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Inactivation of *Listeria innocua* on Frankfurters by Flash Pasteurization and Lauric Arginate Ester

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Abstract

Listeria monocytogenes, a psychrotrophic food-borne pathogen, is a recurring post-process contaminant on ready-to-eat meat (RTE) products including frankfurters. Flash Pasteurization (FP) uses short pulses of steam to decontaminate the surface of precooked sausages such as frankfurters. The antimicrobial lauric-arginate-ester (LAE) has been shown to reduce levels of *L. monocytogenes* and its nonpathogenic surrogate *L. innocua* on frankfurters. In this study the use of FP to inactivate *L. innocua* on frankfurters followed by application of LAE immediately prior to vacuum-packaging in a pilot plant setting was investigated. Use of FP (1.5 s, 120°C steam), LAE (3.33 ml of a 5% volume/volume solution per pack of four frankfurters), or FP followed by application of LAE, resulted in a 2.5, 1.6, and 3.3 of log reductions of *L. innocua* that was surface-inoculated onto frankfurters, respectively. Although FP alone reduced *L. innocua* levels by 2.5 log, the bacterium recovered and grew to a density of >10^6 CFU/g within 2 weeks during refrigerated storage (10°C). LAE inhibited *L. innocua* growth for 8 weeks, but the bacterium was able to recover and grow to a density of >10^6 CFU/g by week 12. The use of FP in combination with LAE effectively inhibited the growth of *L. innocua* for 12 weeks. The use of FP in combination with LAE had little effect on frankfurter color and texture, and was found to be an effective hurdle process for decontamination of frankfurter surfaces.

Introduction

Listeria monocytogenes is an occasional post-process contaminant on ready-to-eat (RTE) meat products, including frankfurters, and while control of this pathogen has improved significantly in recent years, it is responsible for a number of food-borne illness outbreaks and product recalls [1-6]. *L. monocytogenes* is capable of growth at refrigerated temperatures and in high salt environments, which allows it to proliferate during long-term cold storage [7] and readily grows in refrigerated ready-to-eat meat products such as deli meats and frankfurters [8]. Control of this microorganism is important because the lower number of bacterial cells accidentally consumed, the lower the risk of contracting listeriosis, which is important due to the increasing percentage of at risk individuals such as diabetics or the elderly in the U.S. [9,10]. Because of the high mortality rate (20-30%) associated with listeriosis, this pathogen is given zero tolerance in ready-to-eat meat products in the United States [11].

Frankfurters surfaces are typically contaminated by microorganisms, including *L. monocytogenes*, after the cooking process and prior to packaging. There are many intervention technologies that can be used to inactivate foodborne pathogens on pre-cooked sausage and ready-to-eat meat products including antimicrobials [8] high pressure processing [12], infrared light [13], in-package thermal treatments [14], ionizing radiation [15], ultraviolet light [6], pulsed-light [16], etc.

While many technologies are available to improve the microbiological safety and shelf-life of precooked sausages, those actually in commercial use are relatively few because of the need for effectiveness, low cost, and the ability to use the technology at commercial processing line speeds. Flash Pasteurization (FP), uses short pulses of steam (120°C, 1.5s) to decontaminate the surfaces of fine emulsion sausages such as frankfurters or bratwurst immediately before packaging [17]. FP inactivates 2-3 log CFU/g of *L. monocytogenes* and *L. innocua* on precooked sausage surfaces, however, the bacteria are eventually able to recover and proliferate during long-term refrigerated storage. Lauric Arginate Ester (LAE), which is metabolized into natural substances when consumed, is Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration, and is applied to RTE meats and packaging surfaces immediately prior to vacuum-sealing. Porto-Fett et al. [22] found that, while LAE inactivated 1-2 log CFU/g of *L. monocytogenes* on frankfurter surfaces, the food-borne pathogen was able to recover and proliferate to approximately 7 log CFU/g during long-term refrigerated storage at 4°C. This outcome was improved when LAE was combined with the antimicrobials sodium diacetate (SDA) and potassium lactate (PL), a phenomenon also observed by other researchers [6].

The most effective approach to improving the microbial safety and quality of foods is the hurdle approach, which involves the combinatorial use of physical and chemical intervention technologies, to improve microbial inactivation and outgrowth during long-term storage. Physical interventions such as ultraviolet light (UV-C), in combination with heat, are more effective than UV-C or heat alone [5]. FP in combination with antimicrobials including SDA and PL mixtures or organic acids is more effective than either technology used by itself [7,18,19]. Sommers et al. [6] demonstrated that UV-C, when used in combination with LAE, SDA and PL was more effective for controlling

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L. monocytogenes, *Salmonella*, and *Staphylococcus aureus* on frankfurter surfaces than either UV-C or the antimicrobials alone. In summary, the most effective means for controlling *Listeria* spp. in ready-to-eat meat products is the combination of multiple intervention processes. This is the first study that investigates the use of FP in combination with LAE as a food safety intervention technology.

The purposes of this study were to: (1) determine the effect of the combinatorial use of FP and LAE to inactivate the *L. monocytogenes* surrogate *L. innocua* on the surface of frankfurters in a pilot plant setting; (2) to determine the growth potential of *L. innocua* on FP and LAE treated frankfurters during refrigerated storage (10°C); and (3) to determine the effect of FP and LAE on frankfurter color and shear force.

Materials and Methods

Frankfurters

Frankfurters were purchased from a local processor. The frankfurters consisted of beef, water, salt, flavoring, paprika, sodium phosphate, sodium nitrate and were 25% fat. They did not contain antimicrobials such as sodium diacetate and potassium lactate. Frankfurters were stored at -20°C and thawed overnight for experimentation the following day.

***L. innocua*:** Three *L. innocua* isolates (51742, 33090, 33091) were obtained from the American Type Culture Collection (Manassas, VA, USA). The strains were propagated on Tryptic Soy Agar (BD-Difco Laboratories, Sparks, MD, USA) at 37°C and maintained at 0-2°C until ready for use. Identity of *Listeria* was confirmed by Gram Stain followed by analysis with Gram Positive Identification (GPI) cards using the Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood, MO, USA).

***L. innocua* propagation and inoculation:** Each *L. innocua* strain was cultured independently in 30-mL Tryptic Soy Broth (Difco) in 50-mL sterile tubes at 37°C (150 rpm) for 18 h. The cultures were then diluted in Butterfield's Phosphate Buffer (BPB) (Applied Research Institute, Newtown, CT, USA) as a mixture. Refrigerated frankfurters were then placed on a sterile surface, rolled in 1 ml of diluted inoculum, including the ends, to a final concentration of 10^5 CFU/g, and placed in a refrigerator for approximately 30 minutes prior to FP or LAE treatments. The inoculation, 10^5 CFU/g, is relatively high in comparison to the low numbers generally associated with naturally occurring contaminations of frankfurters [20]. Preliminary experiments revealed an inability to recover viable *L. innocua* following treatment at lower inoculations (10^2-10^3 CFU/g) that would typically represent the upper limit of naturally occurring contaminations [20].

Flash pasteurization

To assess the effect of the FP inactivation process, the surface-inoculated frankfurters were loaded into open preformed trays at the inlet of the Flash Pasteurization prototype unit (Alkar-RapidPak, Lodi, WI) as a single layer of 4 frankfurters [19]. The frankfurters were then exposed to steam treatments (120°C) for 1.5 s. Following treatment, the frankfurters were placed in sterile polynylon bags (Uline, Inc., Philadelphia, PA) which were stored in an ice-water bath prior to enumeration of *L. innocua*.

Lauric arginate ester

Lauric Arginate Ester (Cytoguard™) and Cytoguard-STAT-N™ were obtained from A&B Ingredients (Fairfield, NJ). Cytoguard-STAT-N is a mixture of bacterial growth inhibitors made to improve the effectiveness of LAE. LAE was suspended in Cytoguard-STAT-N to a final concentration of 5%. The 5% concentration and volume were based on previous research and manufacturer's recommendations for the concentration and volume. Approximately 3.3 ml were added to four frankfurter packs in polynylon bags (Uline, Inc., Philadelphia, PA, USA) using a Badger Model industrial paint sprayer, and then vacuum sealed (30 mB) using a Multi-Vac A300 packager (MultiVac, Inc., Kansas City, MO, USA).

Flash pasteurization and Lauric arginate ester

The same procedures were used as stated above with the exception that the LAE was added immediately following FP. The frankfurter packages were then vacuum-sealed as described above.

Recovery and plating of *L. innocua*: Following FP the samples were assayed for colony forming units (CFU's) by standard pour plate procedures. Fifty-mL of sterile DE Buffer (Remel, No. R453042, Lenaxa, KS, USA) was added to a polynylon bag that contained 4 frankfurters and shaken manually for 1 min in order to resuspend the bacteria and neutralize the LAE. Failure to neutralize the LAE solution results in an overestimation of the log reduction of microorganisms as a result of inhibition of growth of the pathogens following diluting and plating [6]. Suspension of the frankfurters in BPB, peptone water, or buffered peptone water, as opposed to DE Buffer, yielded unsatisfactory results [6]. The samples were then serially diluted in BPB, using tenfold dilutions, and 0.1-mL of diluted sample was pour plated using Palcam Medium (BD-Difco, Inc., Sparks, MD, USA). Two 0.1-mL aliquots were plated per dilution. The Palcam plates were then incubated for approximately 72 h at 37°C prior to enumeration for CFU, as opposed to 48 h which is typically used for *Listeria* spp., to further mitigate the effect of LAE on growth rate during the incubation period. Each experiment was conducted independently three times (n=3).

Storage study: The same inoculation and recovery procedures were used and described in the previous sections. Single layer packages of frankfurters were held at 10°C for 12 weeks. The temperature for long term storage was selected based on USDA FSIS recommendations for evaluation of post-process interventions and alternative [21]. Frankfurter packs were sampled every two weeks. Each experiment was conducted independently three times (n=3).

Color analysis: Color analysis was performed using a Hunter Lab Miniscan XE Meter (Hunter Laboratory, Inc., Reston, VA, USA) [19]. The meter was calibrated using white and black standard tiles. Illuminate D65, 10° standard observer, and a 2.5-cm port/viewing area were used. Results are from three independent experiments, with three readings taken per experiment.

Shear force: Cutting force of the frankfurters was measured using a Texture Technologies Corp. (Scarsdale, NY, USA) TA-XT2 Texture Analyzer. A TA-7 Warner-Bratzler Blade was used with a test speed of 2.0 mm/s, 55 mm distance, and 20 g auto-trigger [19]. Maximum shear force (g) results are from three independent experiments, with three readings taken per experiment.

Statistical analysis

Descriptive statistics and Analysis of Variance (ANOVA) were performed using the descriptive statistics package of MS Excel (Microsoft Corp., Redmond, WA, USA).

Results and Discussion

Flash pasteurization (FP) is a commercialized process that uses short pulses of steam to inactivate *Listeria* spp. on the surfaces of precooked sausages including frankfurters and bratwurst, and extend product shelf-life [5,19]. The technology can be used to decontaminate 50, 000 kg of precooked sausages in modern meat processing plants per day. Like other post-process intervention technologies, FP is most effective when used in combination with antimicrobials which inhibit the growth of *Listeria* spp. during refrigerated storage [7,18,19].

In this study the use of FP, when used in combination with the emerging GRAS antimicrobial lauric-arginate-ester (LAE), which is now widely used by the meat processing industry in the US, to inactivate *L. innocua* on frankfurter surfaces was investigated. *L. innocua* has been used as a surrogate microorganism in previous pilot-plant scale studies for FP decontamination of frankfurters [5,7,17-19]. This is the first report on the efficacy of FP in combination with LAE as an antilisterial measure.

FP conditions (1.5s steam, 120°C) were used based on previous research and conditions of use in actual commercial practice, and resulted in 2-3 log reductions of *L. monocytogene*s and *L. innocua* [Personal Communication, Seth Pulsfus, Alkar-Rapid-Pak, Inc]. In this study FP reduced *L. innocua* levels by 2.5 log CFU/g. In a previous study by Sommers et al. [6], a 5% solution of LAE reduced populations of *L. monocytogenes* on frankfurter surfaces by 2.1 log CFU/g, and in this study *L. innocua* populations were reduced by 1.6 log CFU/g. Use of FP (1.5s, 120°C steam) followed by application with LAE (5% solution) resulted in a 3.1 log reduction of *L. innocua* on frankfurter surfaces (Figure 1).

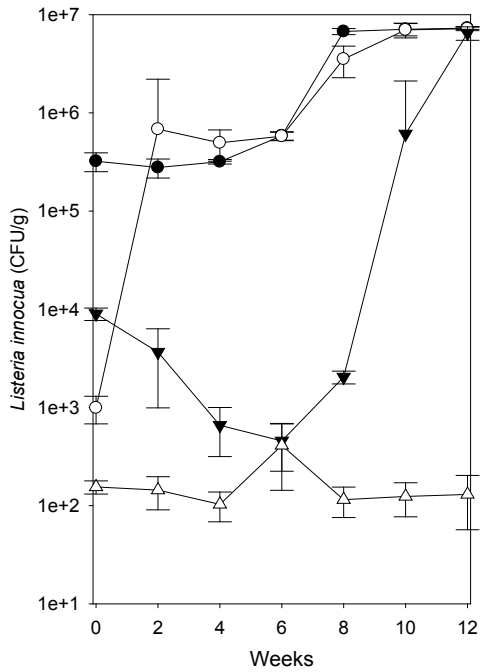

Figure 1: Growth of *L. innocua* on frankfurters (10ºC) treated with Flash Pasteurization and Lauric Arginate Ester. Untreated controls (closed circles); FP only (open circles); 5% LAE only (closed triangles); and FP + LAE (open triangles) Standard error of the mean shown as error bars (n=3).

	Weeks	Untreated control	FP	LAE	FP+LAE
a-value	0	14.8 (±0.26)a	15.4 (±0.14)a	16.9 (±0.12)b	16.9 (±0.18)b
b-value	0	26.7 (±0.51)a	27.7 (±0.34)a	29.5 (±0.23)b	29.9(±0.42)b
L-value	0	55.7 (±0.43)a	54.2 (±0.53)a	51.9 (±0.47)b	51.0 (±0.67)b
Shear	0	1683 (±72.01)a	1993(±376.4)a	1847 (±53.31)a	1898 (±48.97)a
a-value	12	15.3 (±0.35)a	15.8 (±0.31)a	16.2 (±0.28)b	16.5 (±0.26)b
b-value	12	27.2 (±0.62)a	27.0 (±0.59)a	29.8 (±0.33)b	29.5(±0.23)b
L-value	12	59.4 (±1.23)a	57.3 (±0.93)a	52.1 (±0.68)b	50.9 (±0.85)b
Shear	12	1747 (±122.8)a	1841(±242.5)a	1713 (±106.7)a	1802 (±113.4)a

Each experiment was conducted independently 3 times (n=3). Standard error of the mean is shown in parenthesis.

Different letters in rows indicate statistical difference as determined by ANOVA (n=3, α=0.05).

Table 1: Effect of Flash Pasteurization (FP) and Lauric Arginate Ester (LAE) on frankfurter color and shear force.

The effect of FP and LAE on growth of *L. innocua* during long-term refrigerated storage was also investigated. USDA-FSIS [21] recommends use of mild temperature abuse (7-10°C) for evaluation of growth inhibitors and intervention technologies as part of post process lethality assessments. *L. innocua* inoculated onto untreated frankfurters, as noted in previous studies with *Listeria spp.,* ultimately proliferated to > 10^6 CFU/g during long-term storage. As expected, *L. innocua* treated using an intervention (FP) with no antimicrobial was able recover and proliferate during storage at 10°C (Figure 1) [7,18]. Application of LAE prevented proliferation of *L. innocua* for the first eight weeks of storage, however, the bacterium was able to recover and proliferate to >10^6 CFU/g by week 10 of storage at 10°C (Figure 1). This was in agreement with results obtained in other studies [6,22].

The most effective approach for control of *Listeria* was by application of both FP and LAE, with the bacterial growth being inhibited for the full 12 week storage period (Figure 1). We were unable to recover *L. innocua* on frankfurters treated with both FP and LAE when they were inoculated to a lower level of 10^3 CFU/g which would be a more realistic post-process contamination level [10], but were able to recover *L. innocua* from frankfurters treated with FP or LAE alone, using the methods described in this study.

While FP and LAE are both used commercially, and have little effect on frankfurter quality attributes, assessments of frankfurter color and shear force were performed. FP had no effect on color of frankfurters. However, application of LAE resulted in increased redness (a-value) of LAE and FP+LAE treated frankfurters, as well as b-value, which were maintained during 12 weeks of storage. As expected, FP + LAE had no effect on frankfurter shear force (Table 1).

Both processes, FP and application of LAE, have been commercialized. However, the use of FP in combination with LAE improved the inactivation of *Listeria* over that of the processes used individually. In conclusion, FP in combination with LAE is an effective hurdle process that can be used to control *Listeria* in pre-cooked sausages such as frankfurters.

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