

Survival of *Listeria monocytogenes* Inoculated Post-Processing on Frankfurters Formulated with Low Concentrations of Malic Acid, Sodium Citrate, and Sodium Acetate, and Exposed to a Heat Treatment after Packaging

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ABSTRACT

Ready-to-eat meat products may become contaminated with *Listeria monocytogenes* after thermal processing and, thus, there is a need to develop chemical and/or physical hurdle technologies to enhance the safety of these products. This study evaluated the antilisterial effects of antimicrobials added at low concentrations to the formulation of frankfurters, with and without application of a post-packaging heat treatment. Frankfurters (two replications; three samples each) formulated with no antimicrobials (control), malic acid (MA; 0.032% wt/wt), sodium citrate (SC; 0.06% wt/wt), sodium acetate (SA; 0.12% wt/wt), or MA+SC+SA were inoculated (4 log CFU/cm²) with a 10-strain mixture of *L. monocytogenes* and vacuum-packaged (one frankfurter/bag). Inoculated and vacuum-packaged control and MA+SC+SA frankfurters were also immersed into boiling water (93±2°C; 5 or 15 s). Samples were stored (7°C, 60 days) and periodically analyzed for *L. monocytogenes* (PALCAM agar) and total bacterial (tryptic soy agar plus 0.6% yeast extract) populations. Initial (day-0) pH values of all product formulations were similar (5.89±0.11 to 6.04±0.05). The post-packaging heat treatments reduced initial pathogen populations by 1.2-1.4 log CFU/cm² (5 s) and 1.8-2.0 log CFU/cm² (15 s). During storage, growth of *L. monocytogenes* was not different on control, MA and SC samples, and populations exceeded 7.0 log CFU/cm² by day-19. In contrast, growth on SA and MA+SC+SA samples was inhibited, and levels were 1-2 log CFU/cm² lower than the other formulation treatments on day-19. Compared to non-treated samples, post-packaging heat treatments extended the lag phase of *L. monocytogenes* on control and MA+SC+SA samples, with better effects obtained for samples treated for 15 s. Inclusion of MA+SC+SA in the formulation, in combination with the initial reductions resulting from the heat treatment (5 or 15 s), allowed increases of the pathogen of <1.0 log CFU/cm² in 19 or 25 days. Findings of this study could be useful to U.S. meat processors in their efforts to control post-processing contamination of frankfurters with *L. monocytogenes*.

OBJECTIVE

To evaluate the antilisterial effects of low concentrations of malic acid, sodium citrate, and sodium acetate, added alone or in combination to the formulation of frankfurters, with and without application of a post-packaging heat treatment.

INTRODUCTION

There are numerous reports on the potential use of chemical or physical treatments to control post-processing contamination with *L. monocytogenes* on ready-to-eat (RTE) meat and poultry products (Barmapala et al., 2005; Bedie et al., 2001; Glass et al., 2002; Houben and Eckenhausen, 2006; Ingham et al., 2005; Samelis et al., 2002). In comparison, however, there are fewer studies that have evaluated the effect of a combination of control strategies which could potentially bring about synergistic or additive effects, in accordance with the hurdle technology concept (Leistner and Gould, 2002). More specifically, two or more control strategies could be used, each of low intensity so as not to affect the sensory properties of the product, but at individually sublethal levels attacking either the same or different cellular targets to disrupt the cell's homeostasis, which may lead to metabolic exhaustion and death (Leistner and Gould, 2002). Using the principles of the hurdle concept, this study evaluated the antilisterial effects of low concentrations of three antimicrobial agents (i.e., malic acid, sodium citrate, and sodium acetate) added to the formulation of frankfurters, which, after cooking, inoculation and packaging, were subjected to a heat treatment of 93±2°C for 5 or 15 s.

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MATERIALS AND METHODS

Frankfurter Formulations:

Basic frankfurter (60% beef, 40% pork) formulation (Samelis et al., 2002) plus:

1. No antimicrobials (control)
2. Malic acid (0.032% wt/wt)
3. Sodium citrate (0.06% wt/wt)
4. Sodium acetate (0.12% wt/wt)
5. Malic acid (0.032%) + Sodium citrate (0.06%) + Sodium acetate (0.12%)

Inoculum:

- L. monocytogenes* 10-strain composite:
 - > 103M (serotype 1a, pork sausage isolate)
 - > 558 (serotype 1/2, pork meat isolate)
 - > N1-227¹ and N1-225¹ (serotype 4b, food and human isolate, respectively, both associated with the same outbreak)
 - > R2-500¹ and R2-501¹ (serotype 4b, food and human isolate, respectively, both associated with the same outbreak)
 - > R2-763¹, R2-764¹, and R2-765¹ (serotype 4b, human, food and environmental isolate, respectively, all associated with the same outbreak)
 - > Scott A (serotype 4b, human isolate)

¹kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, NY; Fugett et al., 2006)

Inoculation and Packaging:

Frankfurters (56.5 cm²) were surface-inoculated to a target inoculum level of 4 log CFU/cm² and vacuum-packaged (one frankfurter/bag).

Post-Packaging Heat Treatments:

1. No treatment (control)
2. Immersion into boiling water (93±2°C) - 5 s
3. Immersion into boiling water (93±2°C) - 15 s

Storage:

7°C, 60 days

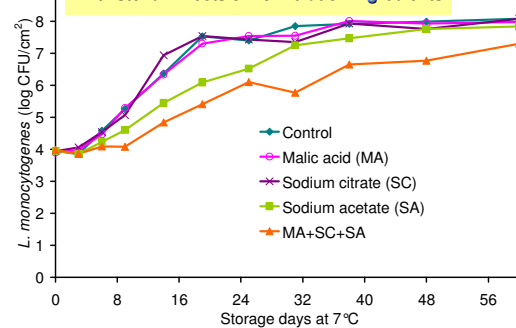
Microbiological, Chemical and Physical Analyses:

- > Two replications; three samples per sampling time and treatment
- > Analyses:
 - Total microbial populations (tryptic soy agar [Difco, Becton Dickinson, Sparks, MD] supplemented with 0.6% yeast extract [Acumedia, Lansing, MI])
 - *L. monocytogenes* populations (PALCAM agar [Difco])
 - pH (Denver Instrument [Arvada, CO] pH meter and glass electrode)
 - Water activity (AquaLab model series 3, Decagon Devices Inc., Pullman, WA)
 - Cooking yields: weight of frankfurters before and after cooking and chilling
 - > *L. monocytogenes* counts (log CFU/cm²) used to calculate lag phase durations and exponential growth rates (Baranyi model [Baranyi and Roberts, 1994] and DMFit [version 2.1; Institute of Food Research, Reading, UK])

TABLE 1: Cooking Yields, Water Activities and Initial pH (±SD)

Formulation	Cooking Yield (%)	Water Activity	Initial pH
Control	85.2±1.3	0.963±0.002	6.04±0.05
Malic acid; MA	85.6±1.4	0.965±0.001	5.89±0.11
Sodium citrate; SC	86.7±1.4	0.963±0.003	5.95±0.10
Sodium acetate; SA	85.9±1.5	0.965±0.003	5.99±0.07
MA+SC+SA	85.2±2.1	0.960±0.005	5.93±0.05

Antilisterial Effects of Formulation Ingredients



Antilisterial Effects of Formulation Ingredients and Post-Packaging Heat Treatments (5 or 15 s)

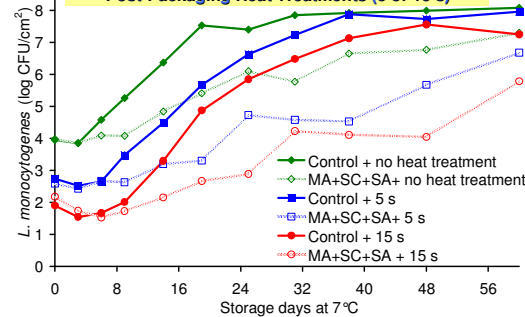


TABLE 2: Lag Phase Durations and Growth Rates

Formulation	Treatment		Lag Phase Duration (days)	Growth Rate ^a (log CFU/cm ² /day)	R ²
	Antimicrobial (%)	Boiling Water (s)			
None	0	0	0 – 2.5	0.140 – 0.300	0.916 – 0.981
		0	0 – 3.0	0.143 – 0.267	0.912 – 0.980
		2.9 – 10.8	0.319 – 0.674	0.879 – 0.980	
		0	0 – 6.2	0.120 – 0.148	0.925 – 0.944
MA+SC+SA	0	0 ^b	0.058 – 0.100	0.798 – 0.898	
		5	4.3 – 9.9	0.200 – 0.301	0.945 – 0.985
MA+SC+SA	5	0 ^b – 18.6	0.081 – 0.088	0.762 – 0.828	
		15	6.7 – 11.9	0.195 – 0.385	0.894 – 0.980
MA+SC+SA	15	0 ^b	0.040 – 0.052	0.602 – 0.724	

^a Exponential growth rate
^b As indicated by the very low growth rates, increases in counts were very slow to allow estimation of lag phase durations by the Baranyi model

RESULTS

Cooking Yields, Water Activities, and pH of Frankfurter Formulations

> Addition of MA, SC, and/or SA to the formulation did not affect the cooking yield, water activity and pH of the product (Table 1).

Effect of Formulation Ingredients on the *L. monocytogenes* Growth during Storage

> Inhibition of growth was obtained on SA and MA+SC+SA samples compared to growth on control (no antimicrobials) samples and those formulated with MA or SC.

✦ The combination treatment had better antilisterial effects than any of the single antimicrobials.

> Growth rates of *L. monocytogenes* on SA and MA+SC+SA samples were lower than for the other formulation treatments (Table 2).

> Bacterial counts on TSA were similar to those obtained on PALCAM agar (data not shown).

Initial Reductions due to Post-Packaging Heat Treatments

> Immersion into boiling water for 5 or 15 s reduced initial pathogen populations by 1.2 to 1.4 log CFU/cm² and 1.8 to 2.0 log CFU/cm², respectively.

Effect of Formulation Ingredients and Post-Packaging Heat Treatments on *L. monocytogenes* Growth during Storage

> Application of post-packaging heat treatments delayed growth of *L. monocytogenes* compared to growth on non heat-treated samples, especially on samples formulated with MA+SC+SA.

✦ Lag phase durations (when estimated) of the pathogen were longer on heat-treated samples.

> Growth rates of the pathogen on heat-treated samples were similar to those on untreated frankfurters.

CONCLUSIONS

> The combination of three antimicrobials added to the product formulation had better antilisterial effects during storage than any of the chemicals added alone.

> Immersion of packaged product into boiling water for 5 or 15 s resulted in significant reductions of initial populations.

> The combination of chemical and physical hurdles significantly inhibited growth of *L. monocytogenes*.

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