ORIGINAL CONTRIBUTION

EFFECT OF DIFFERENT ORGANIC ACID TREATMENTS WITH SIMILAR pH TO CONTROL LISTERIA MONOCYTOGENES ON COOKED FRANKFURTERS

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ABSTRACT

AIM: The antilisterial effect of different organic acid (2.5% acetic acid; 0.5% lactic acid; 0.3% citric acids) treatments with similar pH (2.85; 2.85; 2.78) on commercially produced frankfurters was studied. METHODS: Sausages were inoculated (3 to 4 logs CFU/cm²) with 10-strain mixture of Listeria monocytogenes cultures originating from different sources. Six treatments were applied (hot water 55°C for 10 s; hot water 55°C for 30 s; acid solution 55°C for 10 s; acid solution 55°C for 30 s; hot water 55°C for 10 s and cold water 4°C for 1 min.; acid solution 55°C for 10 s and cold water 4°C for 1 min). RESULTS: Data showed that treatment with acid solutions 55°C for 30 s (2.5% acetic acid and 0.5% lactic acid) had a stronger antilisterial effect. In these cases, ≥2 logs CFU/cm² reduction of L. monocytogenes on surface of experimental frankfurters was observed. No significant differences (P ≥ 0.05) were noted between applied treatments. The pH of all treated frankfurters was similar to that of distilled water. CONCLUSIONS: The practical application of results of the study is discussed. Key words: Listeria monocytogenes, organic acids, pH, frankfurters

INTRODUCTION

Cooked perishable cured meat products such as emulsion-style sausages (e.g. frankfurters) are distributed chilled and may, or may not, be heated before consumption (1). Non-reheated frankfurters, contaminated with Listeria monocytogenes have been implicated in outbreaks of human listeriosis (2). The pathogen does not survive the cooking process (3). According to Tompkin (4) L. monocytogenes is distributed in the environment, may be present in food processing and can contaminate sausages during peeling or packaging. Aymerich et al. (5) noted that consumers demand high quality, natural, nutritious, fresh appearance and convenient meat products with natural flavour and taste and an extended shelf-life. In the European Union, 2006, Listeria monocytogenes provoked 1583 cases of human listeriosis, mainly related to ready-to-eat (RTE) products (6). The Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) has issued a separate opinion on Listeria monocytogenes. It recommended to keep the concentration of Listeria monocytogenes in food below 100 cfu/g. The Scientific Committee on Food (SCF) agreed with these recommendations in its opinion of 22 June 2000 (7).

Some countries as the USA have developed compliance guidelines USDA-FSIS (8) for the extent of L. monocytogenes reduction as a measure of post-lethality treatment effectiveness, and suggest that the intervention must reduce the pathogen by at least 1 log-cycle. A number of interventions exist for sanitizing or decontaminating food products in the United States. These include some chemical solutions, especially organic acids (9). Smulders and Greer (10) reported the antibacterial efficacy of dilute solutions of organic acids (lactic, acetic). An advantage over many other intervention strategies is that the residual antimicrobial activity is demonstrable over extended periods of storage. Dilute organic acid solutions (1 to
3%) are generally without effect on the desirable sensory properties of meat.

The aim of this study was to evaluate the effect of different organic acid solutions with similar pH on *Listeria monocytogenes* inoculated on frankfurter surface and influence of pH on bacterial reduction.

**MATERIAL AND METHODS**

**Preparation of frankfurters**
Frankfurters were prepared and stored for up to 3 months as described by Byelashov et al. (10).

**Inoculum preparation and inoculation**
The following *L. monocytogenes* strains were used for contamination of experimental frankfurters: N1-225, N1-227, 558, NA-1, N-7150, R2-500, R-501, R2-763, R2-764, R2-765 as described by Byelashov et al. (11). Working bacterial cultures of each *Listeria* strain were prepared by inoculation into 10 ml tryptic soy agar (Difco) with 0.6% yeast extract (TSBYE) and incubation at 30°C for 22 hours, and then subcultured twice under the same conditions. Cells were harvested and washed by centrifugation as described by Byelashov et al. (12). After washing each culture deposition was separately resuspended in sterile frankfurter homogenate (10% w/w in distilled water) and then habituated for 72 h at 4°C, as described by Lianou et al. (13). Habituated suspensions were mixed and diluted to approximately 7 log CFU/ml in frankfurter homogenate before product inoculation. Frankfurters were contaminated by spreading 0.2 ml of the diluted bacterial mixture on the surface of each sausage with a sterile bent glass rod. After contamination samples were covered with sanitized aluminium foil and held at 4°C for 10 min., to allow bacterial cell attachment before treatment with organic acid solutions with similar pH.

**Treatments**
The study was performed with three organic acids (acetic, lactic and citric) (Birko Co., Denver, CO) dissolved in sterile distilled water (DW) (w/w) at concentrations of 2.5%; 0.5% and 0.3%, respectively. Values of pH of acid solutions were very close: 2.85; 2.85 and 2.78. The following treatments for evaluating of acid solution's effect on *L. monocytogenes* were applied: 1. Control (no treatment of inoculated frankfurters); 2. Water 55°C for 10 s; 3. Water 55°C for 30 s; 4. Acid solution 55°C for 10 s; 5. Acid solution 55°C for 30 s; 6. Water 55°C for 10 s, then water 4°C for 60 s; 7. Acid solution 55°C for 10 s, then water 4°C for 60 s. The protocols were repeated with each organic acid. Three inoculated frankfurters were studied for each combination. Temperature of treated frankfurters was 4°C when they were put in sterile stainless steel mixing bowls, at the specified temperature and for specified times. The temperature of the solutions in the bowls during sample treatment was monitored with sanitized alcohol thermometers. The temperature of treatment solutions (55°C) decreased by 1-3°C within the first 15 s of sausages' dipping and then stabilized at the same level. After dipping, all samples were drained for 30 s before bacteriological study.

**Microbiological analysis**
Immediately after draining three frankfurters were placed into sterile 24-oz bags (Whirl-Pak, Nasco, Modesto, CA) containing 50 ml of maximum recovery diluent (MRD, Difco), and manually shaken 30 times for 30 s to detach the microorganisms. Appropriate dilutions were prepared in 0.1% buffered peptone water (Difco) and plated on PALCAM agar (Difco) and TSAYE for enumeration of *L. monocytogenes* and total microbial counts. Petri dishes with PALCAM were put in incubator at 30°C for 48 h and typical colonies of *L. monocytogenes* were counted after incubation. The TSAYE plates were kept at 25°C for 72 h and number of colonies was counted. The counts were converted into log CFU/cm².

**Physicochemical analysis**
The pH of samples in MRD was tested after sample pummeling for 2 min in Masticator, IUL Instruments, Barcelona, Spain, using a pH-meter fitted with a glass electrode (Denver Instruments, Arvada, CO). Water activity (aw) values (AquaLab model series 3, Decagon Devices, WA), and fat and moisture contents (AOAC International official methods 960.39 and 950.46.B, respectively; AOAC (14) of frankfurters were also determined.

**Statistical analysis**
Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms. Least-squared means were separated using a protected pairwise t-test of SAS® (v 8.2). All differences were reported at a significance level of 0.05.
RESULTS

The physical parameters and chemical composition of the frankfurters studied were as followed: lipids (% in dry matter) – 15.2 ± 2.4, moisture – 58.9 ± 2.0%, pH – 6.04 ± 0.11 and aw – 0.974 ± 0.005. The initial pH value of untreated frankfurters was reduced (P ≤ 0.05), following some treatments with 2.5% acetic acid (Fig. 4). In case of dipping of samples for 10 and 30 s in 2.5% acetic acid solution at 55 °C, pH decreased from 6.07 to 5.72, 5.55 and 5.8 (P ≤ 0.05). Other treatments (with 0.5% lactic and 0.3% citric acid solutions) did not reduce statistically significantly pH values (Fig. 4).

Initial L. monocytogenes counts of inoculated control frankfurters were 4.15 ± 0.1 log CFU/cm² in the experiment with 2.5% acetic acid solution; 4.03 ± 0.1 log CFU/cm² before treatment with 0.5% lactic acid solution and 4.37± 0.2 log CFU/cm² in trial with 0.3% citric acid solution (Fig. 1, 2, 3). Total microbial counts were very close or equal compared to L. monocytogenes numbers (P ≥ 0.05). Analysis of the results showed (Fig. 1, 2 and 3) that treatments with distilled water (DW) at 55°C or combination with DW at 4°C in all three experiments reduced bacterial cells from 1.3 to 2 log CFU/cm². Dipping in organic acid solutions with similar pH value decreased microbial counts from 1.5 to 2.2 log CFU/cm². Statistically significant difference (P ≤ 0.05) between DW-treatments and acid solutions was found (≥ 0.4 log CFU/cm²) only when 2.5% acetic acid at 55°C for 30 s was used. (Fig. 1). In this case, the lowest pH value of treated frankfurters was estimated – 5.55 (Fig. 4). Experiments with 0.5% lactic acid and 0.3% citric acid solutions did not result in significant differences (P ≥ 0.05) compared to DW treatments.
DISCUSSION
According to Commission Regulation (7) 2073/2005 ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes had a limit up to 100 CFU/g. None-reheated frankfurters are classified as a “high risk” product that can cause listeriosis (USDHHS-FDA-CFSAN and USDA-FSIS, (15). Bacterial population on frankfurters presented by *L. monocytogenes* can be reduced by physical removal and inactivation rather than injury, as shown in the studies of Barmpalia et al. (16) and Byelashov et al. (12). Brul and Coote (17) noted that organic acids disrupt the cytoplasmic membranes of bacterial cells. Our results showed higher reduction of *L. monocytogenes* counts after treatment with 2.5% acetic acid solution at 55°C for 30 s. Other used organic acid solutions had lower concentrations and lower reduction effect. We should emphasize the similar pH values of the three organic acid solutions, used in our experiments and difference of reduction effect only when acetic acid solution was used. Ricke (18) reported that acid shock is a commonly
occurring event experienced by foodborne pathogens during exposure to food processing and preservation. A more recent observation is that acid-sensitive bacteria could adapt to acid stress by induction of an acid tolerance response. Barker and Park (19) proved that citrate, ascorbate, propionate, and acetate solutions at pH 3.0 were the least effective against *L. monocytogenes*, and the most effective compounds were formate, benzoate, malate, lactate, and sorbate, in that order. Durán and Sofos (20) compared the antilisterial effects of low equal molar concentrations (0.083 M) of acetic (0.5% v/v, pH 2.90), lactic (0.75% v/v, pH 2.30) and citric (1.6% v/v, pH 2.05) acid. The citric acid solution, which had the lowest pH, caused the most important inhibition. In our experiments, the used organic acids were with different molar concentrations, but with similar pH values - acetic (0.55 M, 2.5% v/v, pH 2.85); lactic (0.055 M, 0.5%, pH 2.85) and citric (0.0155 M, 0.3%, pH 2.78) acids. Inhibitory effect was related to the acid solution with higher molar concentration - acetic (0.55 M) and longer time of dipping (30 s). Skřivanova and Marounek (21) studied the influence of pH on antimicrobial activity of organic acids against rabbit enteropathogenic strain of *Escherichia coli*. They concluded that low pH played a positive role on bactericidal effect of organic acid solutions. El-Enean Hanan et al. (22) observed a more marked inhibitory effect with lactic acid, followed by acetic and citric acids. The inhibitory effect depended on acid solution concentration (0.5%; 1% or 5%).

In conclusion, our results and literature data showed no significant effect of similar pH, based on different concentrations of used organic acids. The best inhibitory result was observed in case when acetic acid solution 2.5% with pH 2.85 was applied for 30 s. At the same time most authors having studied the inhibitory effects of organic acid solutions agreed that lactic acid had a pronounced effect on foodborne pathogens. Low pH had positive influence in case of treatment with organic acid solutions, but factors as duration of exposure and concentrations predominated.

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REFERENCES


