 500 ppm	2.40 ± 0.43 [a, A]	2.37 ± 0.49 [abcd, A]	2.24 ± 0.63 [ab, A]	2.29 ± 0.66 [a, A]	2.10 ± 0.60 [a, A]
β-acid 1000 ppm	2.35 ± 0.42 [a, A]	1.97 ± 0.43 [ab, A]	1.79 ± 0.45 [a, A]	1.54 ± 0.53 [a, A]	1.80 ± 0.71 [a, A]
Control 0 ppm	2.47 ± 0.36 [a, A]	3.48 ± 0.23 [d, B]	4.57 ± 0.38 [d, C]	5.83 ± 0.41 [b, D]	6.88 ± 0.61 [b, E]

[1]Means ± standard deviation of $n = 6$ observations for each reading.
[2]The minimum detectability of the methodology was > 10 cells per gram.
[a-d] Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
[A-E]Mean values with same letters in the same row were not significantly different ($P \geq 0.05$).

APPENDIX B

Calculation of combination index (CI)

Calculation of combination index (CI)

In Chapter 3, the combination index (CI) was calculated to determine the synergistic effect of hop/PAPD combination in *Listeria* inhibition. CI was the result of sum of log-reduction (comparing to initial inoculation) from individual treatment dividing by log-reduction from combination treatment. The results were interpreted as synergistic (CI < 1), additive (CI = 1), and antagonistic (CI > 1). However, the results showed in Table 3.2 are calculated based on mean values of log reduction, which might lack of degree of uncertainty. To be more realistic, the calculation of CI should use all data from the experiment and the CI should be presented in range of value (Table B.1).

Table B.1 Interpretation possible effects of 25 ppm hop acid extract/0.5% PAPD mixtures on *Listeria monocytogenes* counts in TSBYE broth after incubation at 37°C for 24 h.

Treatment	Log-reduction from hop acid or PAPD alone	Log-reduction from hop/PAPD	Sum of log-reduction from hop acid alone and PAPD alone	Possible CI	Interpretation
α-acid	3.67 - 4.41	4.57 - 4.95	5.04 - 6.36	1.02 - 1.39	AN
β-acid	1.86 - 2.25	4.57 - 4.95	3.23 - 4.20	0.65 - 0.92	SY
Acid-tetra	1.67 - 2.77	4.57 - 4.95	3.04 - 4.72	0.61 - 1.03	AN/SY/AD
K-tetra	1.79 - 2.23	3.47 - 4.95	3.16 - 4.18	0.64 - 1.20	AN/SY/AD
K-hexa	1.43 - 2.07	3.04 - 4.95	2.80 - 4.02	0.57 - 1.38	AN/SY/AD
PAPD	1.37 - 1.95				-

[1] AN: Antagonistic, SY: Synergistic, AD: Additive.

Example of CI calculation:

1. CI from mean value of log reduction.

Given: mean of log reduction from α-acid is 3.94.

mean of log reduction from PAPD is 1.59.

mean of log reduction from α-acid/PAPD is 4.74.

Therefore, sum of log reduction from α-acid alone and PAPD alone is 5.53 (from 3.94+1.59).

CI is 1.17 (from 5.53/4.74).

2. CI from all values of log reduction.

Given: log reduction of from α-acid is 3.67 to 4.41 units (data from experiment).

log reduction of from PAPD is 1.37 to 1.95 units (data from experiment).

log reduction from α-acid/PAPD is 4.57 to 4.95 units (data from experiment).

Therefore, sum of log reduction from α-acid alone and PAPD alone is 5.04 (from 3.67+1.37) to 6.36 (from 4.41+1.95).

Possible CI is 1.02 (from 5.04/4.95) to 1.39 (from 6.36/4.57).

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