FINAL REPORT

AMERICAN MEAT INSTITUTE FOUNDATION

PROJECT TITLE

COMPARISON OF USE OF ACTIVATED LACTOFERRIN WITH USE OF A 'GOLD STANDARD' COMBINATION/CONCENTRATION OF ANTIMICROBIALS FOR POST-PROCESSING CONTROL OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT MEAT PRODUCTS

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SUMMARY OF STUDIES

Contamination of ready-to-eat (RTE) meat products with *Listeria monocytogenes* is a major concern to the meat processing industry and needs to be addressed in order to enhance the safety of these products. The studies described in this report were designed to evaluate lactoferrin, as well as activated lactoferrin (ALF) as additional hurdles for *L. monocytogenes* control in various RTE meat and poultry products. Specifically, the objective of this work was to examine the behavior of *L. monocytogenes* in RTE meat products containing lactoferrin, surface treated (dipped or sprayed) with the activated form of this protein (ALF), or both, in comparison to or in combination with the effective combination of sodium/potassium lactate and sodium diacetate, which is currently used in processed meats. Additional studies were conducted to model the growth/no growth boundaries of the pathogen in RTE meat products as a function of lactic acid concentration (0, 1, 2, 3, and 4%), dipping time (0, 1, 2, 3, or 4 min), and storage temperature (4, 7, or 10^{o}C). One of the studies was designed to investigate the antilisterial activity of lactoferrin as a formulation ingredient in bologna stored at 4 or 7^{o}C in vacuum packages. Lactoferrin was used individually or in combination with potassium lactate and/or sodium diacetate. In addition, another study evaluated ALF as a dipping (30 s) or spraying (10 psi, 2 s per side) solution in comparison or in combination with water or lactic acid against *L. monocytogenes* on bologna stored anaerobically at 7^{o}C. Moreover, a number of studies evaluated the antilisterial activity of ALF, applied as a surface treatment for 60 to 180 s in comparison or in combination with organic acids (lactic acid or acetic acid) or their salts (potassium lactate or sodium diacetate) in various products, including bologna, uncured or cured turkey breast, ham and frankfurters. Finally, the effectiveness of lactoferrin incorporated into frankfurters individually or together with potassium lactate, was evaluated in combination with immersion of the finished product into solutions of acetic acid or ALF.

Overall, the results showed that, under the conditions of this study, lactoferrin added as a formulation ingredient in bologna was not as effective as the combination of potassium lactate and sodium diacetate; also lactoferrin used in combination with potassium lactate and/or sodium diacetate did not enhance, or in some instances reduced, their antimicrobial activity. However, in frankfurters, lactoferrin combined with potassium lactate in the formulation was as effective as the potassium lactate-sodium diacetate combination. Furthermore, ALF as a post-processing antimicrobial solution on products including, frankfurters, and slices of bologna (formulated with beef only or beef and pork), ham, and uncured and cured turkey breast, was found not as effective as organic acid (acetic or lactic acid) or salt (sodium diacetate) solutions. It should be noted that in frankfurters formulated with potassium lactate and lactoferrin, dipping in ALF solution after processing, was as effective as dipping in acetic acid for frankfurters of the same formulation; both combinations did not allow growth for 50 days of storage at 7°C.

INTRODUCTION

Listeria monocytogenes, the causative agent of listeriosis has been associated with major, highly publicized recalls of ready-to-eat (RTE) meat and poultry products contaminated with this pathogen (CDC, 1999; 2000; 2002). According to the quantitative assessment of the relative risk to public health from *L. monocytogenes* that was developed by the Food and Drug Administration (FDA) and the USDA-FSIS, among 23 categories of RTE foods, deli meats and

non-reheated frankfurters were identified as products of highest risk for listeriosis on a per-serving basis (FDA-USDA, 2003).

Listeria monocytogenes is an important post-processing food contaminant and has been isolated from a variety of foods of plant or animal origin (Farber and Peterkin, 1991); however, post-processing contamination of cooked RTE meat products that do not receive further heat treatment before consumption is of particular concern. Although the pathogen is frequently present in product ingredients, it usually does not survive regular commercial heat treatments and, therefore, the main source of food contamination seems to be the processing environment (Kathariou, 2002).

Listeria monocytogenes is a remarkably difficult organism to control in food-processing environments due to its widespread distribution (Weis and Seeliger, 1975), its psychrotrophic nature (Junttila et al., 1988), and its ability to tolerate adverse growth conditions (Lou and Yousef, 1999). Traditional cleaning and sanitation procedures, and implementation of Hazard Analysis Critical Control Point (HACCP) programs often appear to be insufficient to prevent the presence or inhibit the growth of the pathogen in processed meat products (Tompkin et al., 1999; Tompkin, 2002), and this can be supported by various surveys revealing that *L. monocytogenes* may be present in RTE meat and poultry products. Specifically, the USDA-FSIS monitoring activities, conducted from 1990 to 1999 at 1,800 establishments of RTE meat and poultry products, revealed that the pathogen was isolated from as much as 5% of some RTE products, such as sliced luncheon meats (Levine et al., 2001). Also, a two-year survey conducted by Wallace et al. (2003) on the prevalence of *L. monocytogenes* in vacuum-packages of refrigerated frankfurters obtained from twelve commercial manufacturers revealed that the pathogen was recovered from 1.65% of the packages tested, and furthermore, was present in at least one package produced in seven of the plants. Another survey (Gombas et al., 2003) of various RTE foods, including luncheon meats, deli salads, various cheeses and seafood products demonstrated that the overall prevalence of *L. monocytogenes* was 1.82%, whereas prevalence among the product categories ranged from 0.17% (fresh soft cheese) to 4.7% (seafood salads).

In June 2003, the USDA-FSIS published an interim final rule, according to which federally inspected establishments that produce RTE meat or poultry products that support growth of the pathogen and are exposed to the environment after lethality treatment are required to take certain measures in order to prevent product adulteration with *L. monocytogenes* and verify the effectiveness of such measures through testing (FSIS, 2003a). In addition, USDA-FSIS inspectors are instructed to perform verification activities, in order to evaluate the effectiveness of the control programs employed by each processing facility. Establishments are required to select one of three alternatives for *L. monocytogenes* control: (i) Alternative 1- Employ both a post-lethality treatment that reduces or kills *L. monocytogenes* (e.g., steam, pressure or antimicrobial agent) and a growth inhibitor that prevents or limits growth of the pathogen (antimicrobial agent or process that inhibits growth); (ii) Alternative 2- Employ either a post-lethality system or a growth inhibitor; in this case, however, establishments will be subject to more frequent USDA-FSIS verification testing than establishments selecting Alternative 1; and, (iii) Alternative 3- Employ sanitation measures only. The latter alternative also requires testing of food contact surfaces for *L. monocytogenes* or indicator organisms in order to confirm the efficacy of sanitation procedures in the post-lethality processing environment, as well as product-

holding procedures when positive tests are obtained. Establishments that rely only on sanitation measures, however, will be subject to the most frequent USDA-FSIS verification activity (FSIS, 2003a). It is obvious from these new regulatory requirements that there is a need to identify alternatives for control of *L. monocytogenes* in post-processing contaminated products.

Research conducted in recent years has revealed that the addition of chemical compounds (e.g., lactates, acetates, diacetates) in the formulation of RTE meat and poultry products can control *L. monocytogenes* contamination. The antilisterial effects of such compounds employed as formulation ingredients (Schlyter et al., 1993a, 1993b; Wederquist et al., 1994; Blom et al., 1997; Bedie et al., 2001; Islam et al., 2002; Mbandi and Shelef, 2001, 2002; Samelis et al., 2002; Porto et al., 2002; Stekelenburg, 2003; Choi and Chin, 2003; Barmpalia et al., 2004, 2005) or as dipping solutions (Schlyter et al., 1993a, 1993b; Palumbo and Williams, 1994; Ariyapitipun et al., 2000; Samelis et al., 2001; Glass et al., 2002; Barmpalia et al., 2004; Uhart et al., 2004) have been evaluated in various meat products and the results obtained from these studies may be valuable to the meat industry in its effort to find effective methods for prevention or inhibition of *L. monocytogenes* growth in RTE products and meet the recent regulatory requirements. Other preservation factors, such as natural antimicrobials of plant, microbial or other origin (Burt, 2004; Aymerich et al., 2005), packaging materials with immobilized antimicrobials (Hoffman et al., 2001; Franklin et al., 2004; Grower et al., 2004; Luchansky and Call, 2004), thermal pasteurization (Samelis et al., 2002; McCormick et al., 2003; Muriana et al., 2004), irradiation (McCormick et al., 2003; Chen et al., 2004; Foong et al., 2004; Sommers and Boyd, 2005), and/or high pressure treatments (Morgan et al., 2000; Karatzas et al., 2001; Hayman et al., 2004; Aymerich et al., 2005) may also assist against accidental post-processing contamination of RTE meat products with the pathogen. The studies presented here evaluated the effectiveness of lactoferrin, as well as that of a new form of this naturally occurring protein (activated lactoferrin) against *L. monocytogenes* in RTE meat and poultry products in combination or in comparison with potassium lactate, sodium diacetate, and lactic or acetic acid.

Lactoferrin is a glycoprotein found in milk that exerts antimicrobial activity due to its ability to bind iron (Naidu, 2002). The antimicrobial and antilisterial activity of lactoferrin has been demonstrated in milk (Payne et al., 1990, 1994). However, to our knowledge, published literature on the antilisterial activity of lactoferrin on meat products is limited. The activated form of lactoferrin (ALF), which is produced by a patented method developed by Naidu (2001), has the ability to prevent bacteria from attaching and growing on biological surfaces (Naidu, 2002). Recently, the FDA designated ALF as a Generally Recognized as Safe (GRAS) compound (FDA-CFSAN-OFAS, 2003). Also, the USDA-FSIS has approved the use of ALF as a rinse on beef carcasses for control of pathogenic bacteria, including *Escherichia coli* O157:H7, *Salmonella*, *L. monocytogenes*, and *Campylobacter* (FSIS, 2003b). According to GRAS notice No. GRN 00067, the use of ALF is approved only in meat parts (not comminuted meat) and the application of the protein must be declared on the label. According to data provided to FDA by aLF Ventures (Salt Lake City, UT), consumption of lactoferrin at approved levels is most likely safe even for individuals who are allergic to milk (FDA-CFSAN-OFAS, 2003). The status of using ALF in RTE meat products is not clear at this time. However, since FDA has approved it as GRAS, use in such products may be approved by USDA-FSIS if found effective, but labeling may be required due to its potential allergenicity.

Although the antilisterial activity of lactoferrin and ALF in RTE meat products has not been thoroughly investigated, it is possible that this new antimicrobial may offer potential inhibitory effects against *L. monocytogenes* in such products. Except for a preliminary study conducted in our laboratory (Ransom et al., 2003), evaluating ALF as a post-processing dipping solution, to our knowledge, the antilisterial activity of lactoferrin or ALF as a formulation ingredient of a meat product introduced before thermal processing has not been evaluated. For such an application, the potential thermal denaturation of the protein during product cooking should be considered. A review of the literature indicated that although it is known that lactoferrin is denatured at high temperatures (Ford et al., 1977; Rüegg et al., 1977), there is a potential for activity at temperatures employed in processing of meat products. A study by Abe et al. (1991) indicated that under acidic conditions (pH 2 or 3), some lactoferrin fragments produced by heat denaturation (100 or 120^{o}C for 5 min) may have antimicrobial properties. However, heat-treated lactoferrin (100^{o}C, 5 min) completely lost its activity in solutions of near neutral or alkaline pH (6-10). Saito et al. (1994) demonstrated that the antibacterial activity of lactoferrin fragments, derived from thermal treatment at pH 2-3, was greater than that of lactoferrin. Also, a study by Hoek et al. (1997) showed that fragments of lactoferrin (produced by hydrolyzing the protein with chymosin at low pH) exerted antimicrobial activity against several Gram-positive and Gram-negative bacteria, including *L. monocytogenes*, *E. coli*, and *Staphylococcus aureus*. In general, the results of the studies discussed above suggest that heat denaturation of lactoferrin does not necessarily mean absence of antimicrobial properties, and considering that the heat stability of lactoferrin or the antimicrobial activity of its heat-induced fragments is affected by various parameters, other than temperature and time (e.g., pH, concentration of protein, type of acidulant), the antilisterial effect of lactoferrin, as a formulation ingredient, needs to be evaluated under conditions that simulate processing and cooking of meat products.

PROJECT OBJECTIVES

The objective of this project, as stated in the original proposal, was to examine the behavior of *L. monocytogenes* in RTE meat products treated with ALF (as a formulation ingredient or a post-processing dipping solution) in comparison or in combination with other antimicrobials including the effective and widely used combination of sodium lactate and sodium diacetate.

In addition to the objective stated in the original proposal, the following objectives were also investigated:

1. Evaluation of the antilisterial properties of ALF applied as a dipping solution individually or as a sequential treatment on the surface of uncured poultry products (turkey breast was selected following a request by AMIF).

2. Comparison of the performance of dipping vs. spraying as methods for applying antimicrobial solutions (including ALF) on the surface of RTE meat products at the post-processing stage.

3. Evaluation of whether and to what degree surface treatment (dipping) duration may affect the antilisterial properties of ALF.

4. Comparison of growth kinetics of two inocula consisting of different *L. monocytogenes* strains on products treated with ALF and other compounds, in order to investigate potential strain-dependent differences in antimicrobial susceptibility.

5. Determination of whether meat product formulation (beef versus beef and pork) may affect antimicrobial properties.

6. Modeling the growth/no growth boundaries of *L. monocytogenes* in RTE meat and poultry products dipped into solutions of lactic acid, as a function of different lactic acid concentrations, dipping times and storage temperatures.

Moreover, a few modifications to the submitted proposal were made after its approval or during the course of the studies. These include:

1. Use of 0.125%, instead of 0.25% sodium diacetate, in the product formulation as requested by AMIF; 0.25% sodium diacetate in the product formulation was only used in a preliminary study.

2. Use of non-activated lactoferrin instead of ALF as a formulation additive based on inputs of Verdis N. Norton, President of aLF Ventures (the reason is discussed below).

3. Modeling of *L. monocytogenes* data as a function of storage time, using the model of Baranyi and Roberts, was conducted as a more efficient way of comparing treatment effects.

I. PRELIMINARY STUDIES

A. Effect of heat treatment on the antilisterial activity of ALF

In this preliminary study, two factors that could potentially affect the antilisterial activity of ALF were tested: (i) heat (71^{o}C) and (ii) pH. Tryptic soy broth (TSB; Difco, Becton Dickinson Co., Sparks, MD) was formulated to contain ALF (aLF Ventures, Salt Lake City, UT) at 0, 0.1, 0.3, or 0.5%, and was heated in a water bath to a final temperature of 71^{o}C. The ALF containing TSB was prepared by adding 1 ml of a 1 (0.1%), 3 (0.3%) or 5% (0.5%) ALF solution to tubes containing 9 ml of TSB. Tubes containing 10 ml of TSB served as controls (0% ALF). The effect of pH on the antilisterial properties of ALF was determined by using TSB (containing 0, 0.1, 0.3, or 0.5% ALF) with pH values adjusted to 5, 6, and 6.5 with HCl (5N). The inoculum, which consisted of 10 *L. monocytogenes* strains, was added (0.1 ml) to heated (after cooling), as well as unheated TSB containing ALF (0, 0.1, 0.3, 0.5%). The 10 strains included in the inoculum were Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known). All the strains were available as frozen stock cultures (-70^{o}C) and were activated by transferring a loopful of stock culture into 10 ml of TSB (Difco) supplemented with 0.6% yeast extract (Acumedia, Baltimore, MD; TSBYE) and incubating at 30^{o}C for 24 h. The strains were subcultured twice in TSBYE before use in the experiment. The TSBYE cultures were centrifuged (five strains in each conical centrifuge tube; 4629 x *g*, 15 min, 4^{o}C), washed with 10

ml of sterile phosphate-buffered saline (PBS) and then centrifuged again (4629 x *g*, 15 min, 4°C). The resulting pellet was resuspended in 20 ml of PBS and serially diluted to a concentration estimated to yield 2-3 log CFU/ml. Inoculated broths (n=3) were incubated at 25±2°C and analyzed for *L. monocytogenes* populations after 0, 24, and 48 h by plating on tryptic soy agar (Difco) supplemented with 0.6% yeast extract (TSAYE). Following incubation (48 h, 30°C) colonies were counted and data were transformed to log CFU/ml.

Results of this study (Table 1) suggest that heat treatment at 71°C may not affect the antilisterial properties of ALF (compared to unheated ALF) and, therefore, its use as a meat additive prior to heat treatment may be considered. However, later discussions with Verdis N. Norton, President of aLF Ventures, indicated to us that ALF is not recommended as an additive in processed meat products due to its interaction with other chemical ingredients, which may result in decreased antimicrobial activity. Thus, regular lactoferrin (instead of ALF, originally proposed) was used as an ingredient in subsequent studies discussed below.

B. Antilisterial effects of lactoferrin, potassium lactate, and sodium diacetate added individually or in combination in the formulation of bologna

A second study was conducted to evaluate the antilisterial activity of various levels of lactoferrin added individually or in combination with potassium lactate and/or sodium diacetate in the formulation of a processed meat product. This study was also performed to test the heat stability of lactoferrin under realistic meat processing conditions.

Fresh pork (approximately 30% fat) and beef trimming (approximately 25% fat) were obtained from Swift Co. (Greeley, CO). The basic bologna formulation (Samelis et al., 2001) consisted of (%, wt/wt): meat (40% pork and 60% beef) trimmings (82.2), ice (10), sodium chloride (2), dextrose (2), dry mustard (0.9), corn syrup solids (2), polyphosphate (0.4; sodium tripolyphosphate and sodium hexameta-phosphate; Heller Inc., Bedford Park, IL), sodium nitrite (0.0156), sodium erythorbate (0.05), paprika (0.25), onion powder (0.05), garlic powder (0.05), coriander (0.05), and white pepper (0.05). Spices and seasonings were purchased from AC Legg Co. (Birmingham, AL). Nine batches were formulated separately to contain:

1. No antimicrobials (control)
2. Potassium lactate (3% of a 60% [wt/wt] commercial product; equivalent to 1.8% pure potassium lactate; Purac Inc., Lincolnshire, IL)
3. Sodium diacetate (0.25%; Niacet, Niagara Falls, NY)
4. Lactoferrin (0.25%; aLF Ventures)
5. Lactoferrin (0.5%)
6. Potassium lactate (1.8%) combined with sodium diacetate (0.25%)
7. Potassium lactate (1.8%) combined with lactoferrin (0.25%)
8. Sodium diacetate (0.25%) combined with lactoferrin (0.25%)
9. Potassium lactate (1.8%) combined with sodium diacetate (0.25%) and lactoferrin (0.25%).

Bologna was processed according to procedures described in a study by Samelis et al. (2001). Specifically, the ingredients of each batch were emulsified in a 35-L bowl chopper (RMF, Kansas City, MO) for 3-5 min to a final temperature of 15.5°C. The mixture was then extruded (Handtmann Inc., Buffalo Grove, IL) into 65 mm diameter fibrous cellulose casings (Koch,

Table 1: Mean (n=3) *Listeria monocytogenes* populations (log CFU/ml ± standard deviation) in inoculated, unheated or heated (prior to inoculation), to a final temperature of 71°C, tryptic soy broth containing various concentrations of activated lactoferrin and acidified to various pH levels, followed by storage at 25±2°C for up to 48 h.

Storage for:		0 hours				24 hours				48 hours			
		Concentration of activated lactoferrin (%)											
	pH	0	0.1	0.3	0.5	0	0.1	0.3	0.5	0	0.1	0.3	0.5
Heated	5	2.0±0.2	2.1±0.1	3.1±0.2	3.0±0.1	5.8±0.5	5.6±0.0	4.9±0.1	3.3±0.0	8.5±0.2	7.9±0.3	6.8±0.0	5.0±0.4
	6	2.1±0.2	3.1±0.1	3.0±0.6	2.9±0.1	8.0±0.0	7.5±0.2	7.9±0.1	7.6±0.3	8.7±0.0	8.4±0.2	8.5±0.1	8.7±0.3
	6.5	2.4±0.2	2.4±0.2	3.0±0.5	2.9±0.2	7.7±0.5	7.7±0.2	8.0±0.1	7.7±0.1	8.8±0.2	8.6±0.0	8.6±0.2	8.7±0.3
Not heated	5	2.1±0.1	2.0±0.2	3.1±0.3	3.1±0.1	7.2±0.5	5.9±0.5	4.2±0.0	2.5±0.1	8.3±0.1	5.9±0.0	5.8±0.2	4.8±0.0
	6	2.2±0.1	2.0±0.0	3.0±0.0	3.3±0.1	8.7±0.2	7.9±0.1	8.0±0.1	7.2±0.1	7.7±0.2	7.9±0.1	8.1±0.1	7.9±0.0
	6.5	2.1±0.2	2.1±0.7	2.9±0.2	2.9±0.0	8.6±0.2	8.2±0.2	8.1±0.2	7.4±0.1	8.4±0.1	8.6±0.2	8.8±0.4	8.7±0.2

Kansas City, MO). The bologna was then cooked in a smokehouse (Alkar, DEC International Inc., Lodi, WI). Specifically, bologna was cooked in dry air for 1 hour (smokehouse temperature 60°C), followed by hot smoking (60°C; Zesti liquid smoke, Hickory Specialties Inc., Crossville, TN) for 38 min. After smoking, the bologna was cooked with steam for 1 hour (smokehouse temperature 71°C, relative humidity 50%). Then the smokehouse temperature was increased to 88°C and the bologna was cooked until its internal temperature reached 70°C. After cooking the bologna was showered with cool tap water for 5 min and cooled overnight at 4°C. The casings were then removed and the bologna was sliced into approximately 5 mm thick slices (Globe slicer, Mozley Manufacturing, Stamford, CT). The slices were transferred to the microbiology laboratory for inoculation, vacuum-packaging, storage and analysis.

A *L. monocytogenes* composite was prepared with 10 strains, including Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak). These 10 strains were used for all subsequent studies, unless otherwise specified. The strains were activated and subcultured as described previously. To prepare the inoculum, the TSBYE cultures were centrifuged (five strains in each conical centrifuge tube) at 4629 x *g* for 15 min (4°C). Each of the five strain mixtures was resuspended in 10 ml of sterile PBS (prepared from basic ingredients). The two mixtures were then combined and centrifuged (as described above). The mixed culture was resuspended in 100 ml PBS and serially diluted to a concentration estimated to yield approximately 2 log CFU/cm^2 of bologna, when 0.1 ml of the inoculum was applied to each side of a bologna slice.

Bologna slices were placed on pieces of aluminum foil and inoculated on one side (0.1 ml from the appropriate dilution) under a biological safety cabinet. The inoculum was spread over the entire surface with a sterile bent glass rod. The inoculated slices were left to stand for 15 min at 5°C for attachment and then the procedure was repeated for the other side. After inoculation, two bologna slices from each treatment were placed into vacuum bags (15 by 20 cm, 3 mil std barrier; Nylon/PE vacuum pouch, Koch), vacuum-packaged (Hollymatic, Corp., Countryside, IL), and stored at 7°C.

Microbiological determinations (three samples per sampling time and treatment) were conducted on days 0, 4, 8, 12, 16, 20 and 24. For microbiological analysis, each sample (two slices) was placed in a sterile bag (Whirl-Pak®, Nasco) with 50 ml maximum recovery diluent (MRD, prepared from basic ingredients in the laboratory) and shaken 30 times as described in the United States Meat and Poultry Inspection Regulation (FSIS, 1996). Ten-fold serial dilutions were made with buffered peptone water (Difco; BPW) and surface plated on TSAYE for the enumeration of total microbial populations and PALCAM agar (Difco) for the enumeration of *L. monocytogenes*. After incubation (25°C, 72 h for TSAYE and 30°C, 48 h for PALCAM) the colonies were counted manually and the counts were transformed to log CFU/cm^2. Results of this study are discussed in following paragraphs.

II. MAIN STUDIES

MATERIALS AND METHODS

Product preparation, antimicrobial treatments, and microbiological analyses

Study 1-Antilisterial effects of lactoferrin, potassium lactate, and sodium diacetate applied as formulation ingredients in bologna stored at 4 or 7°C

Bologna was prepared as described previously to contain:
1. No antimicrobials (control)
2. Potassium lactate (3% of a 60% [wt/wt] commercial product; equivalent to 1.8% pure potassium lactate)
3. Lactoferrin (0.5%)
4. Lactoferrin (1%)
5. Potassium lactate (1.8%) combined with sodium diacetate (0.125%)
6. Potassium lactate (1.8%) combined with lactoferrin (0.5%)
7. Sodium diacetate (0.25%) combined with lactoferrin (0.5%)
8. Potassium lactate (1.8%) combined with sodium diacetate (0.125%) and lactoferrin (0.5%)
9. Potassium lactate (1.8%) combined with sodium diacetate (0.0625%) and lactoferrin (0.25%).

Bologna was processed, inoculated (approximately 2 log CFU/cm^2) and stored at 4°C or 7°C, as described above. Microbiological analyses for total microbial counts and *L. monocytogenes* were performed on days 0, 10, 20, 43, 75, and 95 for samples stored at 4°C, and on days 0, 4, 8, 12, 16, 20, 28, 43, and 57 for samples stored at 7°C, as described previously.

Study 2-Antilisterial effects of ALF and lactic acid applied as dipping or spraying solutions on the surface of bologna

The present study investigated whether the method of application (dipping or spraying) of surface treatments may influence their antilisterial activity. Bologna (without antimicrobials in the formulation) was prepared, as described above. Following inoculation (composite of 10 *L. monocytogenes* strains, approximately 3 log CFU/cm^2) slices were either left untreated (control) or dipped (30 s, 16 slices in 200 ml of solution) into or sprayed (under a biosafety hood for 2 s per side, 10 psi) with the following:
1. Distilled water
2. 2% lactic acid (88%; Purac, Barcelona, Spain)
3. 2% ALF (aLF Ventures).

The spraying system used in this study consisted of a stainless steel sprayer, custom-built by Chad Co. (Lenexa, KS) using a compressor (YI000, Husky Professional Tools, Atlanta, GA) as a vacuum pump and a spray gun (TriggerJet, MI 22650, Mfr. Spraying Systems Co., Wheaton IL). Bologna slices were sprayed on each side with the nozzle (H1/8vvss80015, Mfr. Spraying Systems Co.; flow rate at 20 psi-0.11 gmp) held at a 90° angle (approximately 10 cm distance from the product), then turned and the procedure was repeated for the other side. All solutions used for dipping or spraying were used at ambient temperature. Treated and untreated (control) slices were then vacuum-packaged (2 per bag) and stored at 7°C. Microbiological analyses were conducted on days 0, 4, 8, 12, 16, 22, 28, and 43 as described previously.

Study 3-Control of *Listeria monocytogenes* on commercial ham with antimicrobial solutions applied pre- or post-inoculation

Commercially prepared 97% fat-free ham (cured with water, salt, sugar, sodium phosphates, sodium erythorbate, and sodium nitrite; no sodium/potassium lactate-sodium diacetate) was sliced into 3-4 mm thick slices in the CSU meat laboratory. Slices were then cut into pieces having a surface area of approximately 30 cm^2 and inoculated with a composite of 10 *L. monocytogenes* strains (~2 log CFU/cm^2) before or after dipping (16-17 pieces into 300 ml of solution) into the following:

1. Nothing (control)
2. Distilled water (2 min)
3. 1% acetic acid (glacial; Mallinckrodt and Baker, Paris, KY) (30 s)
4. 2% ALF (2 min).

Samples were vacuum-packaged (2 pieces per bag), stored (7^oC) and microbiologically analyzed on days 0, 5, 15, 22, and 30 as described previously.

Study 4-ALF and organic acids or salts applied as dipping solutions for *Listeria monocytogenes* control on commercial cured turkey breast

Commercially prepared 98% fat-free turkey breast (ingredients: turkey breast, turkey broth, salt, sugar, sodium phosphates, sodium erythorbate, sodium nitrite; no sodium/potassium lactate-sodium diacetate) was sliced into 3-4 mm thick slices in the CSU meat laboratory. Slices were then cut into pieces having a surface area of approximately 30 cm^2 prior to inoculation with a composite of 10 *L. monocytogenes* strains (~3 log CFU/cm^2). Pieces were then subjected to dipping into one of the following treatments:

1. Nothing (control)
2. Distilled water
3. 3% potassium lactate
4. 3% sodium diacetate
5. 1% lactic acid
6. 1% acetic acid
7. 2% ALF
8. 1% ALF
9. 1% ALF followed by 3% potassium lactate
10. 1% ALF followed by 3% sodium diacetate
11. 1% ALF followed by 1% lactic acid
12. 1% ALF followed by 1% acetic acid.

Activated lactoferrin was selected to be applied before the other antimicrobials based on results by Ransom et al. (2003). Treatments were applied by immersion of 20 pieces into 200 ml of the solution for 1 min, followed by draining, vacuum-packaging (2 slices per bag) and storage at 7^oC. Microbiological analyses were conducted on days 0, 4, 8, 12, 16, 22, 28, and 43 as described previously.

Study 5-Different concentrations of ALF and/or organic acids or salts applied as dipping solutions for *Listeria monocytogenes* control on commercial uncured turkey breast

Uncured turkey breast (99% fat-free and formulated without sodium/potassium lactate-sodium diacetate) was obtained from a commercial manufacturer and was used for experiments within three days of production. The ingredients of the product included turkey breast meat, turkey broth, modified food starch, salt, sugar, sodium phosphates and flavoring. The turkey breast was first sliced into 3-4 mm thick slices in the CSU meat laboratory, and then each slice was cut into 5 x 5 cm pieces (surface area of 50 cm^2). Each sample was subsequently inoculated with a 10-strain mixture of *L. monocytogenes*, prepared and diluted as described above, to obtain and inoculum level of approximately 2 log CFU/cm^2 when 0.1 ml was spread on each side of the sample. Following inoculation, turkey breast pieces were subjected to one of the following dipping treatments and times of exposure:

1. No treatment (control)
2. Distilled water (1 min)
3. 1.5% sodium diacetate (1 min)
4. 1% lactic acid (1 min)
5. 2% lactic acid (1 min)
6. 1% ALF (1 min)
7. 2% ALF (1 min)
8. 2% ALF (2 min)
9. 2% ALF (1 min) followed by 1.5% sodium diacetate (1 min)
10. 1% ALF (1 min) followed by 2% lactic acid (1 min)
11. 2% ALF (1 min) followed by 1% lactic acid (1 min).

Treatments were applied by immersion of 20 pieces into 250 ml of the solution, followed by draining (1 min), vacuum-packaging (2 slices per bag) and storage at 7^oC. Microbiological analyses were conducted on days 0, 4, 8, 12, 16, 24 and 34, as described previously.

Study 6-Different concentrations of ALF and/or lactic acid applied as dipping solutions for *Listeria monocytogenes* control on commercial uncured turkey breast

Some of the treatments applied in Study 5 were repeated in this study, and an additional treatment was also added (2% ALF [1 min] followed by 2% lactic acid [1 min]). The treatments applied were, thus:

1. No treatment (control)
2. 2% lactic acid (1 min)
3. 1% ALF (1 min)
4. 2% ALF (1 min)
5. 2% ALF (2 min)
6. 1% ALF (1 min) followed by 2% lactic acid (1 min)
7. 2% ALF 1 min) followed by 2% lactic acid (1 min).

Samples were microbiologically analyzed on the same storage days given above.

Study 7-Application of antimicrobial dipping solutions for *Listeria monocytogenes* control on frankfurters and effect of treatment duration on antilisterial properties of ALF

The frankfurter formulation used in the study was identical to the basic (no antimicrobials included) bologna formulation described previously. The meat and non-meat ingredients of each batch were emulsified as described previously and extruded into 24 mm cellulose casings

(Koch). The frankfurters were then weighed and cooked in a smokehouse (Alkar). Specifically, the frankfurters were cooked first in dry air for 30 min (smokehouse temperature 80°C), followed by hot smoking (60°C; Zesti liquid smoke) for 30 min. The frankfurters were cooked with steam for 30 min (smokehouse temperature 80°C, relative humidity 26%), showered with cool tap water for 5 min and cooled overnight at 4°C (Bedie et al., 2001). After cooking, the frankfurters were reweighed for cooking yield determination, peeled manually and cut into 10 cm length links.

Product was inoculated (~3 log CFU/cm^2) and vacuum-packaged as follows: two frankfurter links from each treatment were transferred into a vacuum bag (15 by 20 cm, 3 mil std barrier, Nylon/PE vacuum pouch, Koch) and inoculated (0.25 ml from the appropriate dilution applied on each frankfurter) with the mixture of *L. monocytogenes* strains used in the previous studies, under a biological safety cabinet. The frankfurters were then massaged in order to spread the inoculum uniformly on their surface. The inoculated frankfurters were left to stand for 15-30 min at 5°C for attachment before treating with one of the treatments described below.

Frankfurters from each treatment were removed from the bags in which they were inoculated and immersed (32-34 links into 1 liter of solution) into:
1. Nothing (control)
2. Distilled water (30 s)
3. 2% lactic acid
4. 3% sodium diacetate
5. 2% ALF (30 s)
6. 2% ALF (60 s)
7. 2% ALF (90 s)
8. 2% ALF (120 s).

Samples were then drained, vacuum-packaged (2 links per bag) and stored at 7°C. Microbiological analyses were performed on days 0, 4, 8, 12, 16, 20, 24, and 32 as described previously.

Study 8-Effect of bologna and inoculum composition on the antilisterial properties of preservative solutions applied by dipping

The basic bologna formulation described previously was used to prepare two batches of product. The first batch was formulated with meat trimmings consisting of 40% pork (approximately 30% fat) and 60% beef (approximately 25% fat), whereas, the second batch was formulated with meat trimmings consisting of beef (approximately 25% fat) only. Following cooking/cooling and slicing (2-3 mm), product was inoculated with one of two different 10-strain mixtures of *L. monocytogenes*:
Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)
Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known,

food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak). Bologna slices, inoculated with one of the two inocula, were then dipped into:

1. Nothing (control)
2. Distilled water (2 min)
3. 2% acetic acid (1 min)
4. 2% ALF (1 min)
5. 2% ALF (2 min)
6. 2% ALF (3 min).

Samples were drained, vacuum-packaged and stored at 10^{o}C. Microbiological analyses were conducted on days 0, 4, 8, 12, 16, and 26.

Study 9-*Listeria monocytogenes* control with antimicrobials in the formulation of frankfurters and by dipping into solutions of ALF or acetic acid

The basic frankfurter formulation, described previously, was used to prepare four batches of product with:

1. No antimicrobials (control)
2. Potassium lactate (1.8%) and sodium diacetate (0.125%)
3. Lactoferrin (0.5%)
4. Potassium lactate (1.8%) and lactoferrin (0.5%).

Frankfurters were processed, cooked, and cooled overnight as indicated previously. The following day, frankfurters were cut into 8 cm pieces and inoculated as described above. Following inoculation, frankfurters of each formulation treatment were dipped (30 links into 900 ml; 2 min) into:

1. Nothing (control)
2. 2% acetic acid
3. 2% ALF.

Samples were then vacuum-packaged (2 links per bag) and stored at 7^{o}C. Microbiological (days 0, 5, 10, 15, 20, 25, 35, and 50) analyses were conducted as in previous studies.

Study 10-Modeling the growth/no growth interface of *Listeria monocytogenes* in ready-to-eat products as a function of lactic acid concentration and dipping time

Experimental design and evaluation of growth/no growth. Products used in this study included bologna, uncured turkey breast, and frankfurters (for product descriptions refer to Studies 1, 5, and 7, respectively). In this study the effect of five lactic acid concentrations (0-4%), five dipping times (0-4 min), and three storage temperatures (4, 7, 10^{o}C) (75 combinations) on growth/no growth limits of *L. monocytogenes* were evaluated in frankfurters, uncured turkey breast, and bologna. Approximately 2 log CFU/cm^2 was inoculated on samples. Samples inoculated with *L. monocytogenes* were subsequently treated and stored in vacuum packages for up to 60 days, depending on products and storage temperatures. Bacterial populations were determined on TSAYE and PALCAM agar on day-0, and the middle and end point of storage. To determine growth/no growth, least squares means in bacterial populations were separated by the ANOVA mixed model procedure of SAS. All differences were reported at a significance

level of alpha=0.05. Significant ($P \leq 0.05$) increases (0.5-0.9 log CFU/cm^2) in bacterial counts during storage were designated as "growth (1)", while non-significant changes in counts were designated as "no growth (0)".

Model development. The growth response data were fitted to logistic regression models using SAS to determine growth/no growth interfaces. Since vigorous growth of *L. monocytogenes* was observed in all combinations for frankfurters, a model was not developed for this product. The following equation was fitted to datasets corresponding to uncured turkey breast and bologna:

$$Logit\ (P) = a_0 + a_1 \cdot T + a_2 \cdot LA + a_3 \cdot DT + a_4 \cdot T \cdot LA + a_5 \cdot T \cdot DT + a_6 \cdot LA \cdot DT + a_7 \cdot T^2 + a_8 \cdot LA^2 + a_9 \cdot DT^2$$

where, *Logit (P)* is an abbreviation of ln[P/(1-P)], *P* is the probability of growth (range of 0-1), a_i are coefficients to be estimated, *T* is storage temperature, *LA* is lactic acid concentrations, and *DT* is dipping times. The automatic variable selection option with a stepwise selection method was used to choose the most significant effects ($P<0.05$). The predicted growth/no growth interfaces for P=0.1, 0.5 and 0.9 were calculated.

Physical and chemical analyses

The water activity (a_w) of products from each treatment (only for day-0 samples) was determined with an AquaLab (model series 3; Decagon Devices Inc., Pullman, WA) water activity meter. The pH of samples, using a Denver Instrument (Arvada, CO) pH meter and electrode, was determined after plating, by homogenizing samples for 120 s at 8.0 strokes/s (Masticator, IUL Instruments, Barcelona, Spain) and measuring the pH of the resultant slurry. The determination of cooking yields (%) of bologna or frankfurters formulated with or without antimicrobials was based on product weight before and after cooking and chilling (Bedie et al., 2001). Finally, the fat and moisture contents of samples were determined according to the AOAC International official methods (Official methods 950.46.B and 930.39 respectively; AOAC, 2000).

Statistical analyses

For each experiment, three to six individual samples from one or two experiments were analyzed at each sampling day and for each treatment. The collected microbiological data were converted to log CFU/cm^2 and means and standard deviations were calculated. In addition, *L. monocytogenes* counts were modeled as a function of time using the model of Baranyi and Roberts (1994). For curve fitting the in-house program DMFit of IFR (Institute of Food Research, Norwich, UK), which was kindly provided by Dr. J. Baranyi, was used. The Baranyi model is a non-autonomous, separable, first order ordinary differential equation (Baranyi et al., 1993). The lag phase is formally separated from the exponential and the stationary phase, which can be regarded as parts of the potential growth defined by the autonomous model. The model (Baranyi and Roberts, 1994) contains four parameters: a parameter expressing the lag phase (corresponds to time elapsed from inoculation to the intercept of the tangent with the inoculum level); μ, the exponential growth rate ($\log_{10}$ CFU/cm^2.day^{-1}); y_0, which represents the lower asymptote of the bacterial growth curve (initial bacterial counts; $\log_{10}$ CFU/cm^2); and y_{end}, which represents the upper asymptote of the growth curve (final bacterial counts; $\log_{10}$ CFU/cm^2). Two more parameters, m and n, are included in the Baranyi and Roberts equation corresponding to the behavior of the growth curve at the "transition" regions (lag to exponential phase and

exponential to stationery phase). The exponential growth rate derived from fitting the $\log_{10}$ values was transformed to maximum specific growth rate, μ_{max}, (day^{-1}) by multiplying it with ln(10).

RESULTS AND DISCUSSION

PRELIMINARY STUDY B-Antilisterial effects of lactoferrin, potassium lactate, and sodium diacetate added individually or in combination in the formulation of bologna

Listeria monocytogenes growth on bologna formulated with or without antimicrobials is described using growth kinetics (Table 2). Examples of fitted curves using the Baranyi and Roberts model are shown in Figure 1. The results of this study showed that addition of lactoferrin in the bologna formulation provided slight inhibition (P>0.05) of *L. monocytogenes* growth compared to the control as suggested by lag phases (period after inoculation with no or very slow bacterial growth) obtained for samples containing 0.25 or 0.5% lactoferrin (2.7 and 3.1 days, respectively) and that obtained for the control (0.4 days). Maximum specific growth rates (μ_{max}; maximum slope of the curve; high values correspond to rapid bacterial growth) for samples with 0.25% and 0.5% lactoferrin were 0.285 and 0.325 $days^{-1}$, respectively and were lower than those observed for single potassium lactate or sodium diacetate (0.830 and 0.740 $days^{-1}$, respectively). However, the lag phase durations for the lactoferrin treatments were much shorter (2.7 and 3.1 days; P>0.05) than that for the potassium lactate (34.3 days) and sodium diacetate (24.2 days) treatments, applied individually. The combinations of 0.25% lactoferrin with 1.8% potassium lactate or 0.25% sodium diacetate exhibited lower (P>0.05) μ_{max} values (0.111 and 0.085 $days^{-1}$, respectively) compared to those observed when each one of these antimicrobials was used individually. Both μ_{max} and the lag phase values observed in samples containing the combination of 0.25% lactoferrin with 0.25% sodium diacetate were not significantly different (P>0.05) than those observed in samples containing the very effective combination of 1.8% potassium lactate and 0.25% sodium diacetate. These results indicate that although lactoferrin was less effective than single potassium lactate or sodium diacetate, it provided slight inhibition of *L. monocytogenes* (compared to the control) suggesting that its antilisterial activity may not be lost after heat treatment of processed meat products. Furthermore, these results gave an indication of appropriate concentrations of antimicrobials to be used in subsequent studies.

MAIN STUDIES

Study 1-Antilisterial effects of lactoferrin, potassium lactate, and sodium diacetate applied as formulation ingredients in bologna stored at 4 or 7°C

Tables 3 and 5 present growth kinetics of *L. monocytogenes* inoculated onto slices of bologna formulated with or without (control) antimicrobials and stored at 4 or 7°C, respectively. *L. monocytogenes* and total microbial populations (log CFU/cm^2) obtained for this study are presented in Figures 2 and 3, and Tables 4 and 6.

Table 2: Mean (n=3) growth kinetics of *Listeria monocytogenes* growth on the surface of bologna slices with or without antimicrobials in the formulation, inoculated with the pathogen after slicing and stored at 10°C for 24 days.

Treatment (formulation)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[2] (log CFU/cm^2)	Y_{end}[3] (log CFU/cm^2)	R^2
Control	0.4 A	0.303 A	1.8	7.7	0.995
PL (1.8%)	34.3 B	0.830 A	1.6	-	0.868
SD (0.25%)	24.2 B	0.740 A	1.6	-	0.877
LF (0.25%)	2.7A	0.285 A	1.8	7.7	0.994
LF (0.5%)	3.1 A	0.325 A	1.7	7.9	0.992
PL (1.8%) + SD (0.25%)	-[1]	-0.015 AB	1.5	-	0.268
PL (1.8%) + LF (0.25%)	11.4 AB	0.111 A	1.7	-	0.887
SD (0.25%) + LF (0.25%)	13.5 AB	0.085 AB	1.6	-	0.481
PL (1.8%) + SD (0.25%) + LF (0.25%)	16.3 B	-0.280 B	1.6	1.3	0.820

[1] no growth observed
[2]Lower asymptote estimated by the Baranyi model
[3]Upper asymptote estimated by the Baranyi model; no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponds to stationary phase
PL: potassium lactate, SD: sodium diacetate, LF: activated lactoferrin
Means within the same column that have a common letter are not significantly different (P>0.05)

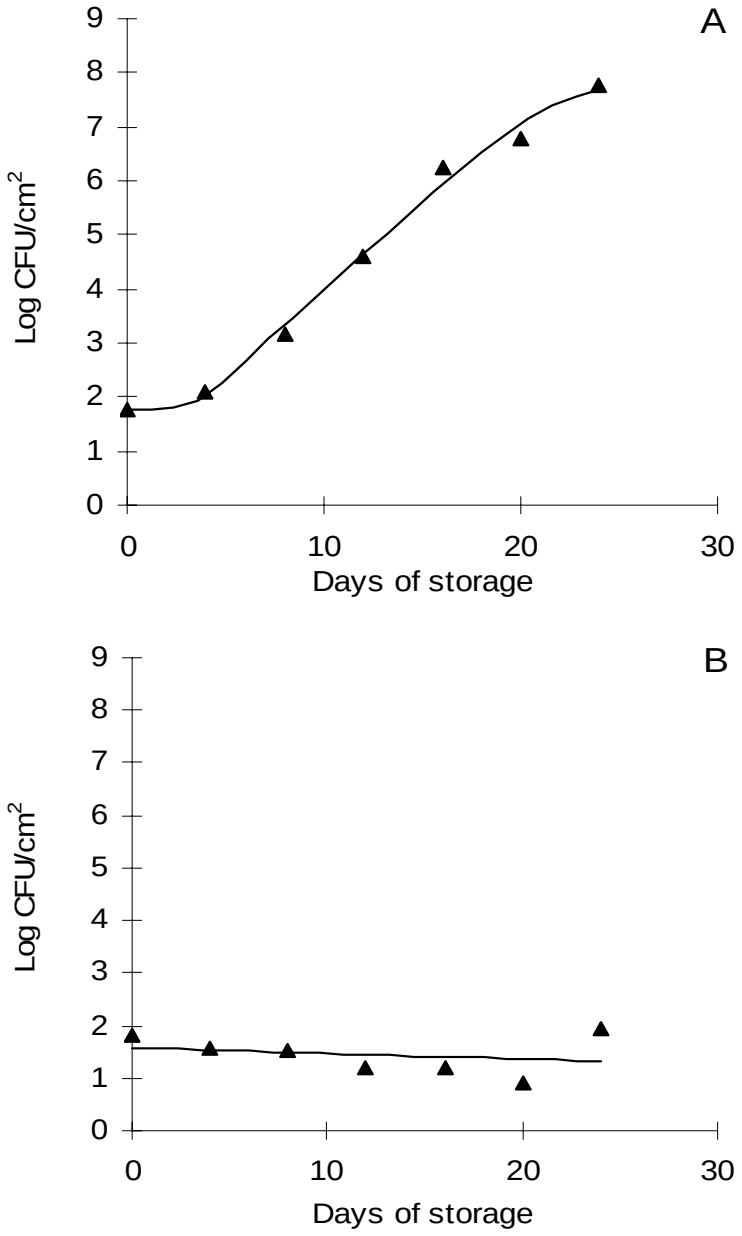


Figure 1: *Listeria monocytogenes* counts (log CFU/cm^2; triangles) on bologna formulated to contain 0.5% lactoferrin (A) or 1.8% potassium lactate and 0.25% sodium diacetate (B) during storage at 10^{o}C under vacuum and predicted growth curves by the Baranyi and Roberts model (line).

During storage at 4°C, lag phases in samples that contained antimicrobials ranged from 1.5 (1% lactoferrin) to 85.7 days (1.8% potassium lactate combined with 0.125% sodium diacetate and 0.5% lactoferrin). No lag phase was observed for control samples. Maximum specific growth rates ranged from 0.047 $days^{-1}$ (1.8% potassium lactate combined with 0.0625% sodium diacetate and 0.25% lactoferrin) to 0.951 $days^{-1}$ (1.8% potassium lactate combined with 0.125% sodium diacetate and 0.5% lactoferrin). Of all single treatments, 1.8% potassium lactate exhibited the greatest antilisterial effects, as it resulted in a slower ($P>0.05$) growth (μ_{max}: 0.048 $days^{-1}$) and a more extended ($P<0.05$) lag phase than treatments that consisted of single lactoferrin. The combination of 1.8% potassium lactate with 0.125% sodium diacetate and 0.5% lactoferrin exhibited a higher ($P<0.05$) μ_{max} value compared to control and other treatments. However, this treatment provided a lag phase of 85.7 days, suggesting that *L. monocytogenes* populations remained constant for the greater part of the storage period before exhibiting an increase (also see Figure 2). This treatment also inhibited total microbial growth as shown in Table 4. Other treatments that caused extended lag phases included 1.8% potassium lactate combined with 0.0625% sodium diacetate and 0.25% lactoferrin (53.9 days), 1.8% potassium lactate combined with 0.5% lactoferrin (45.6 days), 1.8% potassium lactate (70.3 days) and 1.8% potassium lactate combined with 0.125% sodium diacetate (76.7 days). Lactoferrin used individually (0.5% or 1%) or in combination with sodium diacetate (0.125% sodium diacetate and 0.5% lactoferrin) resulted in lag phases (3.1, 1.5, and 2.7 days, respectively) of shorter duration ($P<0.05$) compared to the other treatments during storage at 4°C. Upper asymptotes (y_{end}; *L. monocytogenes* counts at the end of the storage period) varied from 1.8 log CFU/cm^2 (1.8% potassium lactate combined with 0.125% sodium diacetate) to 8.0 log CFU/cm^2 (1.8% potassium lactate combined with 0.5% lactoferrin). Lactoferrin (0.5 or 1%) resulted in higher y_{end} values (7.3 and 7.1 log CFU/cm^2, respectively) compared to that of the control (6.8 log CFU/cm^2).

At 7°C, samples that permitted immediate *L. monocytogenes* growth included the control and samples that contained 0.5 or 1% lactoferrin or the combination of 1.8% potassium lactate with 0.0625% sodium diacetate and 0.25% lactoferrin (Table 5). The combination of 1.8% potassium lactate with 0.125% sodium diacetate provided complete inhibition of the pathogen's growth, whereas, the rest of the treatments caused lag phases that ranged from 6.4 (1.8% potassium lactate combined with 0.5% lactoferrin) to 28.7 (1.8% potassium lactate combined with 0.125% sodium diacetate and 0.5% lactoferrin) days. As expected, lag phases observed during storage at 7°C were generally shorter compared to those at 4°C across treatments. Maximum specific growth rates varied from -0.023 $days^{-1}$ (1.8% potassium lactate combined with 0.125% sodium diacetate) to 0.299 $days^{-1}$ (control). Potassium lactate (1.8%) combined with 0.125% sodium diacetate did not allow growth of the pathogen and, therefore, no lag phase value is provided for this treatment. The combination of 1.8% potassium lactate with 0.125% sodium diacetate was the most effective treatment as it resulted in a μ_{max} of -0.023 day^{-1} (complete inhibition/reduction of growth throughout the storage period). Lactoferrin used individually or in combination with potassium lactate and/or sodium diacetate provided inhibition of *L. monocytogenes* growth as these treatments resulted in lower ($P<0.05$) μ_{max} values compared to that of control samples. Lactoferrin used individually at 0.5% or 1% and the combination of 0.25% sodium diacetate with 0.5% lactoferrin did not cause a lag phase, whereas, the combination of 1.8% potassium lactate with 0.5% lactoferrin resulted in a lag phase of 6.4 days.

These results indicate that, under the conditions of this study, lactoferrin used individually or in combination with potassium lactate in the formulation of bologna resulted in slight inhibition of *L. monocytogenes* growth during the first days of the storage period; however, populations of the pathogen reached high counts, eventually. Moreover, lactoferrin did not appear to enhance the antilisterial activity of potassium lactate and sodium diacetate. On the contrary, bologna samples with lactoferrin (0.5%) included in their formulation together with 1.8% potassium lactate and 0.125% sodium diacetate resulted in higher *L. monocytogenes* populations compared to those of samples formulated with 1.8% potassium lactate and 0.125% sodium diacetate.

Growth on TSAYE and PALCAM agar followed similar patterns at both temperatures (Figures 2 and 3, Tables 4 and 6) especially during the first days of storage, suggesting that the majority of colonies that grew on TSAYE were *L. monocytogenes*. The higher counts obtained on the nonselective medium, especially during the last days of the storage reflect growth of spoilage microorganisms.

The pH of untreated bologna (no antimicrobials in the formulation) on day-0 was 6.32 (Tables 7 and 8). Addition of antimicrobials to the product formulation caused reductions of 0.01 (1.8% potassium lactate) to 0.11 (1.8% potassium lactate and 0.125% sodium diacetate) units on day-0. By the end of storage (day-95) at 4^{o}C (Table 7), no substantial changes in pH values were observed in samples that contained 1.8% potassium lactate individually or in combinations with sodium diacetate, lactoferrin, or both. On the contrary, pH values of samples that contained single lactoferrin (0.5 or 1%) or the combination of 0.125% sodium diacetate with 0.5% lactoferrin was reduced (0.56, 0.60, and 1.25 units, respectively) by the end of the storage period suggesting microbial growth. Similar trends were observed during storage at 7^{o}C (Table 8), as samples containing 0.5 or 1% lactoferrin and 0.125% sodium diacetate with 0.5% lactoferrin had pH values of 5.89, 5.82, and 5.42, respectively by the end of storage (day-57).

Water activity values obtained for samples formulated without antimicrobials on day-0 was 0.966 (Table 9). Treatments that led to the greatest reductions of product a_w on day-0 were single 1.8% potassium lactate and its combination with 0.5% lactoferrin (0.958 and 0.959, respectively).

Cooking yields and moisture and fat contents of bologna formulated with or without antimicrobials are presented in Table 10. The cooking yield of untreated bologna was 87.9%. Potassium lactate (1.8%) was the treatment with the highest increase in cooking yield (90.2%). Addition of other antimicrobials in the product formulation did not affect cooking yield considerably. The moisture content of bologna ranged from 58.8 (1.8% potassium lactate combined with 0.0625% sodium diacetate and 0.125% lactoferrin) to 65.7% (0.125% sodium diacetate combined with 0.5% lactoferrin). Potassium lactate (1.8%) combined with 0.0625% sodium diacetate and 0.125% lactoferrin and 1.8% potassium lactate combined with 0.125% sodium diacetate were the treatments that resulted into the lowest (12.5%) and highest (17.4%) fat content, respectively.

Table 3: Mean growth kinetics (n=3) of *Listeria monocytogenes* growth on the surface of bologna slices with or without antimicrobials in the formulation, inoculated with the pathogen after slicing and stored at 4°C for 95 days.

Treatment (formulation)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[2] (log CFU/cm^2)	Y_{end}[3] (log CFU/cm^2)	R^2
Control	-[1]	0.146 A	2.5	6.8	0.985
PL (1.8%)	70.3 A	0.048 A	1.7	6.0	0.930
LF (0.5%)	3.1 B	0.077 A	2.0	7.3	0.997
LF (1%)	1.5 B	0.134 A	1.9	7.1	0.931
PL (1.8%) + SD (0.125%)	76.7 A	0.084 A	1.9	1.8	0.473
PL (1.8%) + LF (0.5%)	45.6 A	0.164 A	2.0	6.7	0.994
SD (0.125%) + LF (0.5%)	2.7 B	0.189 A	2.0	8.0	0.999
PL (1.8%) + SD (0.125%) + LF (0.5%)	85.7 A	0.951 B	2.1	-	0.969
PL (1.8%) + SD (0.0625%) + LF (0.25%)	54.0 A	0.047 A	2.0	4.5	0.904

[1] no lag phase observed

[2]Lower asymptote estimated by the Baranyi model

[3]Upper asymptote estimated by the Baranyi model; no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponds to stationary phase

PL: potassium lactate, LF: lactoferrin, SD: sodium diacetate

Means within the same column that have a common letter are not significantly different (P>0.05)

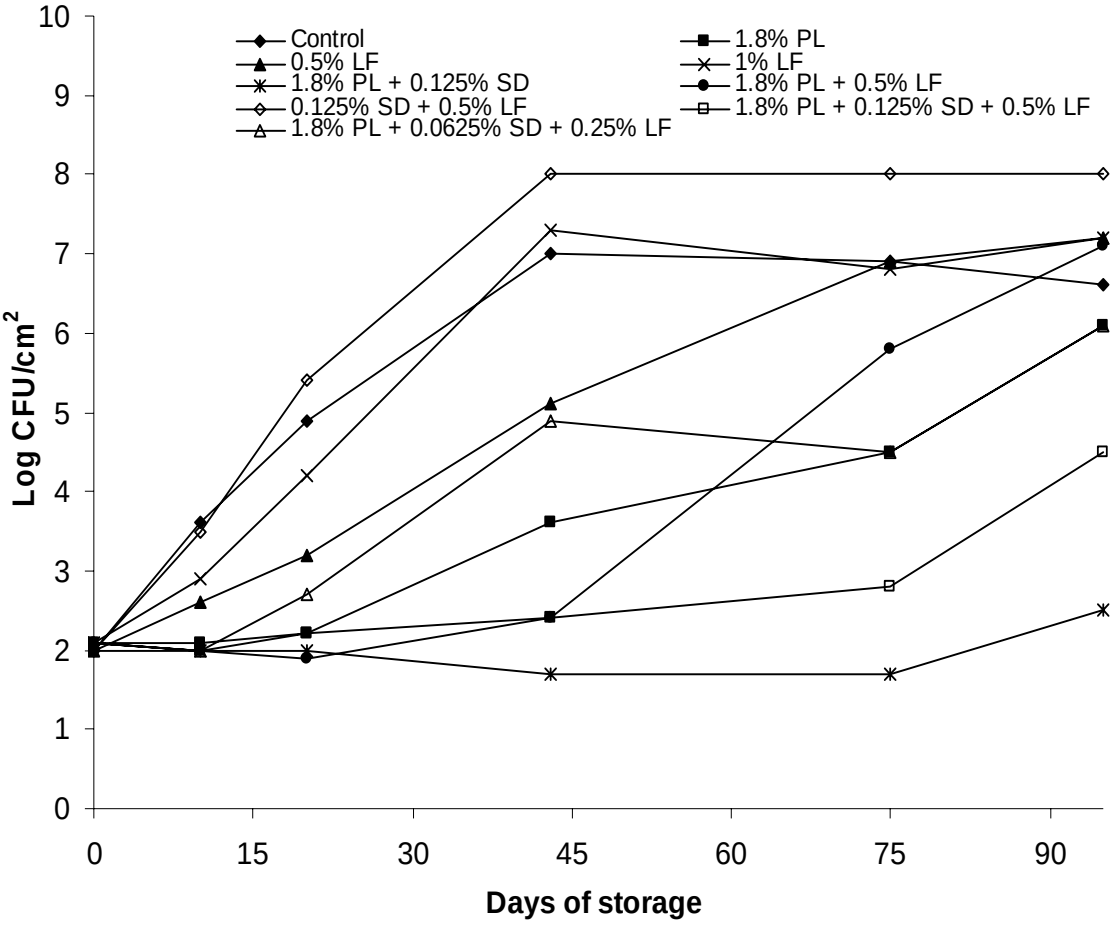


Figure 2: Mean (n=3) populations of *Listeria monocytogenes* (log CFU/cm^2) inoculated onto the surface of bologna slices formulated with or without antimicrobials before vacuum- packaging and storage at 4^{o}C for 95 days. LF, lactoferrin; PL, potassium lactate; SD, sodium diacetate.

Table 4: Mean (n=3) total microbial populations (log CFU/cm^2 ± standard deviation) on the surface of bologna slices formulated with or without antimicrobials, inoculated with *Listeria monocytogenes* after slicing, vacuum-packaged and stored at 4^{o}C.

	Days of storage					
Treatment (formulation)	0	10	20	43	75	95
Control	2.4±0.7	4.9±0.2	6.3±0.4	7.3±0.0	7.2±0.2	7.7±1.5
PL (1.8%)	2.2±0.0	2.2±0.2	2.5±0.1	5.2±0.4	5.8±0.7	7.3±1.4
LF (0.5%)	2.3±0.1	4.0±1.1	3.8±0.3	6.3±0.4	7.2±0.1	7.8±0.8
LF (1%)	2.3±0.2	3.4±0.1	5.3±0.2	7.5±0.2	7.2±0.1	7.8±1.0
PL (1.8%) + SD (0.125%)	2.5±0.4	2.0±0.0	2.1±0.1	2.0±0.1	2.4±1.2	2.6±1.2
PL (1.8%) + LF (0.5%)	2.2±0.1	2.2±0.1	1.9±0.1	2.7±0.5	5.8±0.4	6.9±0.2
SD (0.125%) + LF (0.5%)	2.2±0.1	3.8±0.4	5.5±0.1	8.0±0.1	8.0±0.1	8.1±0.1
PL (1.8%) + SD (0.125%) + LF (0.5%)	2.2±0.2	2.1±0.0	3.2±0.5	3.6±0.6	4.7±0.8	4.9±0.4
PL (1.8%) + SD (0.0625%) + LF (0.25%)	3.0±1.1	2.1±0.2	3.7±0.5	5.5±0.3	5.6±0.5	6.4±0.5

PL, potassium lactate; LF, lactoferrin; SD, sodium diacetate

Table 5: Mean (n=3) growth kinetics of *Listeria monocytogenes* growth on the surface of bologna slices with or without antimicrobials in the formulation, inoculated with the pathogen after slicing and stored at 7°C for 57 days.

Treatment (formulation)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[3] (log CFU/cm^2)	Y_{end}[4] (log CFU/cm^2)	R^2
Control	-[1]	0.299 A	3.1	7.2	0.934
PL (1.8%)	14.2 A	0.106 BE	2.1	5.2	0.866
LF (0.5%)	-[1]	0.148 BC	2.1	6.2	0.781
LF (1%)	-[1]	0.162 CF	2.2	7.2	0.971
PL (1.8%) + SD (0.125%)	-[2]	-0.023 D	2.1	1.9	0.628
PL (1.8%) + LF (0.5%)	6.4 A	0.069 E	2.1	-	0.985
SD (0.125%) + LF (0.5%)	-[1]	0.208 FG	2.3	7.9	0.975
PL (1.8%) + SD (0.125%) + LF (0.5%)	28.7 B	0.142 BC	1.9	-	0.946
PL (1.8%) + SD (0.0625%) + LF (0.25%)	-[1]	0.299 A	2.1	7.2	0.994

[1] no lag phase observed; [2] no growth observed

[3]Lower asymptote estimated by the Baranyi model

[4]Upper asymptote estimated by the Baranyi model; no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponds to stationary phase

PL: potassium lactate, LF: lactoferrin, SD: sodium diacetate

Means within the same column that have a common letter are not significantly different (P>0.05)

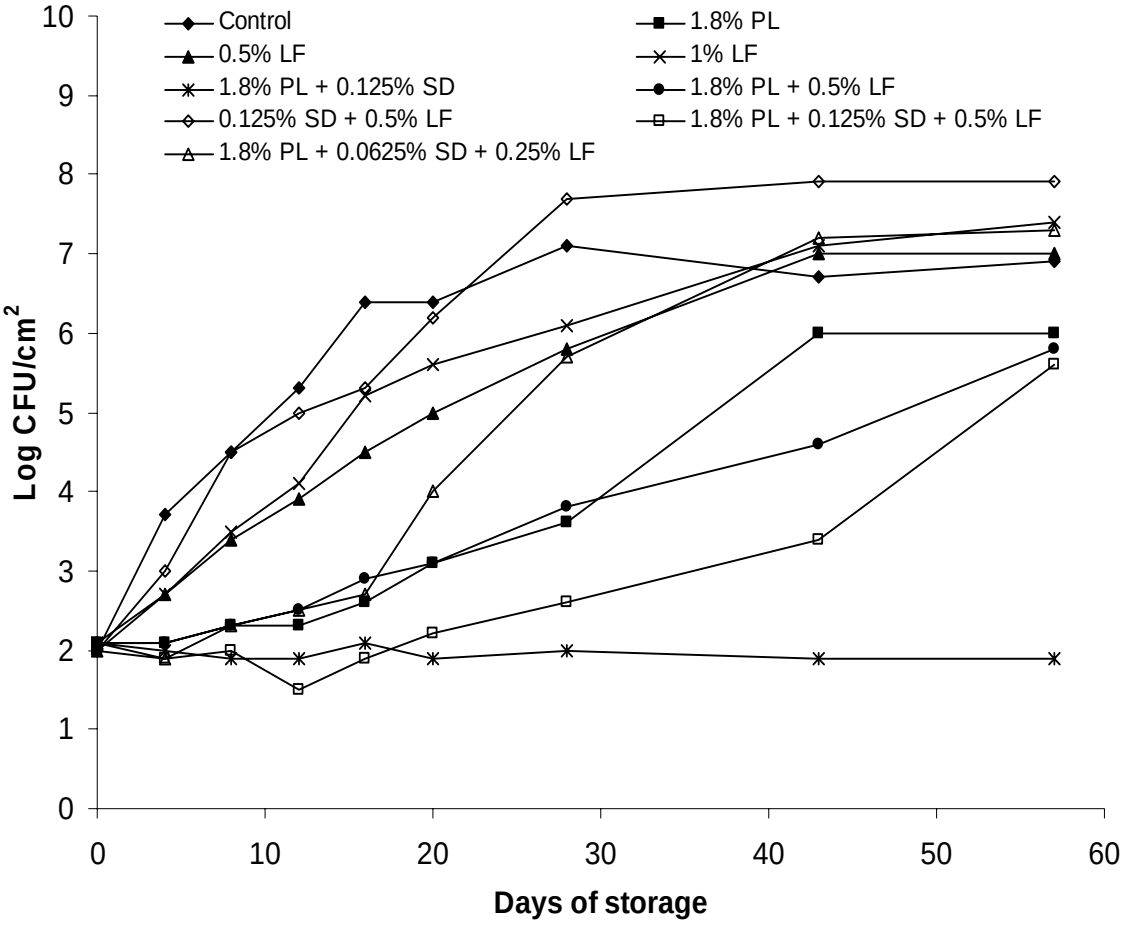


Figure 3: Mean (n=3) populations of *Listeria monocytogenes* (log CFU/cm^2) inoculated onto the surface of bologna slices formulated with or without antimicrobials before vacuum-packaging and storage at 7^{o}C for 57 days. LF, lactoferrin; PL, potassium lactate; SD, sodium diacetate.

Table 6: Mean (n=3) total microbial populations (log CFU/cm^2 ± standard deviation) on the surface of bologna slices formulated with or without antimicrobials, inoculated with *Listeria monocytogenes* after slicing, vacuum-packaged and stored at 7^{o}C.

	Days of storage								
Treatment (formulation)	0	4	8	12	16	20	28	43	57
Control	2.4±0.7	4.5±0.5	5.3±0.2	5.8±0.2	6.8±0.1	6.8±0.1	7.4±0.0	7.1±0.0	7.3±0.2
PL (1.8%)	2.2±0.0	2.2±0.0	2.5±0.1	2.4±0.3	2.9±0.2	3.5±0.5	5.3±0.2	5.9±0.1	6.2±0.3
LF (0.5%)	2.3±0.1	2.9±0.0	3.8±0.3	4.5±0.4	5.3±0.2	5.7±0.1	6.3±0.1	7.1±0.1	7.2±0.2
LF (1%)	2.3±0.2	3.1±0.2	3.8±0.1	4.5±0.3	5.1±0.6	5.6±0.1	6.3±0.2	7.2±0.2	7.5±0.1
PL (1.8%) + SD (0.125%)	2.5±0.4	2.1±0.0	1.9±0.1	2.0±0.0	2.2±0.2	1.8±0.4	2.1±0.1	2.3±0.4	3.1±1.0
PL (1.8%) + LF (0.5%)	2.2±0.1	2.3±0.1	2.2±0.2	2.5±0.1	3.3±0.1	3.1±0.1	4.1±0.6	4.6±0.3	5.8±0.4
SD (0.125%) + LF (0.5%)	2.2±0.1	3.0±0.2	4.5±0.1	5.1±0.2	5.5±0.2	6.1±0.2	7.2±0.1	7.9±0.0	8.2±0.6
PL (1.8%) + SD (0.125%) + LF (0.5%)	2.2±0.2	1.9±0.0	2.1±0.2	1.7±0.0	2.6±0.7	2.2±0.0	3.0±0.8	3.4±0.2	5.3±0.9
PL (1.8%) + SD (0.0625%) + LF (0.25%)	3.0±1.1	2.1±0.1	2.8±0.3	2.8±0.1	4.1±0.0	4.6±0.2	5.9±0.2	7.3±0.1	7.4±0.2

PL, potassium lactate; LF, lactoferrin; SD, sodium diacetate

Table 7: Mean (n=3) pH values (pH ± standard deviation) of bologna slices formulated with or without antimicrobials, inoculated with the pathogen after slicing, vacuum-packaged and stored at 4°C.

	Days of storage					
Treatment (formulation)	0	10	20	43	75	95
Control	6.32±0.04	6.34±0.00	6.23±0.02	5.81±0.02	5.57±0.04	5.52±0.06
PL (1.8%)	6.31±0.01	6.34±0.01	6.25±0.02	6.36±0.02	6.28±0.02	6.27±0.02
LF (0.5%)	6.26±0.01	6.28±0.00	6.20±0.02	6.25±0.05	5.84±0.02	5.70±0.02
LF (1%)	6.27±0.03	6.30±0.00	6.23±0.02	6.11±0.00	5.82±0.07	5.67±0.05
PL (1.8%) + SD (0.125%)	6.21±0.00	6.23±0.00	6.14±0.00	6.26±0.01	6.26±0.02	6.26±0.01
PL (1.8%) + LF (0.5%)	6.30±0.00	6.32±0.00	6.23±0.01	6.32±0.04	6.29±0.03	6.29±0.05
SD (0.125%) + LF (0.5%)	6.27±0.04	6.22±0.01	6.11±0.02	5.41±0.11	5.10±0.01	5.02±0.06
PL (1.8%) + SD (0.125%) + LF (0.5%)	6.20±0.01	6.20±0.02	6.11±0.01	6.23±0.02	6.18±0.04	6.26±0.05
PL (1.8%) + SD (0.0625%) + LF (0.25%)	6.28±0.00	6.28±0.01	6.15±0.04	6.32±0.02	6.20±0.03	6.22±0.05

PL, potassium lactate; LF, lactoferrin; SD, sodium diacetate

Table 8: Mean (n=3) pH values (pH ± standard deviation) of bologna slices formulated with or without antimicrobials, inoculated with *Listeria monocytogenes* after slicing, vacuum-packaged and stored at 7°C.

	Days of storage								
Treatment (formulation)	0	4	8	12	16	20	28	43	57
Control	6.32±0.04	6.32±0.03	6.34±0.02	6.32±0.02	6.29±0.01	6.21±0.12	5.87±0.06	5.66±0.01	5.57±0.06
PL (1.8%)	6.31±0.01	6.32±0.01	6.33±0.02	6.32±0.02	6.33±0.02	6.41±0.01	6.34±0.03	6.29±0.01	6.31±0.02
LF (0.5%)	6.26±0.01	6.25±0.03	6.29±0.01	6.27±0.02	6.30±0.02	6.33±0.01	6.28±0.00	6.08±0.07	5.89±0.01
LF (1%)	6.27±0.03	6.29±0.01	6.29±0.01	6.27±0.01	6.29±0.01	6.30±0.01	6.29±0.01	6.00±0.12	5.82±0.02
PL (1.8%) + SD (0.125%)	6.21±0.00	6.22±0.01	6.11±0.07	6.17±0.06	6.22±0.01	6.23±0.04	6.22±0.04	6.19±0.02	6.26±0.01
PL (1.8%) + LF (0.5%)	6.30±0.00	6.30±0.01	6.34±0.02	6.21±0.01	6.32±0.01	6.37±0.06	6.35±0.01	6.29±0.04	6.33±0.00
SD (0.125%) + LF (0.5%)	6.27±0.04	6.20±0.01	6.16±0.05	6.06±0.02	6.20±0.00	6.26±0.02	6.18±0.01	5.68±0.02	5.42±0.04
PL (1.8%) + SD (0.125%) + LF (0.5%)	6.20±0.01	6.18±0.01	6.20±0.01	6.06±0.03	6.21±0.01	6.24±0.03	6.24±0.02	6.19±0.02	6.23±0.01
PL (1.8%) + SD (0.0625%) + LF (0.25%)	6.28±0.00	6.22±0.06	6.24±0.05	6.06±0.05	6.25±0.01	6.31±0.01	6.30±0.01	6.17±0.02	6.08±0.01

PL, potassium lactate; LF, lactoferrin; SD, sodium diacetate

Table 9: Mean (n=2) a_w values (a_w ± standard deviation) of bologna slices formulated with or without antimicrobials and inoculated with *Listeria monocytogenes*

Treatment (formulation)	Water activity on day-0
Control	0.966±0.001
PL (1.8%)	0.958±0.002
LF (0.5%)	0.965±0.004
LF (1%)	0.965±0.000
PL (1.8%) + SD (0.125%)	0.960±0.001
PL (1.8%) + LF (0.5%)	0.959±0.004
SD (0.125%) + LF (0.5%)	0.969±0.000
PL (1.8%) + SD (0.125%) + LF (0.5%)	0.963±0.001
PL (1.8%) + SD (0.0625%) + LF (0.25%)	0.964±0.000

PL, potassium lactate; LF, lactoferrin; SD, sodium diacetate

Table 10: Mean (n=2) values (± standard deviation) of cooking yields, and moisture and fat content of bologna formulated with or without antimicrobials.

Treatment (formulation)	Cooking yield (%)	Moisture content (%)	Fat content (%)
Control	87.9±7.7	62.7±1.3	13.4±0.0
PL (1.8%)	90.2±3.0	60.7±0.1	15.7±1.0
LF (0.5%)	89.2±5.4	65.1±0.7	12.7±0.6
LF (1%)	87.2±5.0	64.3±2.4	12.9±1.4
PL (1.8%) + SD (0.125%)	88.2±2.2	63.1±0.7	17.4±0.4
PL (1.8%) + LF (0.5%)	87.2±4.3	59.1±0.1	13.8±0.2
SD (0.125%) + LF (0.5%)	89.0±1.6	65.7±0.3	17.2±0.6
PL (1.8%) + SD (0.125%) + LF (0.5%)	87.2±2.2	61.6±0.4	13.7±0.5
PL (1.8%) + SD (0.0625%) + LF (0.25%)	88.9±3.4	58.8±1.2	12.5±0.3

PL, potassium lactate; LF, lactoferrin; SD, sodium diacetate

Study 2-Antilisterial effects of ALF and lactic acid applied as dipping or spraying solutions on the surface of bologna

Kinetics of *L. monocytogenes* growth obtained for this study are presented in Table 11. Additionally, *L. monocytogenes* and total microbial populations (log CFU/cm^2) on inoculated bologna slices treated with water or antimicrobial solutions are shown in Figure 4 and Table 12, respectively.

Lower asymptote (y_0; *L. monocytogenes* counts at the beginning of storage) values show that dipping into water or antimicrobial solutions led to initial reductions of 0.5 log CFU/cm^2 (ALF) to 0.9 log CFU/cm^2 (water) in *L. monocytogenes* populations; spraying caused reductions of 0.4 log CFU/cm^2 (water) to 1.0 log CFU/cm^2 (lactic acid). Final populations (y_{end}) of the pathogen exceeded 7 log CFU/cm^2 for all treatments. The majority of treatments allowed immediate growth of *L. monocytogenes* and only dipping into 2% lactic acid caused complete inhibition for 6.5 days before allowing increases in pathogen levels. Maximum specific growth rates varied from 0.158 day^{-1} (spraying with 2% lactic acid) to 0.552 day^{-1} (dipping into water). *Listeria monocytogenes* proliferated at a faster rate ($P>0.05$) in samples dipped into 2% ALF, compared to the control, as suggested by corresponding μ_{max} values; however, when the same solution was applied as a spraying treatment, the μ_{max} value was lower ($P>0.05$) than that of the control. Regarding 2% ALF or water, application of these treatments on bologna by spraying (10 psi for 2 s each side) resulted in lower ($P<0.05$) μ_{max} values compared to dipping for 30 s. However, the method of application of 2% lactic acid did not affect ($P>0.05$) the μ_{max} values obtained for this antimicrobial; in addition, dipping into this solution resulted in a lag phase that lasted for 6.5 days, unlike spraying that allowed growth immediately. Results of this study show that, under the given conditions, spraying appeared slightly more effective than dipping for 2% ALF but not for 2% lactic acid.

The pH of untreated bologna was 6.31 on day-0 (Table 13). Among treatments, dipping into lactic acid applied as a dipping or a spraying solution led to the greatest reductions in product pH (0.64 and 0.43 units, respectively) on day-0, whereas dipping into or spraying with water or ALF cause slight product pH increases. During storage, reductions of pH in treated and untreated product was observed with samples dipped in water having the lowest pH (5.20) on day-43.

The a_w of untreated product was 0.996 on day-0 (Table 14). Most of the dipping or spraying treatments (except both 2% ALF treatments) led to a_w increases of 0.003 (spraying with 2% lactic acid) to 0.009 (dipping into water).

Study 3-Control of *Listeria monocytogenes* on commercial ham with antimicrobial solutions applied pre- or post-inoculation

Growth kinetics of *L. monocytogenes* inoculated onto ham before or after dipping into antimicrobial solutions are shown in Table 15. Also, *L. monocytogenes* and total microbial counts (log CFU/cm^2) obtained for this study are shown in Figure 5 and Table 16, respectively. As expected, no reductions of initial populations of *L. monocytogenes* were obtained for samples that were not treated before inoculation. Of all treatments applied before inoculation, acetic acid exhibited superior residual antilisterial effects during storage, since it inhibited growth of

Table 11: Mean (n=3) growth kinetics of *Listeria monocytogenes* growth on bologna slices inoculated with the pathogen and dipped in (30 s) or sprayed with (10 psi, 2 s each side) antimicrobial solutions or water (except control), vacuum-packaged and stored at 7°C for 43 days.

Application	Treatment	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[2] (log CFU/cm^2)	Y_{end}[3] (log CFU/cm^2)	R^2
	Control	-[1]	0.300 AE	3.5	7.4	0.931
Dipping	Water	-[1]	0.552 D	2.6	7.5	0.958
	2% LA	6.5	0.174 CF	2.7	7.5	0.955
	2% ALF	-[1]	0.348 BE	3.0	7.5	0.925
Spraying	Water	-[1]	0.401 B	3.1	7.6	0.985
	2% LA	-[1]	0.158 C	2.5	7.2	0.967
	2% ALF	-[1]	0.251 AF	3.0	7.5	0.966

[1] no lag phase observed
[2]Lower asymptote estimated by the Baranyi model
[3]Upper asymptote estimated by the Baranyi model
LA, lactic acid; ALF, activated lactoferrin
Means within the same column that have a common letter are not significantly different (P>0.05)

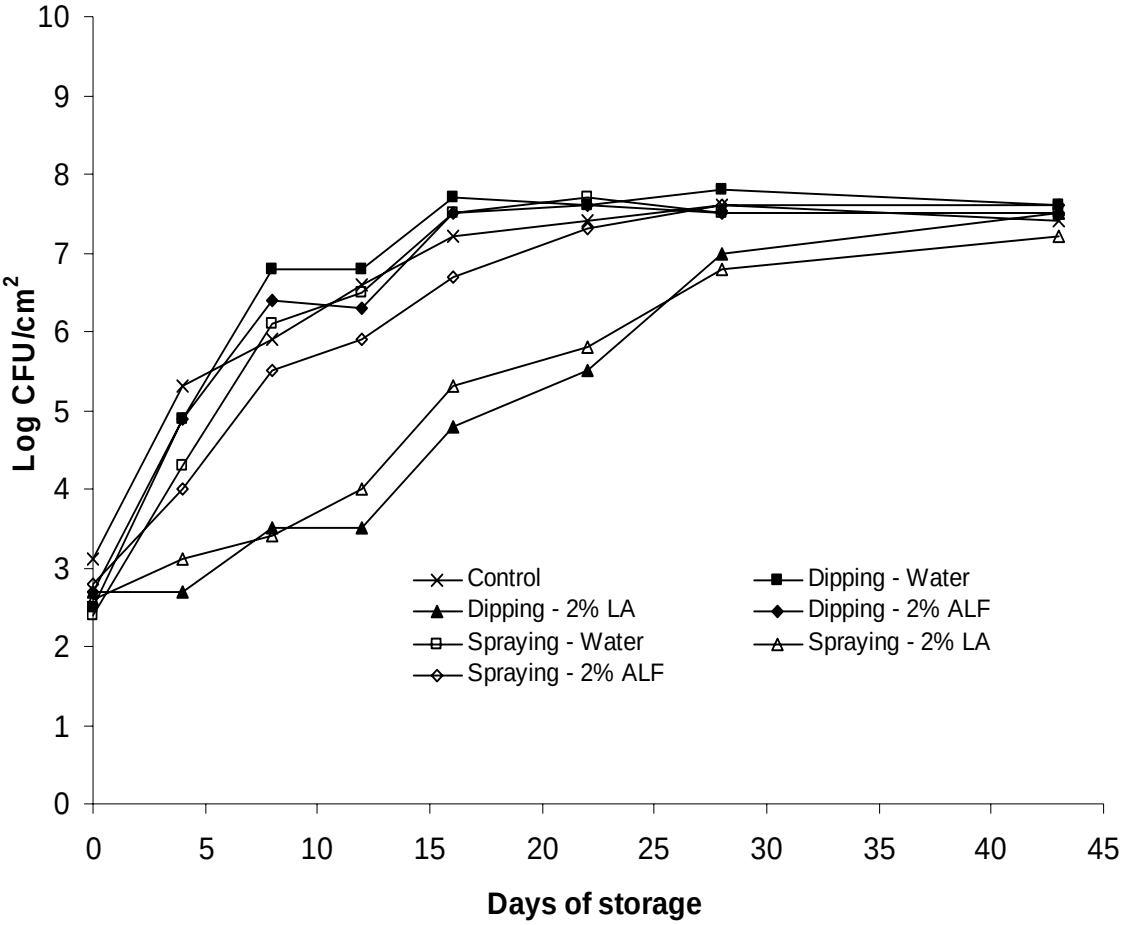


Figure 4: Mean (n=3) populations of *Listeria monocytogenes* (log CFU/cm^2) inoculated onto the surface of bologna before dipping (for 30 s) or spraying (10 psi, 2 s each side) with water or antimicrobial solutions, vacuum-packaging and storage at 7^{o}C for 43 days. LA, lactic acid; ALF, activated lactoferrin.

Table 12: Mean (n=3) total microbial populations (log CFU/cm^2 ± standard deviation) on bologna slices inoculated with *Listeria monocytogenes* and dipped in (30 s) or sprayed with (10 psi, 2 s each side) antimicrobial solutions or water (except control), vacuum-packaged and stored at 7^{o}C.

		Days of storage							
Application	Treatment	0	4	8	12	16	22	28	43
	Control	3.2±0.0	5.2±0.7	6.0±0.1	6.6±0.1	7.3±0.1	6.5±1.8	7.7±0.4	7.8±0.5
Dipping	Water	2.6±0.1	4.9±0.2	6.5±0.5	6.9±0.0	7.7±0.0	7.7±0.1	7.8±0.0	7.7±0.1
	Lactic acid (2%)	2.8±0.0	3.0±0.3	3.2±0.1	3.9±0.0	5.2±0.5	5.7±0.1	7.0±0.1	7.6±0.1
	Activated lactoferrin (2%)	3.1±0.5	4.8±0.1	6.4±0.2	6.8±0.2	7.6±0.1	7.6±0.0	7.6±0.0	7.7±0.0
Spraying	Water	2.5±0.1	4.2±0.1	6.0±0.0	6.6±0.0	7.5±0.2	7.8±0.1	7.6±0.0	7.7±0.1
	Lactic acid (2%)	2.8±0.3	3.1±0.1	3.8±0.2	4.1±0.3	4.9±0.0	5.8±0.0	6.9±0.2	7.4±0.1
	Activated lactoferrin (2%)	2.9±0.0	4.1±0.1	5.7±0.3	6.0±0.1	6.8±0.3	7.3±0.1	7.7±0.4	7.6±0.2

Table 13: Mean (n=3) pH values (pH ± standard deviation) of bologna slices inoculated with *Listeria monocytogenes* and dipped in (30 s) or sprayed with (10 psi, 2 s each side) antimicrobial solutions or water (except control), vacuum-packaged and stored at 7^{o}C.

		Days of storage							
Application	Treatment	0	4	8	12	16	22	28	43
	Control	6.31±0.01	6.31±0.02	6.31±0.01	6.28±0.06	6.97±0.03	5.80±0.03	5.61±0.02	5.48±0.04
Dipping	Water	6.32±0.02	6.33±0.01	6.1±0.01	6.05±0.05	5.64±0.01	5.57±0.03	5.32±0.08	5.20±0.08
	Lactic acid (2%)	5.67±0.01	5.70±0.02	5.72±0.02	5.68±0.02	5.69±0.03	5.76±0.03	5.65±0.02	5.32±0.05
	Activated lactoferrin (2%)	6.38±0.01	6.38±0.02	6.37±0.02	6.34±0.02	5.96±0.09	5.65±0.03	5.42±0.02	5.27±0.04
Spraying	Water	6.34±0.01	6.34±0.01	6.31±0.01	6.26±0.03	5.81±0.06	5.64±0.01	5.42±0.01	5.34±0.04
	Lactic acid (2%)	5.88±0.04	5.85±0.02	5.88±0.02	5.89±0.04	5.85±0.04	5.90±0.02	5.76±0.04	5.40±0.05
	Activated lactoferrin (2%)	6.36±0.03	6.34±0.04	6.37±0.01	6.32±0.01	6.19±0.07	5.87±0.08	5.49±0.03	5.39±0.04

Table 14: Mean (n=2) a_w values (a_w ± standard deviation) of bologna slices inoculated with *Listeria monocytogenes* and dipped in (30 s) or sprayed with (10 psi, 2 s each side) antimicrobial solutions or water (except control).

Application	Treatment	Water activity on day-0
	Control	0.966±0.001
Dipping	Water	0.975±0.001
	Lactic acid (2%)	0.970±0.001
	Activated lactoferrin (2%)	0.966±0.001
Spraying	Water	0.974±0.001
	Lactic acid (2%)	0.969±0.000
	Activated lactoferrin (2%)	0.965±0.002

L. monocytogenes completely for 30.1 days and subsequent growth of the pathogen on samples dipped into 1% acetic acid was slower ($P<0.05$) (μ_{max}: 0.120 $days^{-1}$) compared to that observed on samples treated with water or ALF (0.391 and 0.271 day^{-1}, respectively).

Immersing the samples into antimicrobial solutions or water after inoculation resulted in reductions in *L. monocytogenes* initial counts (y_0) that ranged from 0.2 (acetic acid) to 0.7 (water or ALF) log CFU/cm^2. Although acetic acid caused minor initial reductions of *L. monocytogenes*, it inhibited growth of the pathogen completely for 45.0 days and subsequent growth was slower (μ_{max}: 0.039 $days^{-1}$) ($P<0.05$) than that observed in samples treated with water (μ_{max}: 0.483 $days^{-1}$) or ALF (μ_{max}: 0.299 $days^{-1}$). Water or ALF post-inoculation treatments also inhibited growth of the pathogen, however the lag phases they provided were of shorter ($P<0.05$) duration (2.8 days) than that caused by acetic acid. Final *L. monocytogenes* populations (y_{end}) in product dipped into water or ALF after inoculation reached levels equal to those obtained for samples dipped before inoculation (approximately 8 log CFU/cm^2). On the contrary, populations of the pathogen on samples treated with acetic acid increased by approximately 1 log CFU/cm^2 during the 30-day storage period (Figure 5). This effect is probably due to injury of the cells due to the high acidity of the acetic acid solution. Nevertheless, the antilisterial activity of ALF did not seem to be affected by its pre- or post-inoculation application, and therefore, the solution was applied after inoculation in subsequent studies.

The pH of untreated samples on day-0 was 6.32 on day-0 (Table 17). Dipping into water or 2% ALF for 2 min led to increases in product pH (0.06 and 0.07 units respectively) on day-0. The pH of samples dipped into solutions of 1% acetic acid was 5.92 and 5.91, when the treatment was applied before and after inoculation, respectively. During storage, reductions of product pH were observed for all treatments, whereas, samples treated with acetic acid after inoculation had the lowest pH (5.88) by the end of storage.

Dipping into antimicrobial solutions or water resulted in increases in a_w values on day-0 (Table 18). Water activity values of treated and untreated samples ranged from 0.972 (control) to 0.981 (water applied before or after inoculation of the product).

Study 4-ALF and organic acids or salts applied as dipping solutions for *Listeria monocytogenes* control on commercial cured turkey breast

Growth kinetics of *L. monocytogenes* on commercial, cured turkey breast treated with antimicrobial solutions are presented in Table 19. Additionally, *L. monocytogenes* and total microbial populations (log CFU/cm^2) obtained during the storage period are presented in Figure 6 and Table 20, respectively.

Initial reductions in *L. monocytogenes* counts caused by single treatments ranged from 0.2 (1% lactic acid or 1% ALF) to 0.8 log CFU/cm^2 (water), whereas reductions caused by combination treatments varied from 0.6 (1% ALF followed by 3% sodium diacetate) to 0.8 log CFU/cm^2 (1% ALF followed by 1% acetic acid). Sodium diacetate (3%) used individually or after application of 1% ALF provided complete inhibition of *L. monocytogenes* growth, whereas, water, 3% potassium lactate, and 1% ALF followed by 3% potassium lactate allowed instant growth of the pathogen. Other treatments provided lag phases that ranged from 0.8 (1% ALF) to 42.0 (1%

Table 15: Mean (n=3) growth kinetics of *Listeria monocytogenes* growth on the surface of commercial ham slices inoculated with *Listeria monocytogenes* before or after dipping into water, acetic acid, or activated lactoferrin, vacuum-packaged and stored at 7°C.

Application	Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[1] (log CFU/cm^2)	Y_{end}[2] (log CFU/cm^2)	R^2
Before inoculation	Control	2.7 AC	0.312 A	3.0	7.9	0.988
	Water (2 min)	2.2 A	0.391 AC	3.4	8.1	0.992
	1% AA (30 s)	30.1 B	0.120 B	3.0	-	0.899
	2% ALF (2 min)	3.0 C	0.271 A	2.9	8.1	0.980
After inoculation	Water (2 min)	2.8 C	0.483 C	2.3	8.0	0.966
	1% AA (30 s)	45.0 D	0.039 D	2.8	4.1	0.829
	2% ALF (2 min)	2.8 C	0.299 A	2.3	8.3	0.976

[1]Lower asymptote estimated by the Baranyi model

[2]Upper asymptote estimated by the Baranyi model; no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponds to stationary phase

AA, acetic acid; ALF, activated lactoferrin

Means within the same column that have a common letter are not significantly different (P>0.05)

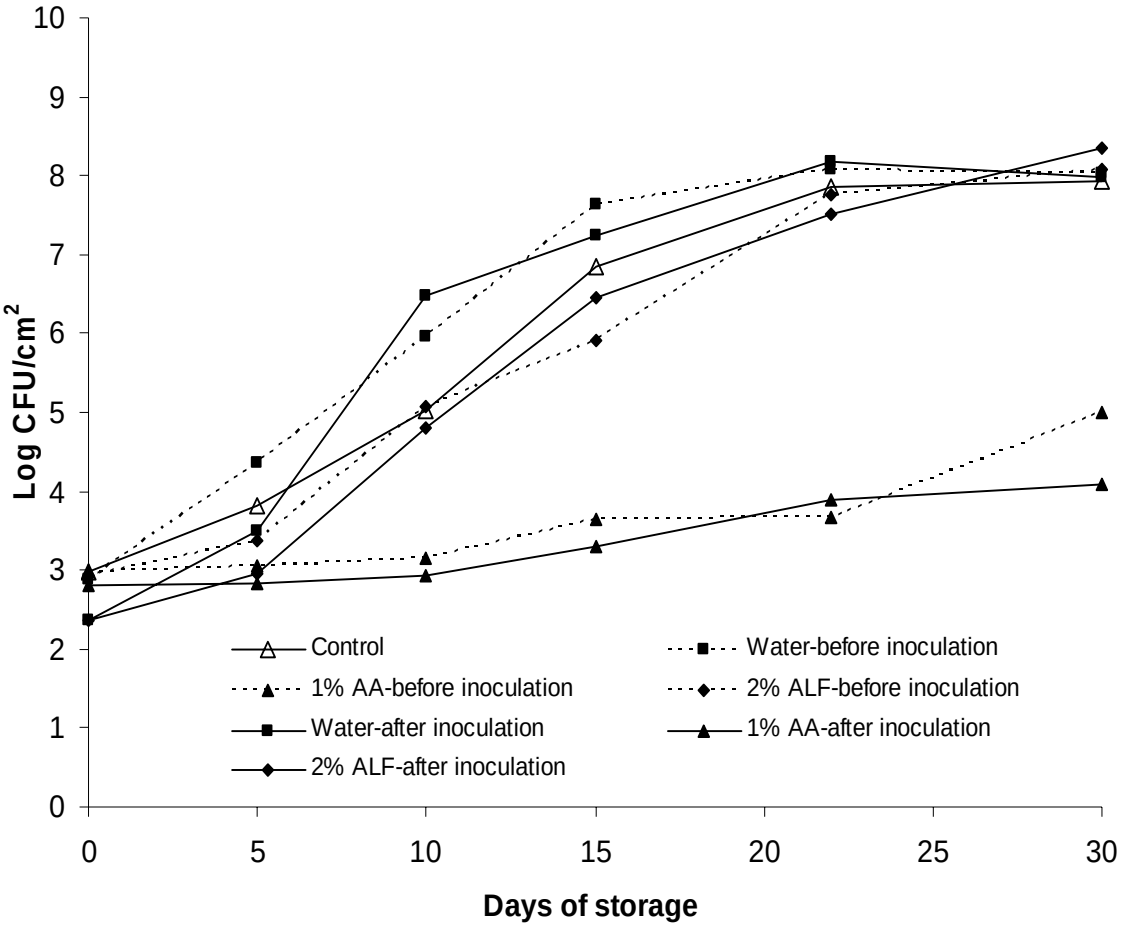


Figure 5: Mean (n=3) populations of *Listeria monocytogenes* (log CFU/cm^2) on the surface of commercial ham slices, inoculated with the pathogen before or after dipping into water (for 2 min), acetic acid (for 30 s), or activated lactoferrin (for 2 min), vacuum-packaged, and stored at 7^{o}C for 30 days. AA, acetic acid; ALF, activated lactoferrin.

Table 16: Mean (n=3) total microbial populations (log CFU/cm^2 ± standard deviation) on the surface of commercial ham slices inoculated with *Listeria monocytogenes* before or after dipping into water (2 min), 1% acetic acid (30 s), or 2% activated lactoferrin (2 min) vacuum-packaged and stored at 7^{o}C.

		Days of storage					
Application	Treatment (dipping)	0	5	10	15	22	30
Before inoculation	Control	3.0±0.0	4.0±0.5	5.0±0.1	6.9±0.2	8.3±0.5	8.0±0.1
	Water (2 min)	3.0±0.0	4.4±0.2	6.0±0.2	7.7±0.3	8.2±0.1	8.2±0.0
	1% AA (30 s)	3.0±0.0	3.1±0.1	3.1±0.0	4.6±0.2	4.7±0.2	4.1±0.4
	2%ALF (2 min)	3.0±0.1	3.3±0.1	5.2±0.1	6.3±0.5	7.9±0.2	8.3±0.1
After inoculation	Water (2 min)	2.6±0.1	4.0±0.4	6.4±0.3	7.8±0.4	8.1±0.0	8.2±0.1
	1% AA (30 s)	2.9±0.0	2.9±0.2	3.0±0.1	4.0±1.3	6.3±0.3	6.0±0.2
	2%ALF (2 min)	2.5±0.1	3.0±0.1	4.6±0.1	6.6±0.2	8.3±1.1	8.2±0.3

AA, acetic acid; ALF, activated lactoferrin

Table 17: Mean (n=3) pH values (pH ± standard deviation) of commercial ham slices inoculated with *Listeria monocytogenes* before or after dipping into water, acetic acid, or activated lactoferrin, vacuum-packaged and stored at 7°C.

		Days of storage					
Application	Treatment (dipping)	0	5	10	15	22	30
Before inoculation	Control	6.32±0.02	6.29±0.01	6.29±0.04	6.24±0.00	6.13±0.05	5.91±0.02
	Water (2 min)	6.38±0.01	6.36±0.01	6.32±0.07	6.20±0.03	5.96±0.01	5.93±0.01
	1% AA (30 s)	5.92±0.05	5.97±0.03	5.91±0.03	5.92±0.06	5.91±0.02	5.90±0.06
	2% ALF (2 min)	6.39±0.02	6.39±0.02	6.39±0.01	6.34±0.03	6.20±0.06	5.92±0.02
After inoculation	Water (2 min)	6.38±0.01	6.35±0.02	6.34±0.02	6.25±0.06	5.96±0.01	5.88±0.05
	1% AA (30 s)	5.91±0.07	5.98±0.02	5.89±0.07	5.88±0.06	5.93±0.08	5.82±0.05
	2% ALF (2 min)	6.39±0.02	6.38±0.02	6.40±0.02	6.34±0.03	6.22±0.01	5.91±0.03

AA, acetic acid; ALF, activated lactoferrin

Table 18: Mean (n=2) a_w values (a_w ± standard deviation) of commercial ham slices inoculated with *Listeria monocytogenes* before or after dipping into water, acetic acid, or activated lactoferrin.

Application	Treatment (dipping)	Water activity on day-0
	Control	0.972±0.003
Before inoculation	Water (2 min)	0.981±0.000
	1% AA (30 s)	0.976±0.000
	2%ALF (2 min)	0.978±0.001
After inoculation	Water (2 min)	0.981±0.000
	1% AA (30 s)	0.978±0.000
	2%ALF (2 min)	0.979±0.002

AA, acetic acid; ALF, activated lactoferrin

acetic acid) days. Maximum specific growth rates for all treatments (single or combinations) ranged from -0.001 $days^{-1}$ (1% ALF followed by 3% sodium diacetate) to 0.904 $days^{-1}$ (1% ALF followed by 1% acetic acid). Of all single treatments, dipping into 3% sodium diacetate caused the slowest growth of the pathogen (μ_{max}: 0.002 $days^{-1}$), whereas, 1% ALF allowed the fastest growth (μ_{max}:0.340 $days^{-1}$). The sequential treatment of activated lactoferrin (1%) followed by potassium lactate (3%), sodium diacetate (3%), or lactic acid (1%) provided similar (P>0.05) antilisterial effects as single 3% potassium lactate, 3% sodium diacetate, or 1% lactic acid, respectively, as suggested by lag phases and μ_{max} values obtained for these treatments. When ALF (1%) was followed by 1% acetic acid, a lag phase duration of 28.9 days was obtained before growth occurred (y_{end}: 4.0 log CFU/cm^2). Acetic acid (1%) applied individually appeared to be more effective that its combination with 1% ALF, as it resulted in slower (P<0.05) growth of the pathogen and a more extended (P>0.05) lag phase. The results indicated that, under the conditions of this study, dipping turkey breast slices into sequential treatments consisting of ALF and sodium diacetate provided complete inhibition and even slight reduction of *L. monocytogenes* growth during storage. However, there was no evidence that ALF enhanced the antilisterial effects of other antimicrobial compounds, as sodium diacetate was very effective even when applied individually.

The pH of untreated product was 6.22 on day-0 (Table 21). Application of sodium diacetate, lactic acid or acetic acid as single or sequential (after dipping into ALF) treatments resulted in pH reductions of 0.29 to 0.54 units. The greatest increase in product pH on day-0 resulted from dipping into 1% ALF (0.09 units). Reductions of pH of dipped or undipped turkey breast were observed during storage with samples treated with 1% ALF followed by 3% lactic acid or 1% ALF having the lowest (5.62) and highest (6.17) pH values, respectively, on day-43.

Water activity values of treated or untreated samples on day-0 ranged from 0.975 (control) to 0.982 (water, 2% ALF, 1% ALF followed 3% potassium lactate or acetic acid) (Table 22).

Study 5-Different concentrations of ALF and/or organic acids or salts applied as dipping solutions for *Listeria monocytogenes* control on commercial uncured turkey breast

Growth kinetics data for *L. monocytogenes* on inoculated uncured turkey breast dipped into antimicrobial solutions and stored for 34 days at 7°C are shown in Table 23. Furthermore, *L. monocytogenes* and total microbial counts (log CFU/cm^2) obtained during the 34-day storage period are presented in Figure 7 and Table 24, respectively.

Most of the antimicrobial treatments resulted in initial reductions of *L. monocytogenes* of 0.7-1.0 log CFU/cm^2, compared to the control (untreated), while the 2% ALF (1 min) treatment and the sequential treatment of 1% ALF followed by 2% lactic acid led to a slightly higher initial reduction of 1.2 log CFU/cm^2 (based on y_0 results). Growth kinetics data (Table 23) indicated that the treatment with the longest lag phase duration (2.4 days) was sodium diacetate (1.5%, 1 min); however, this value was not different (P>0.05) to the lag phase durations (ranging from 0.5 to 1.0 days) exhibited by some of the other treatments. Maximum specific growth rate data indicated that of all the single treatments applied, sodium diacetate (1.5%, 1 min) and lactic acid (2%, 1 min) had the lowest μ_{max} values (0.128 and 0.175 day^{-1}, respectively) (P>0.05). However, the sodium diacetate treatment appeared to have a greater antilisterial activity due to its lag phase

Table 19: Mean (n=3) growth kinetics of *Listeria monocytogenes* growth on the surface of commercial, cured turkey breast slices inoculated with the pathogen, dipped into antimicrobial solutions or water for 1 min (except control), vacuum-packaged and stored at 7°C for 43 days.

Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[3] (log CFU/cm^2)	Y_{end}[4] (log CFU/cm^2)	R^2
Control (undipped)	3.7 A	0.204 A	3.0	7.2	0.974
Water	-[1]	0.240 A	2.2	7.0	0.962
PL (3%)	-[1]	0.254 A	2.4	6.9	0.941
SD (3%)	-[2]	0.002 A	2.7	-	0.045
LA (1%)	9.7 A	0.231 A	2.8	7.0	0.975
AA (1%)	42.0 B	0.060 A	2.8	-	0.481
ALF (2%)	3.3 A	0.250 A	2.5	7.1	0.932
ALF (1%)	0.8 A	0.340 A	2.6	7.2	0.971
ALF (1%) + PL (3%)	-[1]	0.216 A	2.3	7.0	0.985
ALF (1%) + SD (3%)	-[2]	-0.001 A	2.4	-	
ALF (1%) + LA (1%)	9.4 A	0.200 A	2.4	6.6	0.969
ALF (1%) + AA (1%)	28.9 B	0.904 B	2.2	4.0	0.530

[1] no lag phase observed; [2] no growth observed

[3]Lower asymptote estimated by the Baranyi model

[4]Upper asymptote estimated by the Baranyi model; no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponds to stationary phase

PL: potassium lactate, SD: sodium diacetate, LA: lactic acid, AA: acetic acid, ALF: activated lactoferrin

Means within the same column that have a common letter are not significantly different (P>0.05)

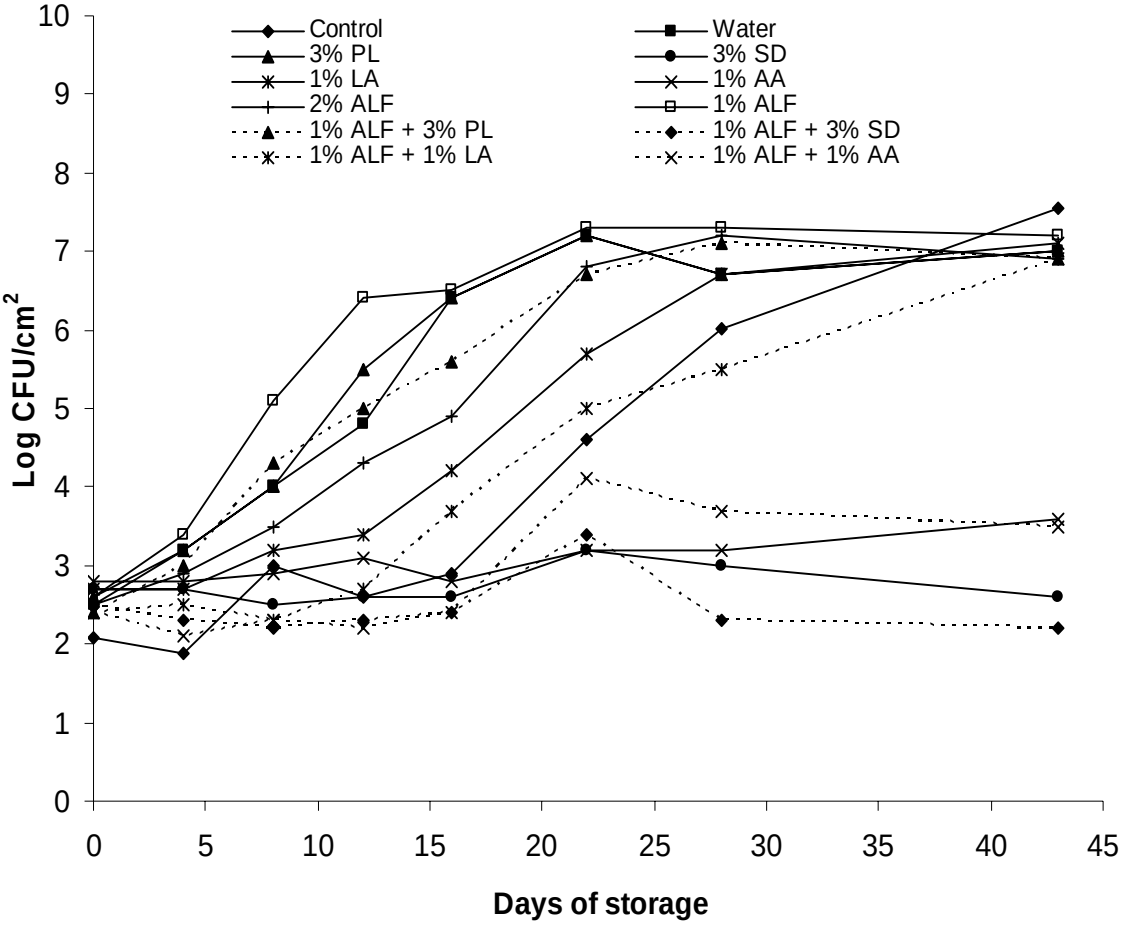


Figure 6: Mean (n=3) populations of *Listeria monocytogenes* (log CFU/cm^2) inoculated onto the surface of commercial, cured turkey breast slices before treating for 1 min with water or antimicrobial solutions (except control), vacuum-packaging and storage at 7^{o}C for 43 days. PL, potassium lactate; SD, sodium diacetate; LA, lactic acid; AA, acetic acid; ALF, activated lactoferrin.

Table 20: Mean (n=3) total microbial populations (log CFU/cm^2 ± standard deviation) on the surface of commercial, cured turkey breast slices, inoculated with *Listeria monocytogenes*, dipped into antimicrobial solutions or water for 1 min (except control), vacuum-packaged and stored at 7^{o}C.

	Days of storage							
Treatment (dipping)	0	4	8	12	16	22	28	43
Control (undipped)	3.1±0.1	3.2±0.2	4.8±0.4	4.8±0.3	5.9±0.3	6.7±0.1	7.1±0.1	7.4±0.1
Water	2.5±0.1	3.3±0.2	4.4±0.5	4.8±0.2	6.4±0.0	7.2±0.3	6.8±0.1	7.0±0.3
PL (3%)	2.7±0.1	3.3±0.2	4.4±0.3	5.6±0.1	6.5±0.4	7.2±0.0	7.2±0.3	6.5±0.2
SD (3%)	2.7±0.1	2.6±0.1	2.6±0.4	2.7±0.1	2.7±0.0	4.3±0.2	4.1±1.0	2.5±0.2
LA (1%)	2.7±0.1	2.7±0.1	3.3±0.1	3.5±0.1	4.2±0.1	5.7±0.4	6.7±0.0	7.2±0.2
AA (1%)	2.8±0.0	2.8±0.1	2.8±0.1	2.9±0.2	2.9±0.2	3.3±0.6	3.4±0.5	3.5±0.8
ALF (2%)	2.6±0.1	2.9±0.1	3.8±0.6	4.3±0.2	5.6±0.6	7.2±1.0	7.2±0.0	7.1±0.4
ALF (1%)	2.6±0.0	3.5±0.1	5.2±0.0	5.9±0.0	6.5±0.2	7.3±0.1	7.3±0.2	7.2±0.6
ALF (1%) + PL (3%)	2.3±0.1	3.1±0.0	4.0±0.5	5.0±0.2	5.7±0.4	6.7±0.3	7.1±0.1	6.9±0.2
ALF (1%) + SD (3%)	2.5±0.0	2.4±0.0	2.5±0.1	2.5±0.2	2.4±0.0	3.9±0.5	2.7±0.3	3.4±1.7
ALF (1%) + LA (1%)	2.4±0.1	2.4±0.1	2.4±0.1	2.5±0.2	3.7±0.3	5.0±0.3	5.6±0.3	6.9±0.7
ALF (1%) + AA (1%)	2.5±0.0	2.1±0.2	2.4±0.0	2.3±0.2	2.4±0.1	4.2±0.0	3.6±1.5	3.0±1.1

PL, potassium lactate; SD, sodium diacetate; LA, lactic acid; AA, acetic acid; ALF, activated lactoferrin

Table 21: Mean (n=3) pH values (pH ± standard deviation) of commercial, cured turkey breast slices, inoculated with *Listeria monocytogenes*, dipped into antimicrobial solutions or water for 1 min (except control), vacuum-packaged and stored at 7°C.

	Days of storage							
Treatment (dipping)	0	4	8	12	16	22	28	43
Control (undipped)	6.22±0.02	6.19±0.05	6.17±0.05	6.23±0.05	6.18±0.03	6.19±0.07	6.17±0.03	6.07±0.07
Water	6.23±0.02	6.22±0.02	6.22±0.03	6.25±0.04	6.20±0.02	6.14±0.04	6.15±0.03	6.10±0.01
PL (3%)	6.21±0.02	6.19±0.07	6.20±0.05	6.20±0.03	6.20±0.03	6.14±0.08	6.11±0.08	6.09±0.06
SD (3%)	5.68±0.05	5.71±0.13	5.80±0.03	5.71±0.05	5.67±0.05	5.73±0.04	5.78±0.04	5.64±0.10
LA (1%)	5.93±0.06	5.90±0.04	5.92±0.11	5.82±0.12	5.94±0.04	5.85±0.02	5.83±0.11	5.82±0.07
AA (1%)	5.73±0.03	5.70±0.05	5.74±0.10	5.71±0.09	5.69±0.11	5.67±0.21	5.76±0.02	5.72±0.09
ALF (2%)	6.19±0.03	6.19±0.03	6.24±0.07	6.29±0.01	6.21±0.03	6.23±0.06	6.15±0.03	6.00±0.17
ALF (1%)	6.31±0.02	6.23±0.03	6.27±0.00	6.28±0.01	6.20±0.02	6.19±0.05	6.18±0.01	6.17±0.01
ALF (1%) + PL (3%)	6.26±0.00	6.23±0.02	6.28±0.03	6.28±0.02	6.25±0.02	6.23±0.01	6.17±0.01	6.16±0.05
ALF (1%) + SD (3%)	5.81±0.05	5.80±0.04	5.82±0.04	5.83±0.10	5.84±0.05	5.88±0.05	5.89±0.02	5.75±0.02
ALF (1%) + LA (1%)	5.84±0.06	5.89±0.01	5.96±0.03	5.86±0.17	5.81±0.18	5.87±0.10	5.94±0.12	5.62±0.13
ALF (1%) + AA (1%)	5.78±0.09	5.72±0.04	5.76±0.02	5.79±0.15	5.77±0.10	5.82±0.11	5.75±0.06	5.71±0.22

PL, potassium lactate; SD, sodium diacetate; LA, lactic acid; AA, acetic acid; ALF, activated lactoferrin

Table 22: Mean (n=2) a_w values (a_w ± standard deviation) of commercial, cured turkey breast slices, inoculated with *Listeria monocytogenes* and dipped into antimicrobial solutions or water for 1 min (except control).

Treatment (dipping)	Water activity on day-0
Control (undipped)	0.975±0.001
Water	0.982±0.001
PL (3%)	0.981±0.001
SD (3%)	0.981±0.001
LA (1%)	0.981±0.000
AA (1%)	0.981±0.001
ALF (2%)	0.982±0.000
ALF (1%)	0.981±0.001
ALF (1%) + PL (3%)	0.982±0.001
ALF (1%) + SD (3%)	0.980±0.000
ALF (1%) + LA (1%)	0.981±0.001
ALF (1%) + AA (1%)	0.982±0.001

PL, potassium lactate; SD, sodium diacetate; LA, lactic acid; AA, acetic acid; ALF, activated lactoferrin

duration of 2.4 days (Table 23). With regards to the ALF treatments that were applied, no lag phase was obtained for 2% ALF (1 min), while similar lag phase durations were noted for 1% ALF (1 min) and 2% ALF (2 min) (0.8 and 1.0 days, respectively). However, a lower ($P<0.05$) μ_{max} value was obtained for the 1% ALF treatment than either (1 or 2 min) of the 2% ALF treatments, possibly implying that 1% ALF was slightly more effective than 2% ALF. This is also reflected in the *L. monocytogenes* counts obtained for these treatments during the storage period (Figure 7). Similar μ_{max} values ($P>0.05$) were obtained for 1% ALF (1 min) and 1% lactic acid (1 min); however, the 1% ALF treatment had a lag phase duration of 0.8 days compared to no lag phase for the 1% LA treatment. Application of 2% ALF for 2 min reduced ($P<0.05$) the μ_{max} value of the corresponding 1 min treatment by 0.100 day^{-1} and also, as seen above, resulted in a lag phase duration of 1.0 days for the 2 min treatment. The μ_{max} values for both 2% ALF treatments, however, were not different ($P>0.05$) from the control and water treatments.

When the ALF treatments (1 or 2%, 1 min) were followed by either 1.5% sodium diacetate (1 min) or 1 or 2% lactic acid (1 min), the μ_{max} values of some of these treatments, as compared to the ALF treatments applied on their own, were reduced. Specifically, the μ_{max} value of 1% ALF was reduced ($P<0.05$) from 0.317 day^{-1} to 0.205 day^{-1} when followed by 2% lactic acid, while the μ_{max} value of 2% ALF (1 min) was reduced ($P<0.05$) from 0.486 day^{-1} to 0.332 day^{-1} when followed by 1.5% sodium diacetate. Thus, the antilisterial activity of the ALF treatments was enhanced when followed by 2% lactic acid or sodium diacetate; however, the sequential treatment with sodium diacetate was less effective ($P<0.05$) than when sodium diacetate was applied on its own, both in the lag phase duration and μ_{max}. In contrast to the above results, the μ_{max} value of 2% ALF applied for 1 min on its own, was almost the same ($P>0.05$) as when it was followed by 1% lactic acid; however, a lag phase of 0.5 days was noted for the sequential treatment. Thus, by applying 2% ALF followed by 1% LA, the μ_{max} value of 2% ALF was not enhanced, and furthermore, the antilisterial effect seen for 1% lactic acid when applied on its own, was lost in the sequential treatment (the μ_{max} value that was obtained for the sequential treatment was not different ($P>0.05$) to that obtained for the control and water treatments).

The pH of untreated, uncured turkey breast samples on day-0 was 6.40 (Table 25). Dipping of product in water and all the ALF treatments had no apparent effect on product pH, while the sodium diacetate and 1% lactic acid treatments, applied alone and in sequence with 2% ALF, led to reductions of 0.21-0.30 pH units. The greatest reductions in product pH were obtained for samples dipped in 2% lactic acid alone and when applied sequentially with 1% ALF (pH reductions of 0.58 and 0.57, respectively). Due to growth of *L. monocytogenes* and spoilage populations, the pH of samples for all treatments was reduced to 5.02-5.82 by the end of storage.

The water activity of control (untreated) samples on day-0 was 0.976 (Table 26). After dipping in water or the antimicrobial treatments, water activity values of 0.980-0.986 were obtained.

Study 6-Different concentrations of ALF and/or lactic acid applied as dipping solutions for *Listeria monocytogenes* control on commercial uncured turkey breast

In this study, some of the treatments applied to uncured turkey breast samples in Study 5 were repeated and the results of both studies are presented here. An additional treatment, ALF (2%, 1

Table 23: Mean (n=3) growth kinetics of *Listeria monocytogenes* growth on the surface of inoculated commercial, uncured turkey breast dipped into antimicrobial solutions, vacuum-packaged and stored at 7°C for 34 days.

Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max}; days^{-1})	Y_o[2] (log CFU/cm^2)	Y_{end}[3] (log CFU/cm^2)	R^2
Control	-[1]	0.444 AB	2.9	6.9	0.957
Water (1 min)	0.7 A	0.452 A	1.9	6.7	0.983
SD (1.5%, 1 min)	2.4 A	0.128 F	2.0	5.2	0.939
LA (1%, 1 min)	-	0.320 CD	2.0	6.6	0.970
LA (2%, 1 min)	-	0.175 EF	2.2	6.8	0.931
ALF (1%, 1 min)	0.8 A	0.317 D	2.0	6.9	0.982
ALF (2%, 1 min)	-	0.486 A	1.7	7.3	0.994
ALF (2%, 2 min)	1.0 A	0.386 BC	1.9	7.3	0.992
ALF (2%, 1 min) - SD (1.5%, 1 min)	1.0 A	0.332 CD	2.0	7.0	0.971
ALF (1%, 1 min) - LA (2%, 1 min)	-	0.205 E	1.7	6.1	0.975
ALF (2%, 1 min) - LA (1%, 1 min)	0.5 A	0.495 A	2.0	6.4	0.950

[1] No lag phase observed
[2] Lower asymptote estimated by the Baranyi model
[3] Upper asymptote estimated by the Baranyi model
SD: sodium diacetate, LA: lactic acid, ALF: activated lactoferrin
Means within the same column that have a common letter are not significantly different (P>0.05)

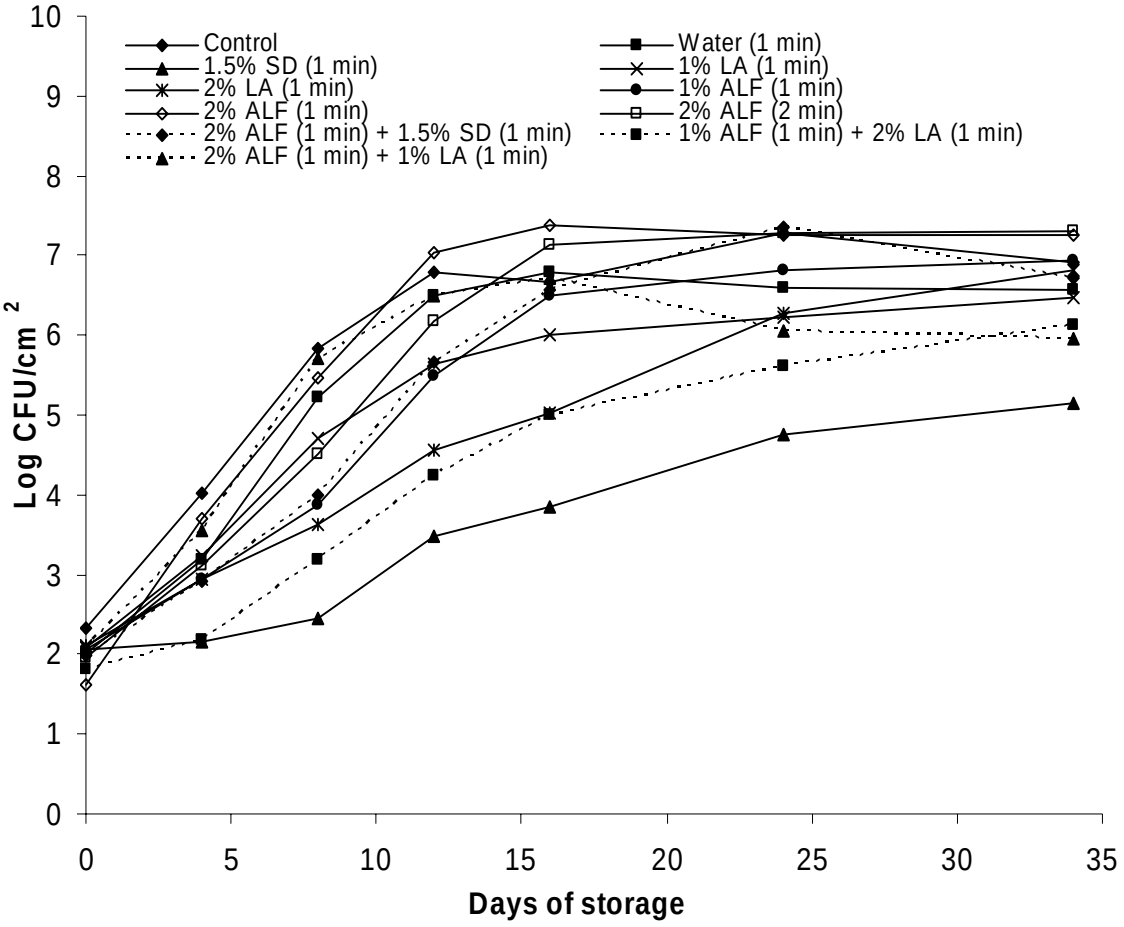


Figure 7: Mean (n=3) populations of *Listeria monocytogenes* (log CFU/cm^2) inoculated onto commercial, uncured turkey breast before dipping into antimicrobial solutions, vacuum-packaging and storage at 7°C for 34 days. The duration of each dipping treatment is provided in the figure legend. SD, sodium diacetate; LA, lactic acid; ALF, activated lactoferrin.

Table 24: Mean (n=3) total microbial populations (log CFU/cm^2 ± standard deviation) on inoculated commercial, uncured turkey breast dipped into antimicrobial solutions, vacuum-packaged and stored at 7°C for 34 days.

Treatment (dipping)	Days of storage						
	0	4	8	12	16	24	34
Control	2.3±0.0	4.6±0.2	7.3±0.1	8.0±0.0	8.0±0.0	8.3±0.2	8.0±0.1
Water (1 min)	2.2±0.0	3.7±0.3	6.6±0.1	7.8±0.1	8.1±0.0	8.0±0.1	8.2±0.1
SD (1.5%, 1 min)	2.2±0.1	2.3±0.1	3.0±0.5	5.3±0.5	5.7±0.5	7.4±0.1	7.7±0.3
LA (1%, 1 min)	2.2±0.1	4.0±0.4	6.9±0.1	7.7±0.0	7.7±0.1	7.7±0.1	7.7±0.1
LA (2%, 1 min)	2.2±0.1	3.1±0.1	4.4±0.5	5.9±0.7	6.5±0.2	7.4±0.4	7.3±0.2
ALF (1%, 1 min)	2.1±0.1	3.0±0.3	4.9±0.4	6.5±0.2	7.9±0.2	7.9±0.1	7.8±0.0
ALF (2%, 1 min)	1.8±0.0	4.3±0.2	6.6±0.2	7.9±0.2	7.9±0.1	7.9±0.1	8.0±0.1
ALF (2%, 2 min)	2.3±0.1	3.8±0.2	5.6±0.2	7.7±0.2	7.9±0.1	7.9±0.1	7.8±0.0
ALF (2%, 1 min) - SD (1.5%, 1 min)	2.1±0.1	3.1±0.4	5.0±0.2	6.8±0.6	7.4±0.4	8.3±0.2	7.8±0.1
ALF (1%, 1 min) - LA (2%, 1 min)	1.9±0.1	3.2±0.3	5.2±0.8	6.6±0.2	7.1±0.6	7.2±0.1	7.4±0.3
ALF (2%, 1 min) - LA (1%, 1 min)	2.3±0.2	4.7±0.1	7.2±0.4	7.6±0.1	7.8±0.3	7.7±0.1	7.6±0.1

SD, sodium diacetate; LA, lactic acid; ALF, activated lactoferrin

Table 25: Mean (n=3) pH values (pH ± standard deviation) of commercial, uncured turkey breast inoculated with *Listeria monocytogenes*, dipped into antimicrobial solutions, vacuum-packaged and stored at 7°C for 34 days.

Treatment (dipping)	Days of storage						
	0	4	8	12	16	24	34
Control	6.40±0.01	6.30±0.01	6.27±0.01	5.95±0.01	5.67±0.07	5.54±0.14	5.52±0.11
Water (1 min)	6.42±0.01	6.31±0.01	6.35±0.01	6.14±0.03	5.88±0.07	5.47±0.14	5.33±0.07
SD (1.5%, 1 min)	6.15±0.06	6.03±0.08	6.06±0.06	6.11±0.02	6.09±0.04	5.69±0.19	5.53±0.01
LA (1%, 1 min)	6.10±0.03	6.00±0.03	5.98±0.07	5.69±0.13	5.62±0.13	5.44±0.17	5.34±0.13
LA (2%, 1 min)	5.82±0.09	5.79±0.07	5.77±0.05	5.87±0.05	5.87±0.11	5.63±0.19	5.54±0.03
ALF (1%, 1 min)	6.45±0.02	6.35±0.01	6.39±0.01	6.40±0.00	6.09±0.16	5.78±0.04	5.82±0.09
ALF (2%, 1 min)	6.44±0.01	6.35±0.01	6.38±0.02	6.19±0.06	5.99±0.13	5.71±0.18	5.50±0.09
ALF (2%, 2 min)	6.48±0.01	6.35±0.03	6.39±0.04	6.26±0.14	6.00±0.09	5.82±0.06	5.63±0.06
ALF (2%, 1 min) - SD (1.5%, 1 min)	6.19±0.03	6.09±0.03	6.13±0.05	6.12±0.02	6.10±0.02	5.72±0.02	5.69±0.09
ALF (1%, 1 min) - LA (2%, 1 min)	5.83±0.06	5.60±0.07	5.65±0.14	5.64±0.02	5.63±0.07	5.41±0.03	5.36±0.13
ALF (2%, 1 min) - LA (1%, 1 min)	6.10±0.06	5.99±0.06	5.95±0.12	5.77±0.05	5.58±0.12	5.28±0.13	5.02±0.05

SD, sodium diacetate; LA, lactic acid; ALF, activated lactoferrin

Table 26: Mean (n=2) a_w values (a_w ± standard deviation) of commercial, uncured turkey breast inoculated with *Listeria monocytogenes* and dipped into antimicrobial solutions.

Treatment (dipping)	Water activity on day-0
Control	0.976±0.001
Water (1 min)	0.981±0.002
SD (1.5%, 1 min)	0.981±0.001
LA (1%, 1 min)	0.982±0.001
LA (2%, 1 min)	0.983±0.003
ALF (1%, 1 min)	0.982±0.001
ALF (2%, 1 min)	0.986±0.002
ALF (2%, 2 min)	0.980±0.001
ALF (2%, 1 min) - SD (1.5%, 1 min)	0.980±0.003
ALF (1%, 1 min) - LA (2%, 1 min)	0.980±0.002
ALF (2%, 1 min) - LA (1%, 1 min)	0.983±0.002

SD, sodium diacetate; LA, lactic acid; ALF, activated lactoferrin

min) followed by LA (2%, 1 min), was also included in this study. Growth kinetics data for *L. monocytogenes* on inoculated product stored for 34 days at 7°C are shown in Table 27. Also, counts (log CFU/cm^2) of *L. monocytogenes* and total microbial populations obtained during the storage period are presented in Figure 8 and Table 28, respectively.

Initial reductions of *L. monocytogenes* after application of the dipping treatments were 0.3 to 0.9 log CFU/cm^2 compared to the control (untreated; based on y_0 results). Lag phase durations ranging between 0.5 and 2.3 days ($P>0.05$) were obtained for all the treatments except for 2% ALF (1 min), which exhibited no lag phase. The lowest μ_{max} values were obtained for 2% LA applied alone (0.200 day^{-1}) and in sequence with 1 and 2% ALF (1 min; 0.236 and 0.268 day^{-1}, respectively). Although 2% LA applied alone had the lowest μ_{max} value, the sequential treatment with 2% ALF had a longer lag phase duration (by 1.3 days). The most effective treatment, however, appeared to be 1% ALF followed by 2% LA since lower pathogen levels were reached by the end of storage (Figure 8).

Application of the single treatments of 1% ALF (1 min) or 2% ALF (1 and 2 min) resulted in μ_{max} values that were not different ($P>0.05$); however; 1% ALF (1 min) exhibited a lag phase duration of 1.7 days compared to no lag phase and 0.5 days for 2% ALF applied for 1 and 2 min, respectively. In contrast to results obtained in Study 5, 2% ALF (2 min) had a lower ($P<0.05$) μ_{max} value than the control, while μ_{max} values for 1 and 2% ALF applied for 1 min were not different ($P>0.05$) to the control. As observed in Study 5, the sequential treatment of 1% ALF (1 min) followed by 2% lactic acid (1 min) enhanced ($P<0.05$) the antimicrobial activity of the ALF treatment applied on its own. Similarly, 2% lactic acid enhanced ($P<0.05$) the antimicrobial activity (lag phase duration and μ_{max}) of 2% ALF (1 min) when these treatments were applied in sequence.

The pH and a_w values for the untreated product on day-0 of storage were 6.38 and 0.975, respectively (Tables 29 and 30, respectively). Changes in pH and a_w values for samples that were treated, as well as for samples that were stored for up to 34 days (pH data only), followed similar trends as those in Study 5 (Tables 25 and 26).

Study 7-Application of antimicrobial dipping solutions for *Listeria monocytogenes* control on frankfurters and effect of treatment duration on antilisterial properties of ALF

Growth parameters obtained for *L. monocytogenes* on frankfurters immersed into antimicrobial solutions are presented in Table 31. Moreover, counts of *L. monocytogenes* and total microbial populations (log CFU/cm^2) obtained during the storage period are shown in Figure 9 and Table 32, respectively.

Activated lactoferrin (applied at 2% for 60, 90, or 120 s) caused the greatest initial reduction in *L. monocytogenes* populations (0.9 log CFU/cm^2) compared to the other antimicrobials and water, as suggested by y_0 values obtained for dipping treatments. All lactoferrin treatments, however, allowed proliferation of the pathogen immediately after dipping, whereas, growth in controls and samples treated with water, 2% lactic acid or 3% sodium diacetate was inhibited for 0.7, 0.6, 5.1, or 3.6 days, respectively. Treatments (including control) resulted in μ_{max} values ranging from 0.239 days^{-1} (control) to 0.370 days^{-1} (water). In general, the pathogen grew readily

Table 27: Mean (n=6) growth kinetics of *Listeria monocytogenes* growth on the surface of inoculated commercial, uncured turkey breast dipped into antimicrobial solutions, vacuum-packaged and stored at 7°C for 34 days.

Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max}; days^{-1})	Y_o[2] (log CFU/cm^2)	Y_{end}[3] (log CFU/cm^2)	R^2
Control	1.7 A	0.469 A	2.5	7.2	0.961
LA (2%, 1 min)	1.0 A	0.200 D	2.2	6.9	0.931
ALF (1%, 1 min)	1.7 A	0.393 AB	2.0	7.1	0.976
ALF (2%, 1 min)	-[1]	0.430 AB	1.9	7.3	0.992
ALF (2%, 2 min)	0.5 A	0.357 BC	2.0	7.3	0.968
ALF (1%, 1 min) - LA (2%, 1 min)	1.1 A	0.236 D	1.6	6.3	0.960
ALF (2%, 1 min) - LA (2%, 1 min)[4]	2.3 A	0.268 D	1.7	7.0	0.937

[1] No lag phase observed
[2] Lower asymptote estimated by the Baranyi model
[3] Upper asymptote estimated by the Baranyi model
[4] n=3
LA: lactic acid, ALF: activated lactoferrin
Means within the same column that have a common letter are not significantly different (P>0.05)

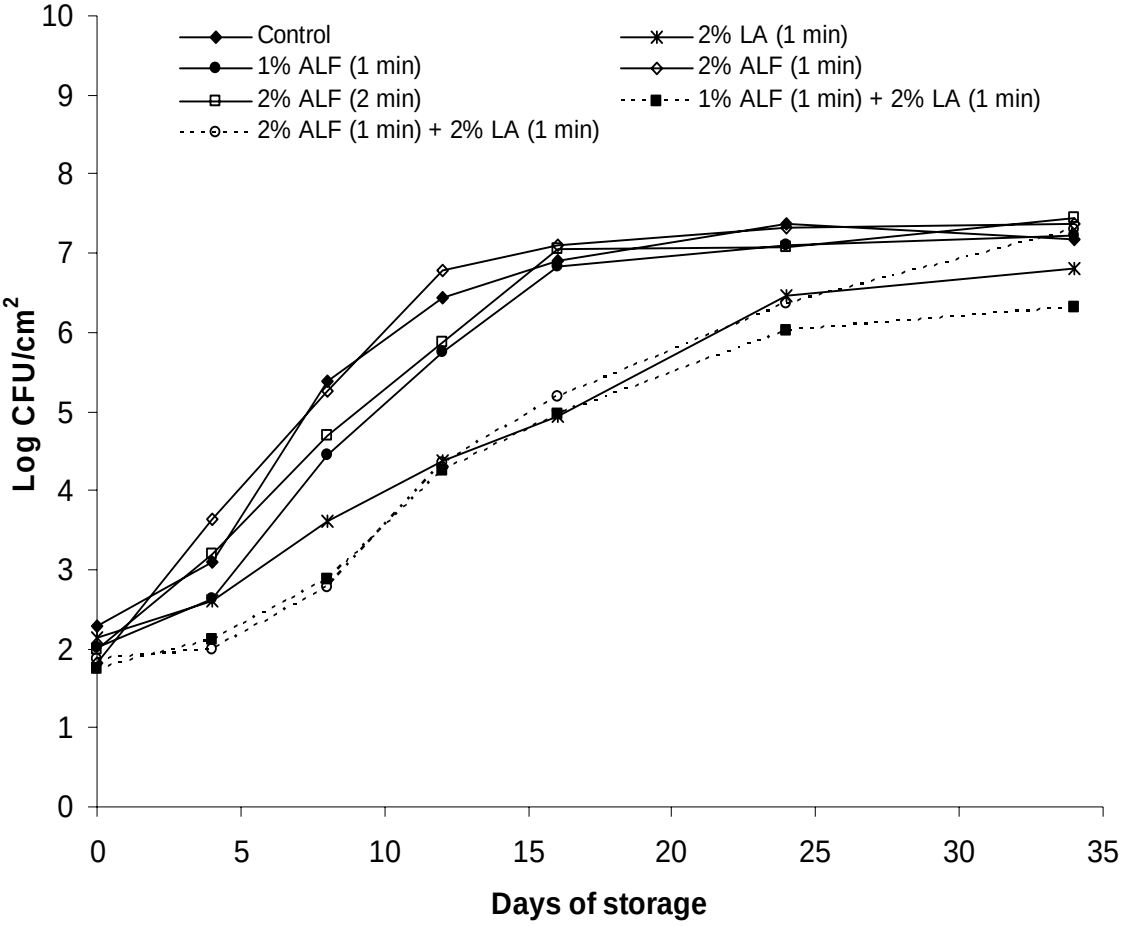


Figure 8: Mean (n=6) populations of *Listeria monocytogenes* (log CFU/cm^2) inoculated onto commercial, uncured turkey breast before dipping into antimicrobial solutions, vacuum-packaging and storage at 7°C for 34 days. The duration of each dipping treatment is provided in the figure legend. LA, lactic acid; ALF, activated lactoferrin.

Table 28: Mean (n=6) total microbial populations (log CFU/cm^2 ± standard deviation) on inoculated commercial, uncured turkey breast dipped into antimicrobial solutions, vacuum-packaged and stored at 7°C for 34 days.

Treatment (dipping)	Days of storage						
	0	4	8	12	16	24	34
Control	2.5±0.1	3.5±1.3	6.1±1.3	7.0±1.1	7.5±0.5	7.9±0.4	7.7±0.3
LA (2%, 1 min)	2.2±0.1	2.7±0.5	3.7±0.6	5.3±1.3	6.4±0.3	7.0±0.5	7.0±0.4
ALF (1%, 1 min)	2.0±0.1	2.7±0.4	4.9±0.3	6.2±0.3	7.5±0.4	7.7±0.2	7.7±0.2
ALF (2%, 1 min)	1.9±0.1	3.9±0.4	5.8±0.8	7.2±0.8	7.4±0.5	7.7±0.3	7.8±0.3
ALF (2%, 2 min)	2.2±0.2	3.5±0.3	5.2±0.5	7.0±0.7	7.5±0.5	7.7±0.3	7.7±0.1
ALF (1%, 1 min) - LA (2%, 1 min)	1.8±0.1	2.5±0.7	3.9±1.6	5.4±1.1	6.0±1.1	7.4±0.1	6.9±0.5
ALF (2%, 1 min) - LA (2%, 1 min)[1]	1.9±0.2	2.0±0.1	2.8±0.5	4.3±0.6	5.1±1.0	6.9±0.1	7.3±0.1

LA, lactic acid; ALF, activated lactoferrin

Table 29: Mean (n=6) pH values (pH ± standard deviation) of commercial, uncured turkey breast inoculated with *Listeria monocytogenes*, dipped into antimicrobial solutions, vacuum-packaged and stored at 7°C for 34 days.

Treatment (dipping)	Days of storage						
	0	4	8	12	16	24	34
Control	6.38±0.05	6.32±0.02	6.28±0.03	6.13±0.21	5.98±0.34	5.84±0.34	5.83±0.35
LA (2%, 1 min)	5.77±0.09	5.69±0.15	5.79±0.08	5.71±0.20	5.67±0.29	5.65±0.13	5.52±0.07
ALF (1%, 1 min)	6.39±0.06	6.36±0.05	6.38±0.02	6.38±0.03	6.17±0.13	5.94±0.21	5.98±0.19
ALF (2%, 1 min)	6.41±0.03	6.35±0.04	6.38±0.01	6.25±0.08	6.14±0.20	5.93±0.26	5.84±0.38
ALF (2%, 2 min)	6.44±0.05	6.39±0.06	6.38±0.03	6.31±0.10	6.11±0.14	6.00±0.20	5.89±0.29
ALF (1%, 1 min) - LA (2%, 1 min)	5.70±0.15	5.65±0.07	5.61±0.18	5.65±0.07	5.62±0.06	5.48±0.08	5.39±0.09
ALF (2%, 1 min) - LA (2%, 1 min)[1]	5.77±0.14	5.71±0.23	5.62±0.14	5.48±0.13	5.56±0.11	5.59±0.03	5.63±0.05

LA, lactic acid; ALF, activated lactoferrin
[1]n=3

Table 30: Mean (n=5) a_w values (a_w ± standard deviation) of commercial, uncured turkey breast inoculated with *Listeria monocytogenes* and dipped into antimicrobial solutions.

Treatment (dipping)	Water activity on day-0
Control	0.975±0.002
LA (2%, 1 min)	0.981±0.004
ALF (1%, 1 min)	0.981±0.001
ALF (2%, 1 min)	0.984±0.003
ALF (2%, 2 min)	0.982±0.003
ALF (1%, 1 min) - LA (2%, 1 min)	0.982±0.003
ALF (2%, 1 min) - LA (2%, 1 min)[1]	0.984±0.001

LA, lactic acid; ALF, activated lactoferrin
[1]n=2

on treated or untreated product, whereas, 2% ALF applied for 120 s was the treatment that resulted in the lowest levels of *L. monocytogenes* at the end of storage (y_{end}: 7.3; see also Figure 9) compared to other treatments and the control. However, up to day-24, samples treated with 2% ALF for 120 s did not exhibit any evidence of superior antilisterial activity compared to the other ALF treatments (Figure 9). Results of this study suggested that the duration of treatment did not cause considerable effects on the inhibitory properties of ALF against *L. monocytogenes* on frankfurters during storage, since 30, 60, 90, and 120 s of dipping resulted in similar ($P>0.05$) μ_{max} values, and all of the ALF treatments resulted in instant proliferation of the pathogen after dipping (no lag phase).

The pH of undipped frankfurters was 6.09 on day-0 (Table 33). Dipping into 2% lactic acid or 3% sodium diacetate led to pH reductions of 0.29 and 0.08 units respectively, whereas, the rest of the treatments caused slight increases of 0.01 (dipping into water for 30 s or 2% ALF for 30 or 60 s) to 0.03 (dipping into 2% ALF for 120 s) units. During storage, decreases in product pH were observed for all treatments. The greatest reduction in product pH was observed in frankfurters dipped into 2% ALF for 120 s (0.68 units).

Water activity values of treated and untreated frankfurters on day-0 are shown in Table 34. The a_w of untreated product was 0.962. Water activity values ranged from 0.954 (frankfurters dipped into 3% sodium diacetate) to 0.967 (frankfurters dipped into 2% ALF for 60 s).

Study 8-Effect of bologna and inoculum composition on the antilisterial properties of preservative solutions applied by dipping

Table 35 presents parameters of *L. monocytogenes* growth inoculated onto slices of beef bologna formulated with or without antimicrobials. *L. monocytogenes* and total microbial populations (log CFU/cm^2) obtained on this product during storage are shown in Figure 10 and Table 36, respectively.

Initial reductions in *L. monocytogenes* populations varied from 0.6 (water) to 1.0 (2% ALF for 2 min) log CFU/cm^2 for inoculum 1 and 0.5 (water) to 0.9 (2% acetic acid) log CFU/cm^2 for inoculum 2. Dipping into 2% ALF for 1 min caused an increase of 0.5 log CFU/cm^2 in *L. monocytogenes* counts on day-0. Treatments that permitted growth of the pathogen immediately after dipping included the control, water, and 2% ALF (1 or 3 min), whereas, acetic acid prohibited *L. monocytogenes* proliferation throughout storage. Dipping into 2% ALF for 2 min caused lag phases of 0.5 (inoculum 1) and 0.2 (inoculum 2) days. Maximum specific growth rates ranged from -0.076 days^{-1} (2% acetic acid, inoculum 2) to 0.842 days^{-1} (water, inoculum 2). Inoculum composition did not appear to have a considerable effect on *L. monocytogenes* growth on this product as similar growth trends were observed for both inocula across treatments. The most effective treatment was 2% acetic acid that resulted in listeriocidal effects during storage as suggested by the negative μ_{max} values obtained for both inocula. The velocity of *L. monocytogenes* growth on samples dipped into ALF solutions was greater ($P<0.05$) than that on undipped samples as indicated by μ_{max} values obtained for samples dipped into ALF for 1, 2, and 3 min (0.592, 0.744, and 0.617 days^{-1}, respectively for inoculum 1 and 0.804, 0.730 and 0.771 days^{-1}, respectively for inoculum 2) and μ_{max} values obtained for the control samples (0.450 and 0.534 days^{-1} for inoculum 1 and 2, respectively). Dipping into ALF for 2 min was the only ALF

Table 31: Mean (n=3) growth kinetics of *Listeria monocytogenes* growth on the surface of frankfurters, inoculated with the pathogen, dipped into antimicrobial solutions for 30, 60, 90 or 120 s, vacuum-packaged, and stored at 7°C for 32 days.

Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[2] (log CFU/cm^2)	Y_{end}[3] (log CFU/cm^2)	R^2
Control	0.7 A	0.239 A	2.8	7.9	0.963
Water (30 s)	0.6 A	0.370 D	2.1	8.1	0.989
LA (2 %; 30 s)	5.1 A	0.282 AB	2.0	8.2	0.976
SD (3%; 30 s)	3.6 A	0.244 AC	2.4	7.8	0.978
ALF (2%; 30 s)	-[1]	0.328 BD	2.1	8.0	0.989
ALF (2%; 60 s)	-[1]	0.322 B	1.9	8.0	0.981
ALF (2%; 90 s)	-[1]	0.291 BC	1.9	8.1	0.988
ALF (2%; 120 s)	-[1]	0.312 B	1.9	7.3	0.988

[1] no lag phase observed
[2]Lower asymptote estimated by the Baranyi model
[3]Upper asymptote estimated by the Baranyi model
LA: lactic acid, SD: sodium diacetate, ALF: activated lactoferrin
Means within the same column that have a common letter are not significantly different (P>0.05)

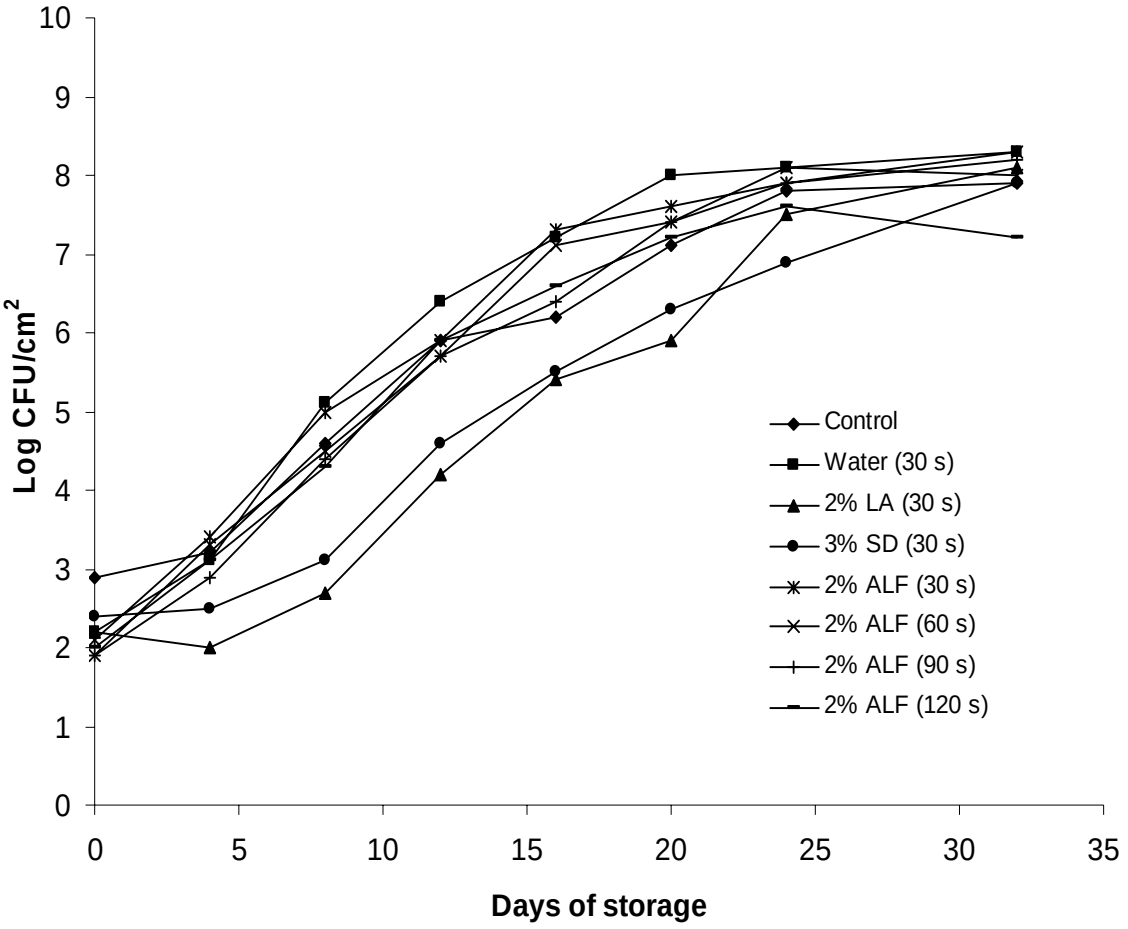


Figure 9: Mean (n=3) populations of *Listeria monocytogenes* (log CFU/cm^2) inoculated onto the surface of frankfurters before immersing into water or antimicrobial solutions, vacuum-packaging and storage at 7^{o}C for 32 days. The duration of each dipping treatment is provided in the figure legend. LA, lactic acid; SD, sodium diacetate; ALF, activated lactoferrin.

Table 32: Mean (n=3) total microbial populations (log CFU/cm^2 ± standard deviation) on the surface of frankfurters, inoculated with *Listeria monocytogenes*, dipped into antimicrobial solutions for 30, 60, 90 or 120 s, vacuum-packaged and stored at 7^{o}C.

	Days of storage							
Treatment (dipping)	0	4	8	12	16	20	24	32
Control	3.2±0.0	3.5±0.1	5.4±0.4	6.5±0.2	6.7±0.5	7.3±0.2	8.0±0.0	7.9±0.1
Water (30 s)	2.2±0.1	3.1±0.3	4.7±0.3	6.4±0.2	7.3±0.2	7.6±0.5	8.2±0.1	8.3±0.1
LA (2 %; 30 s)	3.3±0.0	2.3±0.2	3.0±0.2	4.2±0.3	5.4±0.4	6.1±0.3	7.6±0.0	8.1±0.0
SD (3%; 30 s)	2.5±0.0	2.5±0.1	3.1±0.5	4.5±0.0	6.2±0.1	6.4±0.1	6.8±0.1	7.8±0.1
ALF (2%; 30 s)	2.1±0.1	3.7±0.2	5.0±0.0	5.9±0.2	7.3±0.1	7.6±0.1	8.0±0.0	8.2±0.0
ALF (2%; 60 s)	2.0±0.1	3.3±0.2	4.4±0.2	5.7±0.1	6.6±0.5	7.4±0.3	8.2±0.4	8.1±0.1
ALF (2%; 90 s)	1.9±0.1	2.9±0.0	4.7±0.3	5.6±0.2	6.6±0.3	7.5±0.0	7.6±0.1	8.2±0.1
ALF (2%; 120 s)	2.1±0.4	3.7±0.1	5.4±0.3	4.7±0.0	7.2±0.1	7.8±0.0	7.8±0.2	7.5±0.3

LA, lactic acid; SD, sodium diacetate; ALF, activated lactoferrin

Table 33: Mean (n=3) pH values (pH ± standard deviation) of frankfurters, inoculated with *Listeria monocytogenes*, dipped into antimicrobial solutions for 30, 60, 90 or 120 s, vacuum-packaged and stored at 7^{o}C.

	Days of storage							
Treatment (dipping)	0	4	8	12	16	20	24	32
Control	6.09±0.01	6.15±0.01	6.17±0.03	6.13±0.00	6.16±0.01	6.11±0.04	5.90±0.02	5.61±0.07
Water (30 s)	6.10±0.01	6.17±0.01	6.18±0.02	6.16±0.01	6.16±0.01	5.91±0.06	5.70±0.14	5.63±0.11
LA (2 %; 30 s)	5.80±0.29	6.03±0.00	6.05±0.01	6.04±0.01	6.06±0.03	6.10±0.02	5.87±0.06	5.70±0.07
SD (3%; 30 s)	6.01±0.02	6.09±0.01	6.10±0.01	6.10±0.00	6.10±0.01	6.13±0.02	6.06±0.02	5.83±0.12
ALF (2%; 30 s)	6.10±0.01	6.18±0.01	6.17±0.01	6.14±0.01	6.14±0.02	6.07±0.02	5.85±0.07	5.65±0.05
ALF (2%; 60 s)	6.10±0.02	6.18±0.03	6.20±0.01	6.18±0.01	6.21±0.00	6.11±0.01	5.89±0.04	5.57±0.10
ALF (2%; 90 s)	6.11±0.02	6.16±0.02	6.21±0.02	6.17±0.00	6.20±0.01	6.12±0.07	5.94±0.14	5.57±0.12
ALF (2%; 120 s)	6.12±0.01	6.20±0.01	6.20±0.02	6.13±0.01	5.99±0.07	5.89±0.01	5.81±0.07	5.44±0.14

LA, lactic acid; SD, sodium diacetate; ALF, activated lactoferrin

Table 34: Mean (n=2) a_w values (a_w ± standard deviation) of frankfurters, inoculated with *Listeria monocytogenes*, dipped into antimicrobial solutions for 30, 60, 90 or 120 s, vacuum-packaged and stored at 7°C.

Treatment (dipping)	Water activity on day-0
Control	0.962±0.006
Water (30 s)	0.963±0.001
LA (2 %; 30 s)	0.963±0.001
SD (3%; 30 s)	0.954±0.003
ALF (2%; 30 s)	0.957±0.002
ALF (2%; 60 s)	0.967±0.005
ALF (2%; 90 s)	0.960±0.001
ALF (2%; 120 s)	0.963±0.001

LA, lactic acid; SD, sodium diacetate; ALF, activated lactoferrin

treatment that delayed growth of the pathogen. However, inhibition of *L. monocytogenes* growth caused by dipping into 2% ALF for 2 min was very brief as it lasted for 0.5 and 0.2 days for inoculum 1 and 2, respectively. Final *L. monocytogenes* populations (y_{end} values) exceeded 8 log CFU/cm^2 for all treatments that allowed growth.

Growth kinetics of *L. monocytogenes* on bologna formulated with beef and pork with or without antimicrobials are shown in Table 37. Additionally, *L. monocytogenes* and total microbial populations (log CFU/cm^2) obtained during the storage period are presented in Figure 11 and Table 38, respectively.

Growth kinetics data obtained for product formulated with beef and pork showed trends similar to those presented previously (Table 35). Initial reductions in *L. monocytogenes* counts ranged from 0.8 (2% ALF for 1 or 2 min) to 1.0 (2% acetic acid or 2% ALF for 3 min) log CFU/cm^2 for inoculum 1 and 0.4 (2% ALF for 1 min) to 1.2 (2% acetic acid) log CFU/cm^2 for inoculum 2. A slight increase of 0.2 log CFU/cm^2 in *L. monocytogenes* initial counts was observed in samples dipped into water after being inoculated with inoculum 1. Listeriocidal effects were observed in samples dipped into 2% acetic acid after inoculation with inoculum 1. For inoculum 2, dipping into 2% acetic acid resulted in complete prevention of *L. monocytogenes* growth for 23.1 days before reductions in pathogen levels were observed. Water and 2% ALF (3 min) also resulted in inhibition of *L. monocytogenes* growth for 0.5 and 0.4 days, respectively, in bologna inoculated with inoculum 1. Maximum specific growth rates of *L. monocytogenes* growth varied from -0.226 days^{-1} (2% acetic acid, inoculum 2) to 0.942 days^{-1} (2% ALF, inoculum 2). Dipping into 2% ALF for 1, 2, or 3 min resulted in μ_{max} values greater than that of the control, and furthermore, growth of *L. monocytogenes* occurred immediately or after a short lag phase (0.4 days; 2% ALF, 3 min, inoculum 1) in these treatments.

The growth kinetics data obtained for the two inocula were combined since results of the two experiments discussed above indicated that the effect of inoculum composition on *L. monocytogenes* counts (log CFU/cm^2) was not significant (P>0.05) (not shown) (Tables 39, beef bologna; Table 40, beef and pork bologna). For both products, dipping into acetic acid caused reductions in *L. monocytogenes* populations during storage, whereas the other treatments caused very brief lag phases or allowed immediate proliferation of the pathogen. Dipping into 2% ALF for 2 min was the only ALF treatment that resulted in a lag phase. However, the duration of the lag phase provided by this treatment was very brief for product formulated with beef (0.5 days) or beef and pork (0.4 days). Mean maximum specific growth rates ranged from -0.060 day^{-1} (dipping into 2% acetic acid) to 0.737 (dipping into 2% ALF for 2 min) and from -0.135 (dipping into 2% acetic acid) to 0.876 (dipping into 2% ALF for 3 min) for beef (Table 39) and beef and pork (Table 40) bologna, respectively. Dipping into 2% ALF for 1, 2, or 3 min caused μ_{max} values similar (P>0.05) to those provided by dipping into water, suggesting that proliferation of *L. monocytogenes* during storage is not affected (P>0.05) by the duration of the dipping treatment. In addition, final populations of the pathogen (y_{end}) on beef or beef and pork bologna, reached approximately 8 log CFU/cm^2 in untreated or treated with water or ALF samples.

The pH of undipped beef bologna on day-0 was 6.14 (Table 41). The treatment that led to the greatest pH reductions on day-0 was acetic acid (0.88-1.07 units). Application of other treatments resulted in slight increases in product pH (0.01-0.10 units). During storage, the pH of

untreated or treated with water or ALF bologna decreased, and on day-26, pH values varied from 4.61 (product inoculated with inoculum 2 and dipped into water or 2% ALF for 2 min) to 5.24 (product inoculated with inoculum 1 and dipped into acetic acid).

The pH of bologna formulated with beef and pork followed similar trends to that of beef bologna (Table 42). On day-0, the pH of undipped product was 6.24, whereas, dipping into water or ALF resulted in slight increases of 0.01 to 0.10 units. Product pH was reduced by 1.09 to 1.15 units after dipping into solutions of acetic acid. By the end of storage, the pH of samples treated with water, ALF, as well as that of the control decreased by 1.45 (control inoculated with inoculum 1) to 1.72 (samples treated with 2% ALF for 180 s after inoculation with inoculum 2).

Water activity values of treated and untreated bologna formulated with beef or beef and pork are shown in Table 43. Higher a_w values were obtained for product formulated with beef and pork compared to those of product formulated with beef only. The a_w of untreated samples on day-0 was 0.955 and 0.963, for product formulated with beef or beef and pork, respectively. Dipping into antimicrobial solutions resulted in increases of product a_w. Specifically, the a_w of treated beef bologna ranged from 0.966 (ALF applied for 2 min) to 0.973 (ALF applied for 3 min), whereas treated beef and pork bologna had a_w values of 0.965 (2% acetic acid) to 0.975 (water).

Study 9-*Listeria monocytogenes* control with antimicrobials in the formulation of frankfurters and by dipping into solutions of ALF or acetic acid

Parameters of *L. monocytogenes* growth on frankfurters formulated with or without antimicrobials and left undipped or dipped into antimicrobial solutions are presented in Table 44. *L. monocytogenes* and total microbial counts (log CFU/cm^2) obtained during product storage are also shown in Figure 12 and Table 45, respectively.

Listeria monocytogenes growth in undipped-control samples occurred after a lag phase of 12.0 days, whereas, lactoferrin added at 5% in the product formulation inhibited *L. monocytogenes* growth for 17.7 days. Addition of potassium lactate (1.8%) and 0.125% sodium diacetate or 0.5% lactoferrin to the formulation of frankfurters caused reductions of *L. monocytogenes* populations during storage. Specifically, in samples containing the combination of 1.8% potassium lactate with 0.125% sodium diacetate, *L. monocytogenes* populations remained constant for 42.0 days before reducing rapidly (μ_{max}: -0.488 days^{-1}), whereas, the decrease in *L. monocytogenes* populations in samples with 1.8% potassium lactate and 0.5% lactoferrin in their formulation started immediately upon storage and was less abrupt ($P<0.05$) (μ_{max}: -0.005 days^{-1}). Populations of the pathogen in samples that contained 0.5% lactoferrin reached lower levels (6.6 log CFU/cm^2) compared to those in control (7.9 log CFU/cm^2) samples at the end of the storage period.

Initial reductions in *L. monocytogenes* populations (based on y_0 values) caused by dipping into 2% acetic acid ranged from 0.9 to 1.3 log CFU/cm^2. Slight growth of the pathogen occurred in dipped samples formulated without antimicrobials after 44.6 days of storage (μ_{max}: 0.172 days^{-1}), and final *L. monocytogenes* counts in these samples reached 1.4 log CFU/cm^2 after 50 days of storage (Figure 12). In samples containing antimicrobials and dipped into acetic acid, growth of the pathogen was completely inhibited and/or reduced during storage, suggesting that

Table 35: Mean (n=2) growth kinetics of *Listeria monocytogenes* growth on the surface of beef bologna slices. Slices were inoculated with either one of two 10-strain composites of *Listeria monocytogenes* and dipped into water (2 min), 2% acetic acid (AA; 1 min), or 2% activated lactoferrin (ALF; 1, 2, or 3 min), vacuum-packaged and stored at 10°C for 26 days.

	Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[3] (log CFU/cm^2)	Y_{end}[4] (log CFU/cm^2)	R^2
Inoculum 1	Control	-[1]	0.450 A	3.7	8.0	0.994
	Water	-[1]	0.873 B	3.1	8.1	0.988
	2% AA	-[2]	-0.046 C	2.8	-	0.829
	2% ALF (1 min)	-[1]	0.592 D	4.2	8.2	0.974
	2% ALF (2 min)	0.5 A	0.744 D	2.7	8.2	0.996
	2% ALF (3 min)	-[1]	0.617 DF	2.8	8.0	0.957
Inoculum 2	Control	-[1]	0.534 DA	3.6	8.2	0.983
	Water	-[1]	0.842 BE	3.1	8.3	0.983
	2% AA	-[2]	-0.075 C	2.7	1.4	0.988
	2% ALF (1 min)	-[1]	0.804 BE	3.0	8.1	0.974
	2% ALF (2 min)	0.2 A	0.730 EF	2.8	8.0	0.988
	2% ALF (3 min)	-[1]	0.771 BE	2.9	8.2	0.987

[1] no lag phase observed; [2] no growth observed

[3]Lower asymptote estimated by the Baranyi model

[4]Upper asymptote estimated by the Baranyi model; no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponds to stationary phase

AA: acetic acid, ALF: activated lactoferrin

Means within the same column that have a common letter are not significantly different (P>0.05)

Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)

Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak)

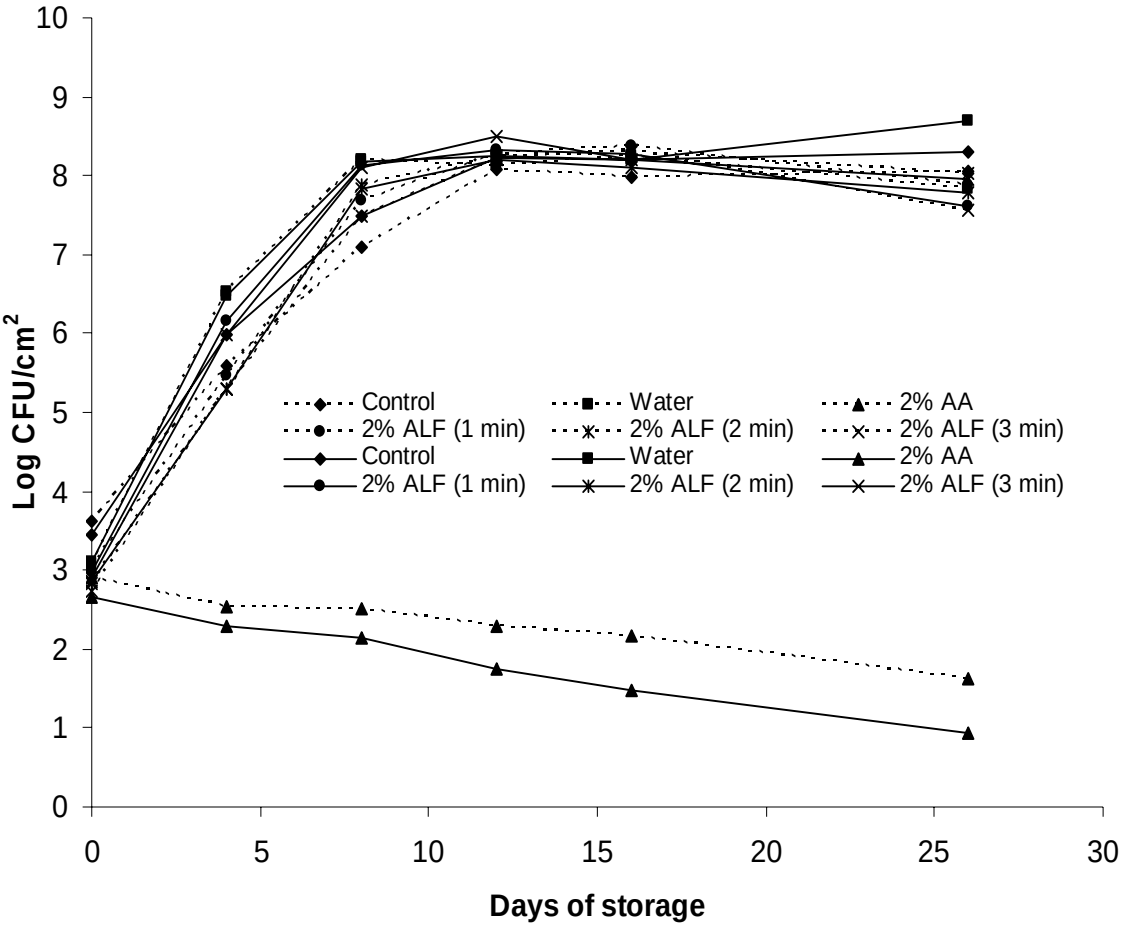


Figure 10: Mean (n=2) populations of *Listeria monocytogenes* (log CFU/cm^2) on the surface of beef bologna slices, inoculated with either one of two 10-strain composites* (inoculum 1: dashed line and inoculum 2: solid line) before dipping into water (for 2 min), 2% acetic acid (for 1 min), or activated lactoferrin (for 1, 2, or 3 min), vacuum-packaging, and storage at 7^{o}C for 26 days. AA, acetic acid; ALF, activated lactoferrin.

*Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)
Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak)

Table 36: Mean (n=2) total microbial populations (log CFU/cm^2 ± standard deviation) on the surface of beef bologna slices. Slices were inoculated with either one of two 10-strain composites of *Listeria monocytogenes*, and dipped into water (2 min), 2% acetic acid (AA; 1 min), or 2% activated lactoferrin (ALF; 1, 2, or 3 min), vacuum-packaged and stored at 10°C.

		Days of storage					
	Treatment (dipping)	0	4	8	12	16	26
Inoculum 1	Control	3.4±0.4	6.6±0.0	7.2±0.0	8.2±0.0	8.0±0.1	8.1±0.1
	Water	3.0±0.1	6.6±0.1	7.7±0.8	8.2±0.1	8.2±0.0	8.0±0.1
	2% AA	3.1±0.1	3.0±0.1	2.8±0.0	2.9±0.1	2.8±0.0	2.4±0.0
	2% ALF (1 min)	3.0±0.0	5.6±0.1	7.7 ±0.3	8.2±0.1	8.4±0.0	8.0±0.0
	2% ALF (2 min)	2.8±0.0	5.4±0.1	7.9±0.0	8.2±0.1	8.3±0.0	8.2±0.1
	2% ALF (3 min)	2.8±0.0	5.7±0.0	7.5±0.1	8.3±0.2	8.3±0.1	8.1±0.1
Inoculum 2	Control	3.4±0.0	6.1±0.0	7.5±0.3	8.3±0.2	8.2±0.1	8.1±0.4
	Water	3.1±0.0	6.7±0.1	8.4±0.2	8.2±0.1	8.1±0.4	7.9±0.0
	2% AA	2.7±0.0	2.8±0.3	2.6±0.1	2.6±0.2	3.9±0.9	2.9±0.3
	2% ALF (1 min)	3.0±0.2	6.0±0.0	8.2±0.0	8.3±0.0	8.1±0.1	7.6±0.1
	2% ALF (2 min)	3.1±0.2	5.3±0.2	7.9±0.1	8.2±0.0	7.8±0.0	8.0±0.0
	2% ALF (3 min)	3.1±0.1	6.0±0.1	8.2±0.2	8.5±0.0	8.3±0.2	7.9±0.1

AA, acetic acid; ALF, activated lactoferrin

Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)
Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak)

Table 37: Mean (n=2) growth kinetics of *Listeria monocytogenes* growth on the surface of bologna slices formulated with beef and pork. Slices were inoculated with either one of two 10-strain composites of *Listeria monocytogenes* and dipped into water (2 min), 2% acetic acid (AA; 1 min), or 2% activated lactoferrin (ALF; 1, 2, or 3 min), vacuum-packaged and stored at 10°C for 26 days.

	Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[3] (log CFU/cm^2)	Y_{end}[4] (log CFU/cm^2)	R^2
Inoculum 1	Control	-[1]	0.543 A	3.6	8.3	0.993
	Water	0.5 A	0.639 A	3.8	8.2	0.997
	2% AA	-[2]	-0.045 B	2.6	-	0.416
	2% ALF (1 min)	-[1]	0.904 C	2.8	7.7	0.770
	2% ALF (2 min)	-[1]	0.805 C	2.8	8.6	0.946
	2% ALF (3 min)	0.4 A	0.880 CD	2.6	8.4	0.979
Inoculum 2	Control	-[1]	0.684 AD	3.5	8.1	0.996
	Water	-[1]	0.917 C	3.0	8.0	0.974
	2% AA	23.1 B	-0.226 E	2.3	1.6	0.789
	2% ALF (1 min)	-[1]	0.621 A	3.1	8.2	0.997
	2% ALF (2 min)	-[1]	0.942 C	2.9	8.1	0.994
	2% ALF (3 min)	-[1]	0.873 C	2.9	8.2	0.995

[1] no lag phase observed; [2] no growth observed

[3]Lower asymptote estimated by the Baranyi model

[4]Upper asymptote estimated by the Baranyi model; no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponds to stationary phase

AA: acetic acid, ALF: activated lactoferrin

Means within the same column that have a common letter are not significantly different (P>0.05)

Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)

Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak)

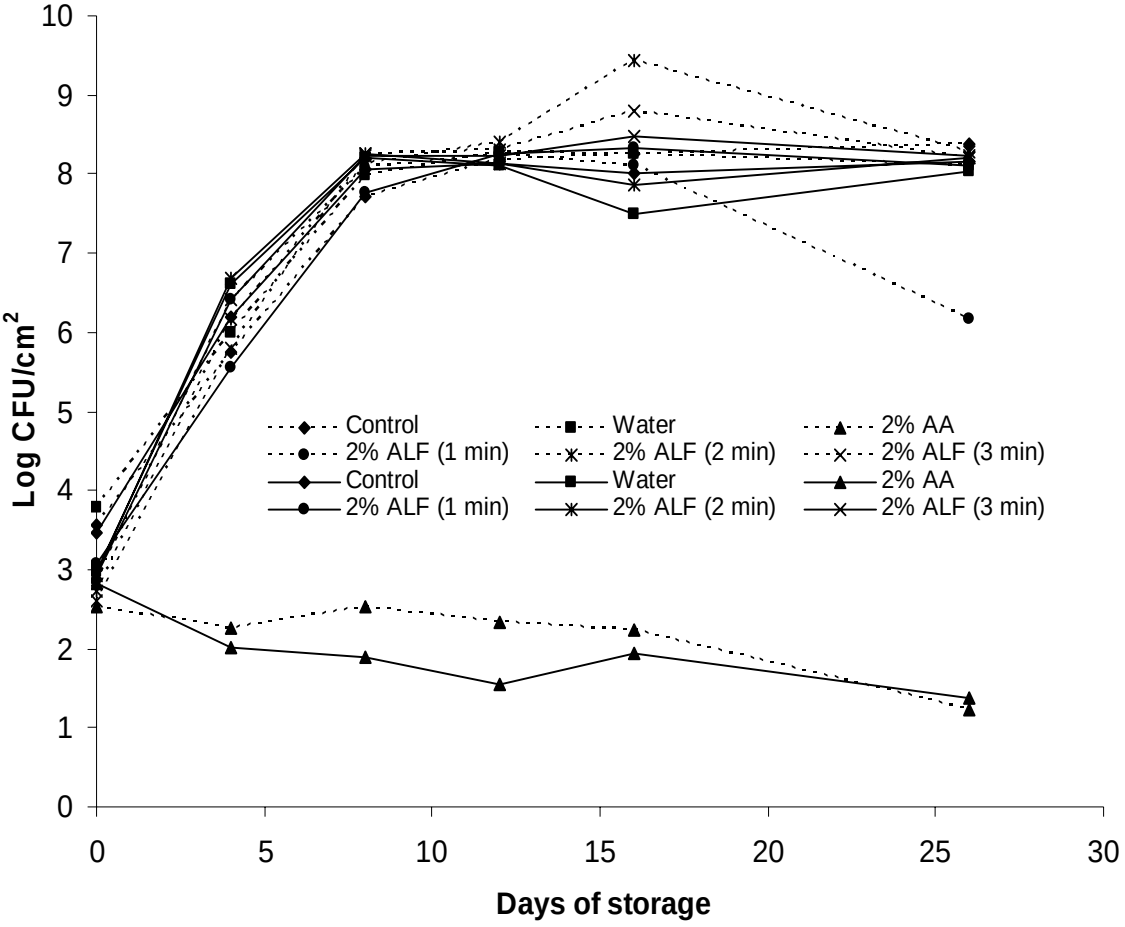


Figure 11: Mean (n=2) populations of *Listeria monocytogenes* (log CFU/cm^2) on the surface of bologna (formulated with beef and pork) slices, inoculated with either one of two 10-strain composites* (inoculum 1: dashed line and inoculum 2: solid line) before dipping into water (for 2 min), 2% acetic acid (for 1 min), or activated lactoferrin (for 1, 2, or 3 min), vacuum-packaging, and storage at 7^{o}C for 26 days. AA, acetic acid; ALF, activated lactoferrin.

*Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)
Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak)

Table 38: Mean (n=2) total microbial populations (log CFU/cm^2 ± standard deviation) on the surface of bologna (formulated with beef and pork) slices. Slices were inoculated with either one of two 10-strain composites of *Listeria monocytogenes*, and dipped into water (120 s), 2% acetic acid (AA; 60 s), or 2% activated lactoferrin (ALF; 60, 120, or 180 s), vacuum-packaged and stored at 10^{o}C.

		Days of storage					
	Treatment (dipping)	0	4	8	12	16	26
Inoculum 1	Control	3.5±0.1	5.9±0.0	7.8±0.0	8.2±0.1	8.2±0.0	8.4±0.2
	Water	3.8±0.1	6.2±0.0	8.0±0.2	8.4±0.2	8.3±0.0	8.2±0.1
	2% AA	3.1±0.1	2.6±0.0	2.8±0.1	3.2±0.0	2.8±0.1	2.0±0.1
	2% ALF (60 s)	2.7±0.2	6.5±0.0	8.2 ±0.1	8.3±0.1	8.1±0.1	8.3±0.0
	2% ALF (120 s)	2.7±0.1	6.2±0.1	8.2±0.0	8.3±0.0	9.1±0.0	8.4±0.1
	2% ALF (180 s)	2.6±0.0	5.6±0.1	8.4±0.1	8.3±0.0	8.6±0.5	8.3±0.0
Inoculum 2	Control	3.6±0.0	6.2±0.0	8.2±0.0	8.2±0.2	7.9±0.1	8.3±0.1
	Water	2.9±0.3	6.5±0.0	8.3±0.1	8.2±0.1	7.5±0.1	8.1±0.1
	2% AA	2.7±0.4	2.5±0.0	2.5±0.0	2.5±0.0	2.4±0.0	2.3±0.0
	2% ALF (60 s)	3.0±0.1	5.5±0.0	8.0±0.0	8.3±0.0	8.4±0.0	8.1±0.0
	2% ALF (120 s)	2.9±0.1	6.8±0.2	8.4±0.0	8.2±0.1	8.9±1.3	8.3±0.1
	2% ALF (180 s)	3.0±0.0	6.4±0.0	8.6±0.2	8.3±0.1	8.6±0.2	8.3±0.1

AA, acetic acid; ALF, activated lactoferrin

Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)
Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak

Table 39: Mean (n=4) growth kinetics of *Listeria monocytogenes* growth on the surface of beef bologna slices inoculated with *Listeria monocytogenes*, dipped into water (2 min), 2% acetic acid (AA; 1 min), or 2% activated lactoferrin (ALF; 1, 2, or 3 min), vacuum-packaged and stored at 10°C for 26 days.

Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} $days^{-1}$)	Y_o[3] (log CFU/cm^2)	Y_{end}[4] (log CFU/cm^2)	R^2
Control	-[1]	0.492 A	3.6	8.1	0.994
Water	-[1]	0.704 B	3.1	8.2	0.986
2% AA	-[2]	-0.060 C	2.8	1.4	0.829
2% ALF (1 min)	-[1]	0.669 AB	3.6	8.2	0.974
2% ALF (2 min)	0.5	0.737 B	2.8	8.1	0.996
2% ALF (3 min)	-[1]	0.6949 B	2.9	8.1	0.972

[1] no lag phase observed; [2] no growth observed
[3]Lower asymptote estimated by the Baranyi model
[4]Upper asymptote estimated by the Baranyi model
AA: acetic acid, ALF: activated lactoferrin
Means within the same column that have a common letter are not significantly different (P>0.05)

Table 40: Mean (n=4) growth kinetics of *Listeria monocytogenes* growth on the surface of bologna slices formulated with beef and pork, inoculated with *Listeria monocytogenes*, dipped into water (2 min), 2% acetic acid (AA; 1 min), or 2% activated lactoferrin (ALF; 1, 2, or 3 min), vacuum-packaged and stored at 10°C for 26 days.

Treatment (dipping)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} $days^{-1}$)	Y_o[3] (log CFU/cm^2)	Y_{end}[4] (log CFU/cm^2)	R^2
Control	-[1]	0.613 A	3.5	8.2	0.995
Water	0.5 A	0.778 AC	3.4	8.1	0.985
2% AA	23.1 B	-0.135 B	2.5	1.7	0.603
2% ALF (1 min)	-[1]	0.763 AC	3.0	7.9	0.884
2% ALF (2 min)	0.4 A	0.873 C	2.9	8.4	0.970
2% ALF (3 min)	-[1]	0.876 C	2.8	8.4	0.987

[1] no lag phase observed; [2] no growth observed
[3]Lower asymptote estimated by the Baranyi model
[4]Upper asymptote estimated by the Baranyi model
AA: acetic acid, ALF: activated lactoferrin
Means within the same column that have a common letter are not significantly different (P>0.05)

Table 41: Mean (n=2) pH values (pH ± standard deviation) of beef bologna slices, inoculated with either one of two 10-strain composites of *Listeria monocytogenes*, dipped into water (2 min), 2% acetic acid (AA; 1 min), or 2% activated lactoferrin (ALF; 1, 2, or 3 min), vacuum-packaged, and stored at 10°C.

		Days of storage					
	Treatment (dipping)	0	4	8	12	16	26
Inoculum 1	Control	6.14±0.01	6.11±0.00	6.09±0.03	5.37±0.14	5.32±0.00	5.16±0.08
	Water	6.15±0.01	6.17±0.01	5.27±0.08	4.85±0.03	4.83±0.05	4.63±0.04
	2% AA	5.26±0.10	5.23±0.00	5.18±0.06	5.27±0.01	5.25±0.03	5.24±0.03
	2% ALF (1 min)	6.18±0.03	6.09±0.01	6.04±0.00	5.14±0.00	5.04±0.05	4.72±0.01
	2% ALF (2 min)	6.21±0.08	6.13±0.01	6.02±0.03	5.17±0.04	5.02±0.02	4.73±0.01
	2% ALF (3 min)	6.19±0.00	6.18±0.00	6.14±0.01	5.12±0.01	4.96±0.02	4.68±0.01
Inoculum 2	Control	6.19±0.03	6.12±0.01	6.06±0.06	5.10±0.11	5.14±0.02	4.82±0.02
	Water	6.19±0.01	6.17±0.00	5.39±0.06	4.88±0.01	4.84±0.08	4.61±0.03
	2% AA	5.07±0.00	5.17±0.10	5.16±0.01	5.16±0.12	5.16±0.09	5.11±0.00
	2% ALF (1 min)	6.20±0.01	6.16±0.03	5.80±0.01	5.03±0.04	4.95±0.09	4.61±0.01
	2% ALF (2 min)	6.19±0.01	6.20±0.01	6.01±0.01	5.00±0.05	4.92±0.01	4.67±0.01
	2% ALF (3 min)	6.24±0.06	6.21±0.02	5.84±0.11	4.99±0.01	4.94±0.09	4.62±0.00

AA, acetic acid; ALF, activated lactoferrin

Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)
Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak)

Table 42: Mean (n=2) pH values (pH ± standard deviation) of bologna (formulated with beef and pork) slices, inoculated with either one of two 10-strain composites of *Listeria monocytogenes*, dipped into water (2 min), 2% acetic acid (AA; 1 min), or 2% activated lactoferrin (ALF; 1, 2, or 3 min), vacuum-packaged, and stored at 10°C

		Days of storage					
	Treatment (dipping)	0	4	8	12	16	26
Inoculum 1	Control	6.24±0.07	6.26±0.03	6.11±0.07	5.11±0.01	5.1±0.01	4.79±0.04
	Water	6.25±0.06	6.26±0.02	5.91±0.03	4.93±0.01	4.86±0.01	4.63±0.01
	2% AA	5.15±0.00	5.08±0.00	5.53±0.12	5.21±0.01	5.22±0.00	5.18±0.01
	2% ALF (60 s)	6.29±0.01	6.24±0.04	5.69±0.01	4.97±0.00	4.88±0.01	4.66±0.01
	2% ALF (120 s)	6.34±0.04	6.24±0.08	5.54±0.13	4.95±0.02	4.89±0.01	4.66±0.00
	2% ALF (180 s)	6.31±0.04	6.26±0.01	5.69±0.06	4.89±0.00	4.85±0.00	4.63±0.03
Inoculum 2	Control	6.27±0.05	6.15±0.07	5.78±0.04	5.08±0.06	4.94±0.02	4.67±0.11
	Water	6.24±0.05	6.23±0.03	5.16±0.04	4.88±0.03	4.75±0.04	4.54±0.04
	2% AA	5.09±0.10	5.23±0.01	5.21±0.11	5.11±0.04	5.12±0.06	5.09±0.07
	2% ALF (60 s)	6.26±0.05	6.26±0.01	5.69±0.02	5.04±0.01	4.94±0.01	4.66±0.00
	2% ALF (120 s)	6.27±0.01	6.28±0.01	5.40±0.07	4.89±0.02	4.80±0.00	4.58±0.02
	2% ALF (180 s)	6.30±0.01	6.30±0.01	5.72±0.00	4.89±0.04	4.80±0.01	4.58±0.01

AA, acetic acid; ALF, activated lactoferrin

Inoculum 1: Scott A (serotype 4b, human isolate), NA-3 (serotype 4b), NA-19 (serotype 3b), 101M (serotype 4b), and 103M (serotype 1a), all isolated from pork sausage, 558 (serotype 1/2, pork meat isolate), and PVM1, PVM2, PVM3 and PVM4, (all pork variety meat isolates, serotype not known)
Inoculum 2: Scott A (serotype 4b, human isolate), 103M (serotype 1a, pork sausage isolate), 558 (serotype 1/2, pork meat isolate), N1-227, N1-225 (serotype 4b, food and human isolate, respectively, both associated with the same outbreak), R2-500, R2-501 (serotype not known, food and human isolate, respectively, both associated with the same outbreak), and R2-763, R2-764, R2-765 (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak

Table 43: Mean (n=2) a_w values (a_w ± standard deviation) of bologna, formulated with beef or beef and pork, inoculated with *Listeria monocytogenes* and dipped into water (2 min), 2% acetic acid (AA; 1 min), or 2% activated lactoferrin (ALF; 1, 2, or 3 min).

Product	Treatment (dipping)	Water activity on day-0
Beef bologna	Control	0.955±0.003
	Water	0.970±0.001
	2% AA	0.970±0.001
	2% ALF (1 min)	0.967±0.001
	2% ALF (2 min)	0.966±0.001
	2% ALF (3 min)	0.973±0.001
Beef and pork bologna	Control	0.963±0.001
	Water	0.975±0.001
	2% AA	0.965±0.001
	2% ALF (1 min)	0.970±0.001
	2% ALF (2 min)	0.972±0.001
	2% ALF (3 min)	0.974±0.004

AA, acetic acid; ALF, activated lactoferrin

antimicrobial additives may increase the antilisterial effectiveness of the acid solution. Among the acetic acid treatments, the steepest (P>0.05) reduction in *L. monocytogenes* populations was observed in samples that contained 1.8% potassium lactate and 0.125% sodium diacetate (μ_{max}: -0.117 $days^{-1}$), whereas, 0.5% lactoferrin added individually in the product formulation before dipping into acetic acid caused complete inhibition of *L. monocytogenes* growth for 49.1 days.

Dipping into 2% ALF reduced the initial counts of the pathogen by 1.3 to 1.7 log CFU/cm^2 (based on y_0 values). Among the 2% ALF treatments, growth of *L. monocytogenes* was permitted immediately in samples containing no antimicrobials or 0.5% lactoferrin, whereas, in samples with 1.8% potassium lactate and 0.125% sodium diacetate in their formulation, *L. monocytogenes* proliferation was inhibited for 42.5 days. Listeriocidal effects were observed in samples that contained 1.8% potassium lactate and 0.5% lactoferrin and were dipped into 2% ALF. The μ_{max} value obtained for samples formulated without antimicrobials and dipped in 2% ALF was 0.165 $days^{-1}$. For the same dipping treatment, growth of the pathogen was also observed in samples that contained 1.8% potassium lactate combined with 0.125% sodium diacetate (μ_{max}: 0.180 $days^{-1}$; increase of 0.7 log CFU/cm^2 by the end of storage; Figure 12) or 0.5% lactoferrin (μ_{max}: 0.114 $days^{-1}$; by approximately 6 log CFU/cm^2 by the end of storage; Figure 12). However, as indicated above, the combination of 1.8% potassium lactate with 0.125% sodium diacetate delayed *L. monocytogenes* growth unlike 0.5% lactoferrin that allowed growth immediately. A negative μ_{max} value was obtained for samples containing 1.8% potassium lactate and 0.5% lactoferrin and that were dipped into 2% ALF, suggesting listeriocidal activity of the treatment. Comparing the effectiveness of antimicrobial ingredients against *L. monocytogenes* in undipped or dipped into ALF samples suggests that the antilisterial activity of certain combination treatments included in the product formulation may be enhanced (1.8% potassium lactate and 0.5% lactoferrin) by dipping into ALF solutions.

The pH of untreated (no antimicrobials, no dipping) frankfurters on day-0 was 6.04 (Table 46). The pH of samples containing antimicrobials ranged from 5.95 (1.8% potassium lactate and 0.125% sodium diacetate) to 6.03 (0.5% lactoferrin used singly or together with 1.8% potassium lactate). Dipping into solutions of acetic acid caused reductions of 0.19 (samples formulated with 1.8% potassium and 0.5% lactoferrin) to 0.97 (samples formulated with 0.5% lactoferrin) units in product pH. On the contrary, ALF applied as a dipping solution resulted in no change in pH (product formulated with 0.5% lactoferrin) or slight increases of up to 0.05 pH units. On day-50, the pH of treated or untreated frankfurters ranged from 5.76 (product formulated with 0.5% lactoferrin and dipped into acetic acid) to 6.04 (product formulated with 1.8% potassium lactate and 0.5% lactoferrin and dipped into ALF).

The a_w of untreated (no antimicrobials in the formulation, no dipping) frankfurters was 0.949 on day-0 (Table 47). Addition of 0.5% lactoferrin in the product formulation caused a slight increase in product a_w (0.002 units), whereas, combination treatments resulted in reductions of 0.008-0.009 units. The a_w of dipped samples ranged from 0.940 (product formulated with 1.8% potassium lactate and 0.5% lactoferrin and dipped into ALF) to 0.956 (product formulated with 0.5% lactoferrin and dipped into acetic acid).

Results for cooking yields and moisture and fat contents of product formulated with or without antimicrobials are presented in Table 48. The cooking yield of untreated product was 79.3%.

Addition of 0.5% lactoferrin in the formulation of frankfurters caused a decrease of 1.1% in cooking yield, whereas, the other formulation treatments did not cause considerable changes in that product property. Moisture contents ranged from 61.0% (0.5% lactoferrin) to 68.3% (1.8% potassium lactate combined with 0.125% sodium diacetate). Control samples had the highest fat content (15.9%), whereas, the lowest fat content was observed in samples that contained 0.5% lactoferrin (13.1%).

Study 10-Modeling the growth/no growth interface of *Listeria monocytogenes* in ready-to-eat products as a function of lactic acid concentration and dipping time

The growth/no growth response of *L. monocytogenes* was monitored in 75 combination treatments of storage temperatures, lactic acid concentrations, and dipping times in uncured turkey breast and bologna (Figure 13). The parameter estimates with significant ($P \leq 0.05$) effects removed are shown in Table 49. The parameter estimates with significant ($P \leq 0.05$) effects were used to produce a model of the growth/no growth interface (Figures 14 and 15). Since dramatic growth was observed in all combinations for frankfurters, a model was not developed for this product. The growth potential and inhibitory activity varied with product type (increasing growth: frankfurters>turkey breast>bologna), while the minimum inhibitory concentration of lactic acid decreased with increasing dipping time for turkey breast and bologna (Figures 14 and 15). Storage at low temperatures (4 or 7^{o}C) allowed inhibition at shorter dipping times; however, predicted growth/no growth interfaces were not different ($P>0.05$) among storage temperatures because the narrow range of storage temperatures (4 -10^{o}C) did not cause large variation of growth responses. The predicted growth/no growth interface with combinations of lactic acid concentrations and dipping times for bologna was lower or shorter, respectively, than those for turkey breast (Figures 14 and 15).

The study provides quantitative data on the antimicrobial effect of lactic acid, and the model developed may be useful in selecting appropriate lactic acid concentrations and dipping times in ready-to-eat product processing for adequate control of *L. monocytogenes*.

Table 44: Mean (n=3) growth kinetics of *Listeria monocytogenes* growth on the surface the surface of frankfurters formulated with or without antimicrobials, inoculated with *Listeria monocytogenes*, left undipped or dipped into solutions of 2% acetic acid or 2% activated lactoferrin for 2 min, vacuum-packaged and stored at 7°C.

Treatment (dipping)	Treatment (formulation)	Lag phase duration (days)	Maximum specific growth rate (μ_{max} days^{-1})	Y_o[3] (log CFU/cm^2)	Y_{end}[4] (log CFU/cm^2)	R^2
No dipping	Control	12.0 A	0.155 A	1.9	7.9	0.935
	PL (1.8%) + SD (0.125%)	[5]42.0 B	-0.488 B	2.1	1.9	0.799
	LF (0.5%)	17.7 C	0.211 A	2.3	6.6	0.973
	PL (1.8%) + LF (0.5%)	-[2]	-0.005 C	2.0	-	0.405
Acetic acid (2%)	Control	44.6 B	0.172 A	0.8	1.9	0.168
	PL (1.8%) + SD (0.125%)	-[2]	-0.117 BC	1.2	0.4	0.796
	LF (0.5%)	[5]49.1 D	-0.040 C	1.0	-	-
	PL (1.8%) + LF (0.5%)	-[2]	-0.021 C	1.0	0.6	0.599
Activated lactoferrin (2%)	Control	-[1]	0.165 A	0.6	6.9	0.968
	PL (1.8%) + SD (0.125%)	42.5 B	0.180 A	0.4	-	0.541
	LF (0.5%)	-[1]	0.114 A	1.0	-	0.827
	PL (1.8%) + LF (0.5%)	-[2]	-0.011 C	0.7	0.0	0.444

[1] no lag phase observed; [2] no growth observed

[3]Lower asymptote estimated by the Baranyi model

[4]Upper asymptote estimated by the Baranyi model; no value could be estimated when the upper part of the growth curve ceased without forming an upper asymptote that corresponds to stationary phase

[5]Lag phase is regarded as shoulder period due to inactivation of *Listeria monocytogenes*

PL: potassium lactate, SD: sodium diacetate, LF: lactoferrin

Means within the same column that have a common letter are not significantly different (P>0.05)

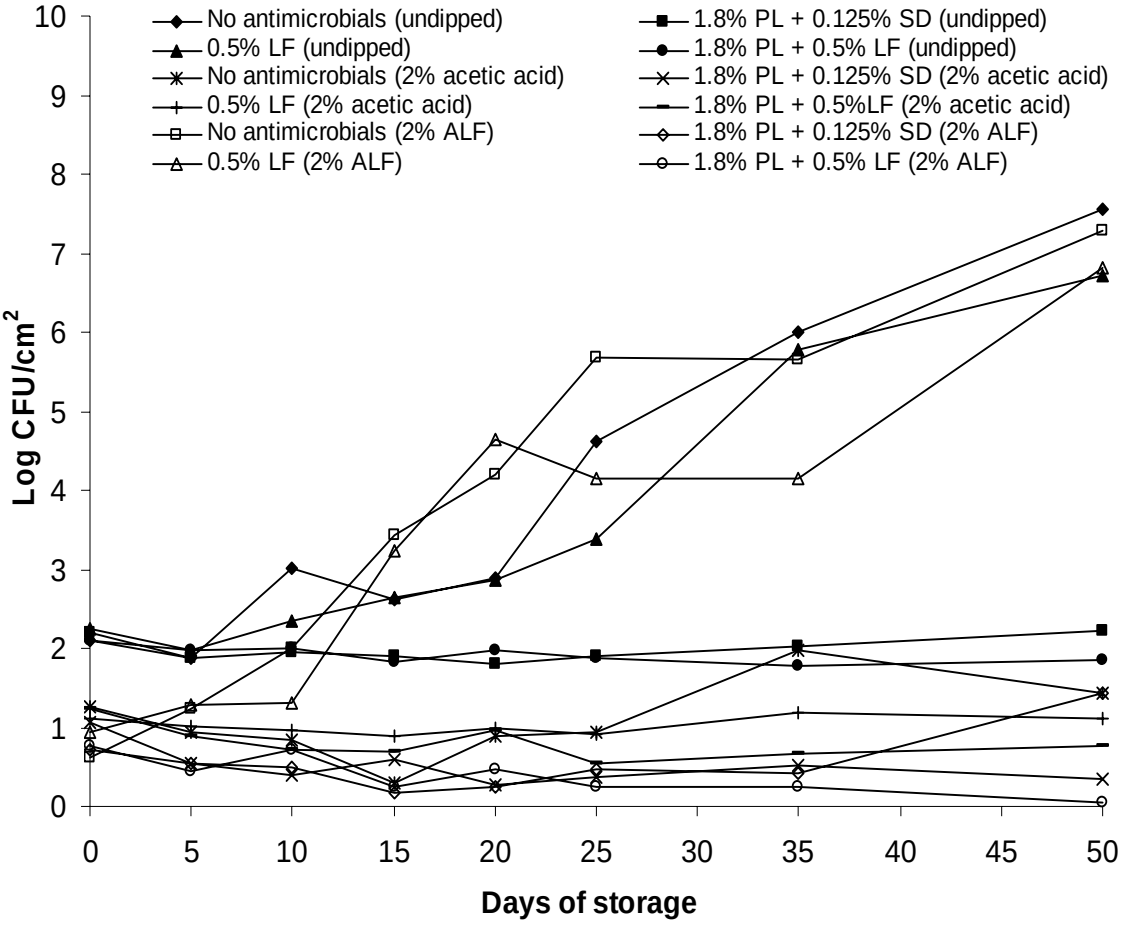


Figure 12: Mean (n=3) populations of *Listeria monocytogenes* (log CFU/cm^2) inoculated onto the surface of frankfurters formulated with or without antimicrobials. Samples were either left undipped or were immersed into solutions of 2% acetic acid or 2% activated lactoferrin for 2 min before vacuum-packaging and storage at 7^{o}C for 50 days. LF, lactoferrin; ALF, activated lactoferrin.

Table 45: Mean (n=3) total microbial populations (log CFU/cm^2 ± standard deviation) on the surface of frankfurters formulated with or without antimicrobials, inoculated with *Listeria monocytogenes*, left undipped or dipped into solutions of 2% acetic acid or 2% activated lactoferrin for 2 min, vacuum-packaged and stored at 7^{o}C.

Treatment (dipping)	Treatment (formulation)	Days of storage							
		0	5	10	15	20	25	35	50
No dipping	Control	2.2±0.1	2.0±0.1	3.1±0.2	3.5±1.4	3.0±0.1	5.1±0.1	5.2±0.5	7.3±0.8
	PL (1.8%) + SD (0.125%)	2.2±0.1	2.1±0.1	2.1±0.1	1.9±0.3	1.9±0.1	1.7±0.3	2.0±0.1	2.4±0.8
	LF (0.5%)	2.3±0.1	2.2±0.1	2.6±0.4	3.1±1.0	3.2±0.6	4.0±1.0	5.4±0.1	6.8±0.7
	PL (1.8%) + LF (0.5%)	2.2±0.1	2.0±0.1	2.1±0.0	1.9±0.1	2.0±0.0	2.0±0.3	2.1±0.1	1.9±0.2
Acetic acid (2%)	Control	1.2±0.1	1.6±1.0	1.0±0.2	1.5±0.7	1.0±0.1	1.6±0.2	2.0±0.3	1.6±0.4
	PL (1.8%) + SD (0.125%)	1.2±0.2	0.7±0.1	0.7±0.1	0.8±0.1	0.8±0.1	0.7±0.2	0.6±0.5	0.7±0.5
	LF (0.5%)	1.5±0.0	1.2±0.2	1.3±0.4	1.5±0.4	1.4±0.4	1.3±0.1	1.2±0.3	1.8±0.2
	PL (1.8%) + LF (0.5%)	1.5±0.1	0.9±0.0	0.9±0.1	1.0±0.1	0.9±0.2	0.8±0.2	0.8±0.3	1.1±0.3
Activated lactoferrin (2%)	Control	0.8±0.1	1.1±0.5	2.0±0.2	3.8±0.4	4.2±0.1	4.8±0.9	5.9±0.4	6.8±0.5
	PL (1.8%) + SD (0.125%)	0.8±0.0	0.6±0.1	0.5±0.3	0.5±0.0	0.3±0.3	0.5±0.3	0.2±0.2	1.6±0.9
	LF (0.5%)	1.1±0.2	1.4±0.5	0.6±0.0	3.3±0.7	4.6±0.3	4.4±0.2	6.2±0.5	7.2±0.7
	PL (1.8%) + LF (0.5%)	1.9±0.1	0.6±0.1	2.0±1.0	0.7±0.4	0.4±0.1	0.5±0.0	0.7±0.6	1.0±0.3

PL, potassium lactate; SD, sodium diacetate; LF, lactoferrin

Table 46: Mean pH (n=3) values (pH ± standard deviation) of frankfurters formulated with or without antimicrobials, inoculated with *Listeria monocytogenes*, left undipped or dipped into solutions of 2% acetic acid or 2% activated lactoferrin for 2 min, vacuum-packaged and stored at 7°C.

Treatment (dipping)	Treatment (formulation)	Days of storage							
		0	5	10	15	20	25	35	50
No dipping	Control	6.04±0.02	5.05±0.02	6.03±0.02	6.02±0.02	6.01±0.03	6.05±0.01	5.94±0.05	5.94±0.05
	PL (1.8%) + SD (0.125%)	5.95±0.07	5.01±0.03	6.01±0.01	5.97±0.02	6.02±0.05	5.97±0.03	5.95±0.05	5.95±0.05
	LF (0.5%)	6.03±0.02	6.01±0.01	6.11±0.02	5.97±0.02	6.02±0.06	5.99±0.01	5.98±0.02	5.98±0.02
	PL (1.8%) + LF (0.5%)	6.03±0.01	6.03±0.04	6.07±0.02	6.05±0.01	6.07±0.02	6.03±0.05	6.03±0.06	6.03±0.06
Acetic acid (2%)	Control	5.40±0.08	5.88±0.04	5.91±0.01	5.83±0.02	5.84±0.05	5.84±0.02	5.79±0.01	5.79±0.05
	PL (1.8%) + SD (0.125%)	5.70±0.01	5.77±0.02	5.71±0.02	5.82±0.03	5.90±0.06	5.73±0.04	5.80±0.06	5.80±0.06
	LF (0.5%)	5.06±0.03	5.75±0.05	5.83±0.03	5.78±0.05	5.84±0.01	5.78±0.03	5.76±0.02	5.76±0.02
	PL (1.8%) + LF (0.5%)	5.84±0.01	5.82±0.01	5.84±0.02	5.86±0.01	5.87±0.13	5.80±0.01	5.83±0.01	5.83±0.01
Activated lactoferrin (2%)	Control	6.09±0.03	6.09±0.06	5.96±0.03	6.05±0.05	6.08±0.03	6.03±0.03	5.95±0.01	5.95±0.01
	PL (1.8%) + SD (0.125%)	5.98±0.01	6.05±0.05	5.93±0.02	5.92±0.02	6.05±0.06	5.99±0.04	5.98±0.01	5.98±0.02
	LF (0.5%)	6.03±0.01	6.07±0.05	6.04±0.02	6.02±0.02	6.13±0.04	6.07±0.03	5.97±0.05	5.97±0.05
	PL (1.8%) + LF (0.5%)	6.09±0.01	6.09±0.05	6.13±0.03	6.03±0.02	6.09±0.04	6.06±0.01	6.04±0.03	6.04±0.03

PL, potassium lactate; SD, sodium diacetate; LF, lactoferrin

Table 47: Mean (n=2) a_w values (a_w ± standard deviation) of frankfurters formulated with or without antimicrobials, inoculated with *Listeria monocytogenes*, left undipped or dipped into solutions of 2% acetic acid or 2% activated lactoferrin for 2 min, vacuum-packaged and stored at 7°C.

Treatment (dipping)	Treatment (formulation)	Water activity on day-0
No dipping	Control	0.949±0.001
	PL (1.8%) + SD (0.125%)	0.940±0.000
	LF (0.5%)	0.951±0.001
	PL (1.8%) + LF (0.5%)	0.941±0.000
Acetic acid (2%)	Control	0.954±0.004
	PL (1.8%) + SD (0.125%)	0.942±0.000
	LF (0.5%)	0.956±0.001
	PL (1.8%) + LF (0.5%)	0.941±0.001
Activated lactoferrin (2%)	Control	0.955±0.001
	PL (1.8%) + SD (0.125%)	0.945±0.001
	LF (0.5%)	0.954±0.002
	PL (1.8%) + LF (0.5%)	0.940±0.000

PL, potassium lactate; SD, sodium diacetate; LF, lactoferrin

Table 48: Mean (n=2) values (± standard deviation), of cooking yields, and moisture and fat content of frankfurters formulated with or without antimicrobials.

Treatment	Cooking yield (%)	Moisture content (%)	Fat content (%)
Control	79.3±0.7	68.2±0.3	15.9±2.0
PL (1.8%) + SD (0.125%)	79.2±0.7	68.3±1.4	15.6±1.2
LF (0.5%)	78.2±1.1	61.0±0.1	13.1±3.1
PL (1.8%) + LF (0.5%)	79.0±0.1	62.2±0.5	13.5±0.4

PL, potassium lactate; SD, sodium diacetate; LF, lactoferrin

Table 49: Parameter estimates (P≤0.05) of the logistic regression models for the growth/no growth interface of *Listeria monocytogenes*.

Coefficient	Uncured Turkey breast		Bologna	
	Estimate	St. error	Estimate	St. error
Intercept	18.9039	6.2650	12.7121	3.5723
Lactic acid concentrations	-4.6142	1.5418	-3.3037	0.9185
Dipping time	-3.3843	1.1587	-2.4366	0.7329

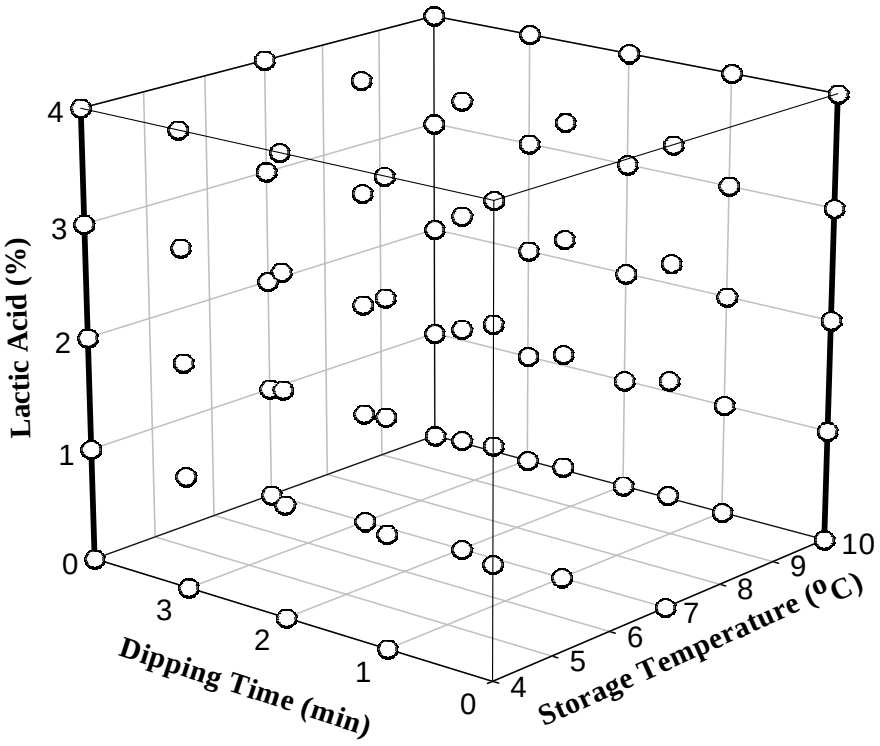


Figure 13: Combinations of storage temperature, dipping time, and lactic acid concentration to generate the model.

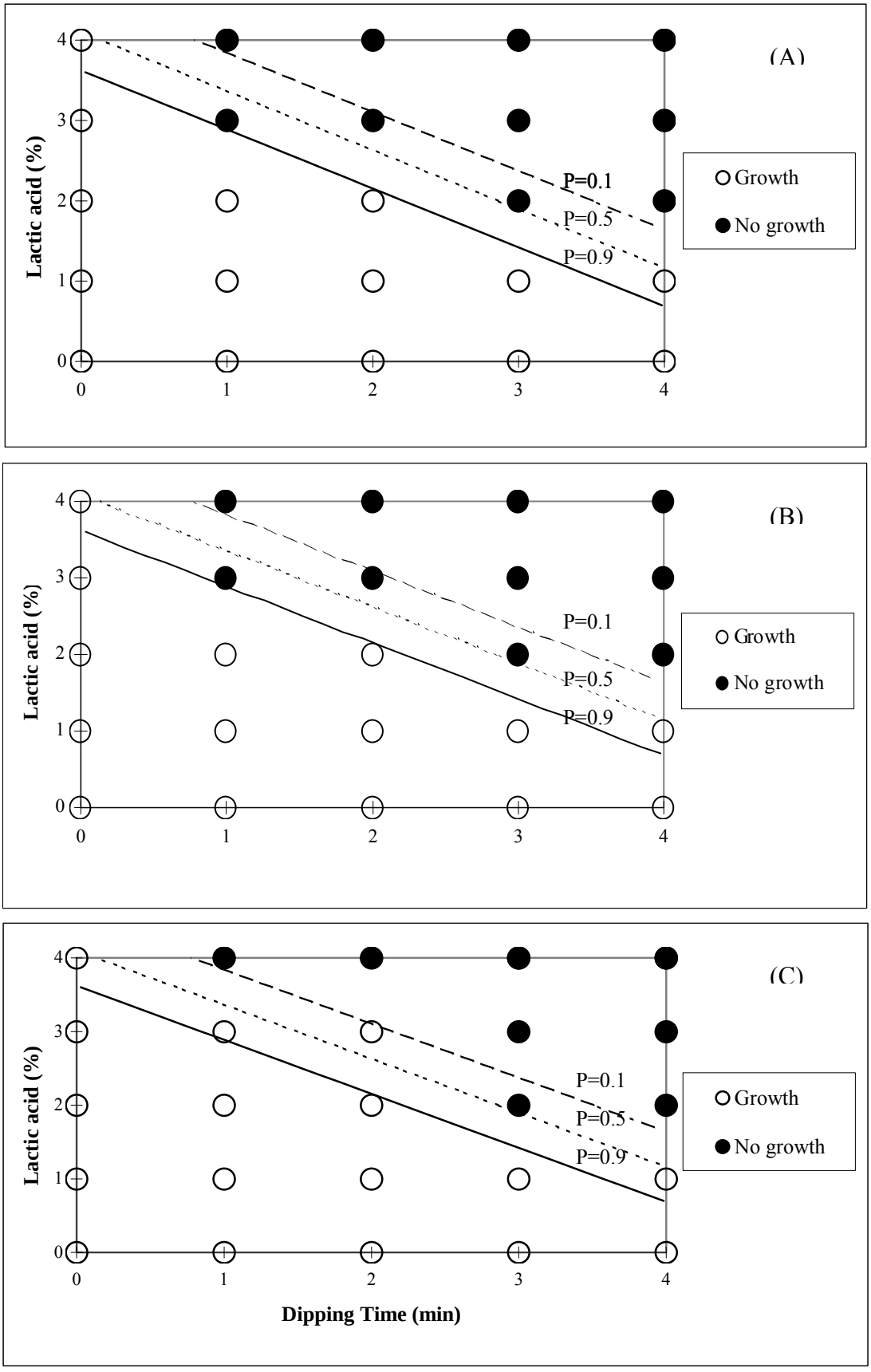
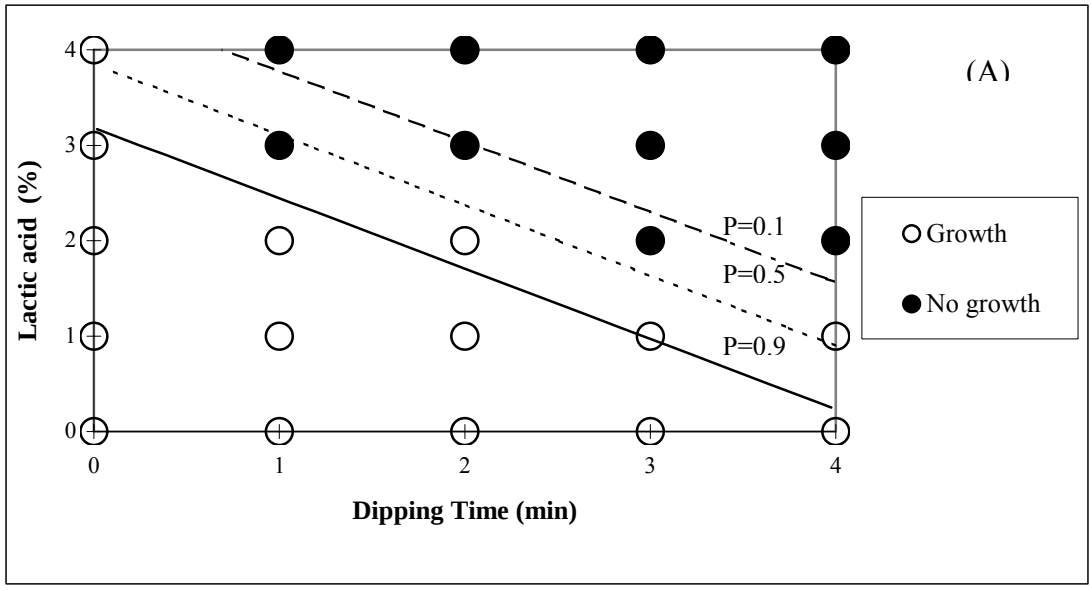
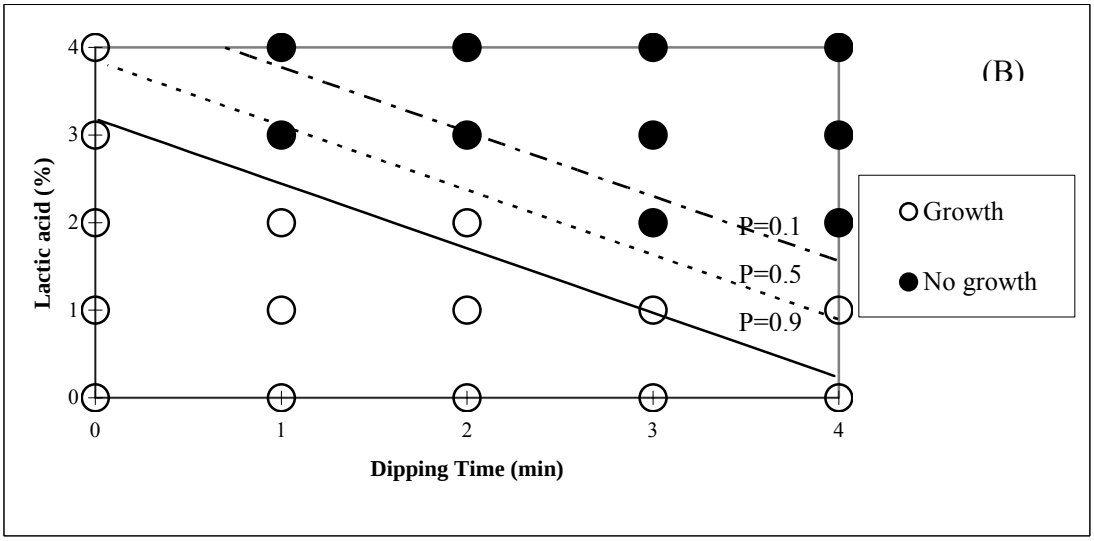


Figure 14: Growth/no growth interface of *Listeria monocytogenes* in turkey breast at 4 (A), 7 (B), and 10°C (C) with respect to lactic acid concentrations and dipping times predicted by the model at probabilities 0.1, 0.5, and 0.9.





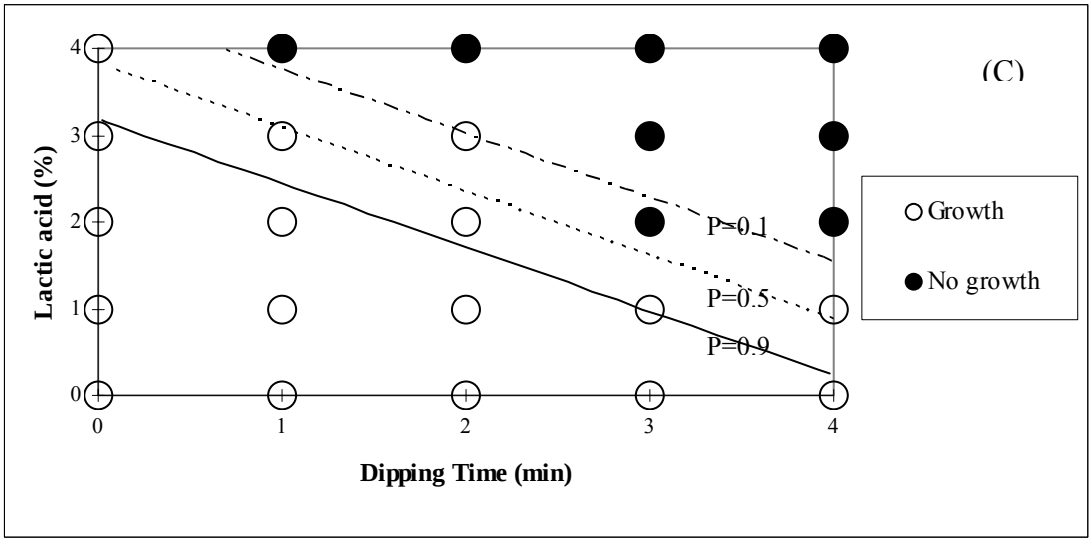


Figure 15: Growth/no growth interface of *Listeria monocytogenes* in bologna at 4 (A), 7 (B), and 10^{o}C (C) with respect to lactic acid concentrations and dipping times predicted by the model at probabilities 0.1, 0.5, and 0.9.

CONCLUSIONS

The studies described here tested the effectiveness of lactoferrin in the formulation of two products, bologna and frankfurters, together and in comparison with known antilisterial chemical compounds (potassium lactate and sodium diacetate). To evaluate the antimicrobial activity of ALF as a post-processing treatment, alone or followed by organic acids (acetic or lactic acid) or salts (potassium lactate, sodium diacetate), studies were conducted using different RTE meat and poultry products, obtained commercially or formulated in the CSU meat laboratory. These products were chosen to include comminuted and restructured or intact, sausage-type and sliced, and cured and uncured varieties. In addition, products formulated with different meat types (beef vs beef and pork) were compared. Thus, the products selected for the post-processing antimicrobial studies included: bologna (beef, beef and pork), ham, turkey breast (cured and uncured) and frankfurters. In addition to the testing of different products, antimicrobial treatments were applied by dipping or spraying to compare the effect of application methods. Also, dipping treatments, with different exposure times (0.5 to 180 s), were tested alone or in sequence to potentially enhance the activity of the single treatments. The potential contribution of inoculum composition on the antimicrobial effectiveness of the treatments was also evaluated by using two different 10-strain *L. monocytogenes* composites.

Results showed that under the conditions of these studies, certain antimicrobials, when incorporated in the formulation of bologna or frankfurters, may provide considerable inhibition of *L. monocytogenes* growth during storage at both 4°C and 7°C. Specifically, the combination of 1.8% potassium lactate with 0.125% sodium diacetate, which is widely used in commercial processed meat or poultry products for *L. monocytogenes* control, provided substantial or complete inhibition of the pathogen in both bologna and frankfurters. Lactoferrin added in the formulation of bologna resulted in slight inhibition of the pathogen during the beginning of the storage period; however, the compound did not seem to sustain its antilisterial effects as counts in samples that contained lactoferrin reached high numbers, eventually. Moreover, no substantial enhancement in the antilisterial activity of potassium lactate or sodium diacetate was observed due to addition of lactoferrin in the product. Lactoferrin provided greater inhibition of *L. monocytogenes* growth when added as an ingredient (0.5%) in frankfurters rather than in bologna. In general, greater inhibition of *L. monocytogenes* proliferation is expected on frankfurters whose surface (skin) is less supportive of bacterial growth than that of sliced product, such as bologna. The combination of 1.8% potassium lactate with 0.5% lactoferrin in the formulation of frankfurters was listeriostatic, whereas the same combination included in the bologna formulation allowed growth of the pathogen.

Application of certain organic acid/salt solutions as surface treatments provided different degrees of inhibition of *L. monocytogenes* growth. In general, acetic acid and sodium diacetate exhibited greater antilisterial effects compared to lactic acid and potassium lactate. In addition, the type and composition of product affected *L. monocytogenes* proliferation in treated products, with chemical antimicrobials being generally more efficient when applied on cured vs. uncured turkey breast or on product of lower vs. higher pH (beef vs. beef and pork bologna). Activated lactoferrin applied as a 1 or 2% dipping (or spraying) solution was generally less effective than organic acid or sodium diacetate solutions. Although product type (sliced vs. non sliced) and composition (cured vs. uncured, formulated with beef vs. beef and pork) affected the rapidity of

L. monocytogenes proliferation in treated samples, as well as final counts of the pathogen, similar trends were observed across treatments in different products used in these studies. Moreover, results obtained for sequential treatments (1 or 2% ALF followed by organic acids or salts) gave no indication that ALF enhanced the antilisterial activity of other antimicrobial compounds. On the contrary, 2% ALF applied as a surface treatment on frankfurters formulated with 1.8% potassium lactate combined with 0.5% lactoferrin appeared to enhance the activity of the additives against *L. monocytogenes*, suggesting that combined incorporation of antimicrobials in the formulation of the product and dipping in ALF solutions may control *L. monocytogenes* in that product. Incorporation of lactoferrin in the formulation of frankfurters slightly enhanced the antilisterial effectiveness of acetic acid applied as a surface treatment as it resulted in small reductions of *L. monocytogenes* populations during storage.

Overall, the results showed that, under the conditions of this study, lactoferrin added as a formulation ingredient in bologna was not as effective as the combination of potassium lactate and sodium diacetate; also lactoferrin used in combination with potassium lactate and/or sodium diacetate did not enhance, or in some instances reduced, their antimicrobial activity. However, in frankfurters, lactoferrin combined with potassium lactate in the formulation was as effective as the potassium lactate-sodium diacetate combination. Furthermore, ALF as a post-processing antimicrobial solution on products, including frankfurters, and slices of bologna (formulated with beef only or beef and pork), ham, and uncured and cured turkey breast, was found not as effective as organic acid (acetic or lactic acid) or salt (sodium diacetate) solutions. It should be noted that in frankfurters formulated with potassium lactate and lactoferrin, dipping in ALF solution after processing, was as effective as dipping in acetic acid for frankfurters of the same formulation; both combinations did not allow growth for 50 days of storage at 7°C.

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