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ARTICLE

Use of Antimicrobial Food Additives as Potential Dipping Solutions to Control *Pseudomonas* spp. Contamination in the Frankfurters and Ham

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Abstract

This study evaluated the effect of sodium diacetate and sodium lactate solutions for reducing the cell count of *Pseudomonas* spp. in frankfurters and hams. A mixture of *Pseudomonas aeruginosa* (NCCP10338, NCCP10250, and NCCP11229), and *Pseudomonas fluorescens* (KACC10323 and KACC10326) was inoculated on cooked frankfurters and ham. The inoculated samples were immersed into control (sterile distilled water), sodium diacetate (5 and 10%), sodium lactate (5 and 10%), 5% sodium diacetate + 5% sodium lactate, and 10% sodium diacetate + 10% sodium lactate for 0-10 min. Inoculated frankfurters and ham were also immersed into acidified (pH 3.0) solutions such as acidified sodium diacetate (5 and 10%), and acidified sodium lactate (5 and 10%) in addition to control (acidified distilled water) for 0-10 min. Total aerobic plate counts for *Pseudomonas* spp. were enumerated on Cetrimide agar. Significant reductions (*ca*. 2 Log CFU/g) in *Pseudomonas* spp. cells on frankfurters and ham were observed only for a combination treatment of 10% sodium lactate + 10% sodium diacetate. When the solutions were acidified to pH 3.0, the total reductions of *Pseudomonas* spp. were 1.5-4.0 Log CFU/g. The order of reduction amounts of *Pseudomonas* spp. cell counts was 10% sodium lactate > 5% sodium lactate ≥ 10% sodium diacetate > 5% sodium diacetate > control for frankfurters, and 10% sodium lactate > 5% sodium lactate > 10% sodium diacetate > 5% sodium diacetate > control for ham. The results suggest that using acidified food additive antimicrobials, as dipping solutions, should be useful in reducing *Pseudomonas* spp. on frankfurters and ham.

Keywords: food spoilage, *Pseudomonas* spp., sodium diacetate, sodium lactate

Introduction

Pseudomonas spp. are Gram-negative, aerophilic, and psychrotrophic bacteria, which can proliferate between 3-7°C (Jay, 2000). The bacteria contribute significantly to spoilage in milk, chicken, fish, and meat, especially at low temperatures (Arnaut-Rollier *et al*., 1999; Bajpai *et al*., 2008). In beef stored at low temperatures, *Pseudomonas* spp. constituted 96.8% of psychrotrophic spoilage bacteria, and most of the identified strains were *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas fragi* (Arnaut-Rollier *et al*., 1999; Bajpai *et al*., 2008; Jay, 2000; Jung and Cho, 1991). *Pseudomonas* spp. produce heat-stable lipases, proteases, and lecithinases, which cause food spoilage (Champagne *et al*., 1994; Sorhaug and Stepaniak, 1997). Food spoilage is a major concern in the food industry because of the economic losses that are incurred (Dogan and Boor, 2003). Thus, various antimicrobial food additives have been used to control post-processing contamination of foodborne pathogen (Patel *et al*., 2006; Sallam, 2007; Samelis *et al*., 2001) in food. However, a recent study by So *et al*. (2013) indicated that consumers considered food additives to be a threat to public health. Therefore, the food industry has initiated a reduction in the concentration of food additives in ready-to-eat (RTE) meats; however, this may allow bacterial contamination in foods, resulting in food spoilage. Hence, if food additives are used as dipping solutions, they would be useful in controlling bacterial contamination in foods, and it may satisfy the demands of consumers.

Sodium diacetate and sodium lactate are the most commonly used antimicrobial food additives, which are flavor enhancers in processed meat products (USDA-FSIS, 2000). The effectiveness of these two antimicrobial food

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additives has been established in controlling *Listeria monocytogenes* contamination in processed meats (Skandamis *et al*., 2007). In 2003, USDA-FSIS (2003) enacted three alternatives to control post-processing contamination of *L. monocytogenes* in RTE meat products, and one of the alternatives was to dip RTE meat products into antimicrobial solutions. This method could be used to control *Pseudomonas* spp. in processed meats without the addition of food additives into the products. Therefore, the objective of this study was to evaluate the bactericidal effects of sodium diacetate and sodium lactate solutions on *Pseudomonas* spp. in frankfurters and ham.

Materials and Methods

Bacterial strains and inoculum preparation

Pseudomonas aeruginosa strains NCCP10338, NCCP 10250, and NCCP11229, and *P. fluorescens* strains KA CC10323 and KACC10326 were isolated from colonies grown on Cetrimide agar (Becton Dickinson and Company, USA), and inoculated in 10 mL nutrient broth (NB; Becton Dickinson and Company) followed by incubation at 35°C for 24 h. Culture fractions of 0.1 mL were subcultured in 10 mL NB at 35°C for 24 h. Stationary-phase cells of the five strains were then mixed and centrifuged at 1,912 *g* and 4°C for 15 min, and the cell-pellet was washed twice and resuspended in phosphate-buffered saline (PBS, pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 L of distilled water). The suspension was appropriately diluted with PBS to obtain a count of 7 Log CFU/mL.

Sample preparation and inoculation

Cured cooked frankfurters and ham (no sodium nitrite/sodium lactate/sodium diacetate included) were obtained from a commercial manufacturer and used within a day. Frankfurters were formulated with pork (93.26%), salt, sugar, starch syrup, acidity regulator, celery powder, yeast extract, egg white powder, dextrose, vitamin C, grapefruit seed extract, mixed spice, smoke flavor, Lac color, and vegetable fermentation bacteria. The ham was formulated with pork (92.96%), salt, sugar, egg white powder, soy protein, lactic acid bacteria powder, red horseradish powder, onion powder, garlic powder, mustard powder, DHA (docosahexaenoic acid) powder, colostrum basic protein, white pepper powder, nutmeg powder, meat enhancer, vitamin C, acidity regulator, and cochineal extract. Ham slices (5 g) and frankfurters (5 g) (two samples/45 mL) were completely immersed into the inoculum (9 Log CFU/mL) for 2 min, and left under a laminar flow hood to allow bacterial attachment for 15 min.

Dipping treatments and microbiological analysis

Dipping solutions were prepared with sodium diacetate [5 and 10% (w/v)] (Sigma-Aldrich Chemie, Germany), sodium lactate [5 and 10% (w/v)] (Duksan, Korea), sodium diacetate [5% (w/v)]+sodium lactate [5% (w/v)] and sodium diacetate [10% (w/v)]+sodium lactate [10% (w/v)] in distilled water, were sterilized at 121°C for 15 min. Following inoculation, frankfurters and ham (two samples/20 mL of solution) were completely immersed into control (sterile distilled water) and each solution for 0, 2, 6, and 10 min. Inoculated frankfurters and ham were also immersed into acidified (pH 3.0) solutions with HCl such as acidified sodium diacetate (5 and 10%), and acidified sodium lactate (5 and 10%) in addition to control (acidified distilled water) for 0, 2, 4, and 10 min. All dipped samples were subsequently washed with distilled water for 5 min. The samples were then transferred to filter bags containing 20 mL buffered peptone water (BPW; Becton Dickinson and Company) and homogenized by a pummeler (BagMixer®, Interscience, France) for 60 s. The homogenates were serially diluted with BPW, and 0.1 mL portions of the diluents were then plated on Cetrimide agar (Becton Dickinson and Company) to determine the survivals of *Pseudomonas* spp. These plates were incubated at 35°C for 24 h, and typical colonies were manually counted. The pH values of the homogenates were measured with pH meter (Accument®, Denver Instruments, USA).

Measurement of antimicrobial residual

To measure the concentrations of sodium-based antimicrobial residuals, Na^+ residual on samples was measured alternatively. After dipping ham samples into the antimicrobial solutions and water-washing, the samples were then transferred to filter bags containing 20 mL of distilled water, followed by homogenizing by a pummeler (BagMixer®) for 60 s. The Na^+ concentration of the homogenized samples were then measured by Digital Handheld Salt Test (DMT-20, Korea).

Statistical analysis

All *Pseudomonas* spp. survival data (n=4) were analyzed using a mixed procedure of SAS® version 9.2 (SAS Institute Inc., USA). Least square means among the fixed effects were compared with pairwise *t*-test at alpha=0.05.

Results and Discussion

When frankfurters were immersed into sodium diacetate or sodium lactate solution, the cell counts of *Pseudomonas* spp. were not significantly altered, regardless of solution concentration. However, the cell counts of *Pseudomonas* spp. were slightly lower with a combination of 5% sodium diacetate and 5% sodium lactate than in other single concentration treatments. Moreover, significant reductions (*ca*. 2 Log CFU/g) were observed with a combination of 10% sodium diacetate and 10% sodium lactate, and an additional reduction (*ca*. 0.5 Log CFU/g) then occurred 4 min after dipping (Fig. 1). Geornaras *et al.* (2006) showed that application of 2.5% acetic acid dipping solution efficiently inhibited *Listeria monocytogenes* growth on frankfurters. A study by Yoon *et al.* (2009) also showed the antilisterial effect of lactic acid dipping solution in frankfurters and bologna. As shown in the studies by Geornaras *et al.* (2006) and Yoon *et al.* (2009), dipping frankfurters into organic acid solutions was very effective in controlling *L. monocytogenes* contaminations. Thus, this method was evaluated for controlling post-processing *Pseudomonas* spp. contaminations, and these antimicrobial solutions should be applied in RTE meat plants.

The dipping solution examined in our study, the significant reduction in the bacterial cell counts of *Pseudomonas* spp. was observed only for 2-min dipping, which suggests that dipping frankfurters into the combination solution of 10% sodium diacetate and 10% sodium lactate for 2 min should be sufficient. After 2-min dipping, the pH of frankfurters was 5.09, which was lower than that of untreated frankfurters (6.05). Thus, the frankfurters were additionally washed with distilled water to wash out the residual solution, and the pH value increased up to 6.01, which was similar to the pH of untreated frankfurters (data not shown).

With respect to ham samples, no significant decrease in the cell counts of *Pseudomonas* spp. was observed for a single concentration of sodium diacetate or sodium lactate solution (Fig. 2). In addition, the combination treatment of 5% sodium diacetate and 5% sodium lactate did not reduce the cell counts of *Pseudomonas* spp. during dipping (Fig. 2). However, a significant reduction (*ca*. 2 Log CFU/g) of cell counts was observed in the combination treatment of 10% sodium diacetate and 10% sodium lactate during dipping for 2 min while a secondary reduction was not observed after 2 min (Fig. 2). This reduction in the cell counts of *Pseudomonas* spp. may be caused by

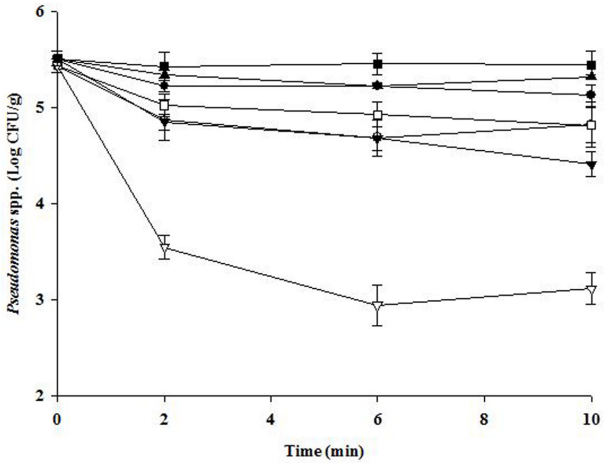

Fig. 1. Survivals of *Pseudomonas* spp. on frankfurters during dipping into antimicrobial food additive solutions for 10 min; ● : 5% sodium diacetate, ○ : 10% sodium diacetate, ■ : 5% sodium lactate, □ : 10% sodium lactate, ▼ : 5% sodium diacetate+5% sodium lactate, ▽: 10% sodium diacetate+10% sodium lactate, ▲ : Sterile distilled water.

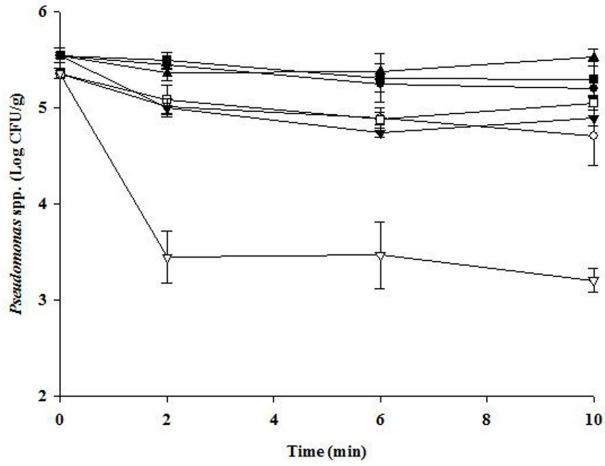

Fig. 2. Survivals of *Pseudomonas* spp. on ham during dipping into antimicrobial food additive solutions for 10 min; ● : 5% sodium diacetate, ○ : 10% sodium diacetate, ■ : 5% sodium lactate, □ : 10% sodium lactate, ▼ : 5% sodium diacetate+5% sodium lactate, ▽ : 10% sodium diacetate+10% sodium lactate, ▲ : Sterile distilled water.

hyperacidification of sodium diacetate and sodium lactate via proton donation at the cytoplasmic membrane interface of the microorganism and intracellular cytosolic acidification of the bacteria as suggested antimicrobial mode of organic acid and their salt by Lin *et al*., 2005, Shetty and Wahlqvist, 2004, and Kwon *et al*., 2007. Moreover, sodium diacetate and sodium lactate may decrease a_w, which also caused the reduction of bacterial cell count (Chirife and Fontan, 1980). The pH of ham samples dec-

reased from 6.32 to 5.12 after 2-min dipping in the combination solutions of 10% sodium diacetate and 10% sodium lactate, but the pH increased to 6.12 after washing with distilled water (data not shown), indicating that the combination treatment can be used to decrease *Pseudomonas* spp. cell counts on ham without adding an acidic flavor. Also, the concentration of Na^+ in untreated or treated ham with the dipping solution, followed by water-washing, was measured to estimate the residual of sodium lactate or sodium acetate on products. As a result, the differences between the levels of Na^+ in treated and untreated products were minimal (*ca*. 0.01-0.09%), indicating that sodium lactate and sodium acetate residuals on samples were washed out by water-washing.

Because the low pH of the solution disrupts metabolic function of bacterial cells, acidified antimicrobial food additive solutions have been suggested to reduce the bacterial cell counts (Vasseur *et al*., 1999). When the pH of control solutions (distilled water) with HCl was decreased to 3.0, the decrease of *Pseudomonas* spp. cell counts in frankfurters was minimal during dipping (Fig. 3). However, when the pH of antimicrobial food additive solutions was adjusted to 3.0, the cell counts of *Pseudomonas* spp. on the samples decreased dramatically after dipping (p<0.05) compared to the control (Fig. 3). The order of reduction in bacterial cell counts for the acidified dipping solutions was as follows: 10% sodium lactate > 5% sodium lactate ≥ 10% sodium diacetate > 5% sodium diacetate > control. Acidified 5% sodium diacetate solution resulted in 2 Log CFU/g, but acidified 10% sodium lactate resulted in 4 Log CFU/g of *Pseudomonas* spp. on frankfurters (Fig. 3), which were higher reductions than the results from Fig. 1. As shown in Figs. 3 and 4, the highest reduction amounts were observed during 2-min dipping. At the time, the pH values of the samples were 4.44-4.96, which was lower than that of untreated frankfurters, but the values were increased up to 5.92 after washing with distilled water. No significant cell count reductions were observed in the control ham samples (Fig. 4). Like frankfurters, acidified dipping solutions caused a dramatic decrease in the cell counts of *Pseudomonas* spp. in ham samples during 2-min dipping. The antimicrobial effect order for acidified dipping solutions in ham was as follows: 10% sodium lactate > 5% sodium lactate > 10% sodium diacetate > 5% sodium diacetate > control (Fig. 4). Acidified antimicrobial food additive solutions caused approximately 1.5-4.0 Log CFU/g, depending on the solution (Fig. 4). After 2-min dipping, pH values of ham samples were 4.49-5.24, but the values increased up to 6.12 after washing with distilled water, which was very similar to the pH of untreated ham (6.32).

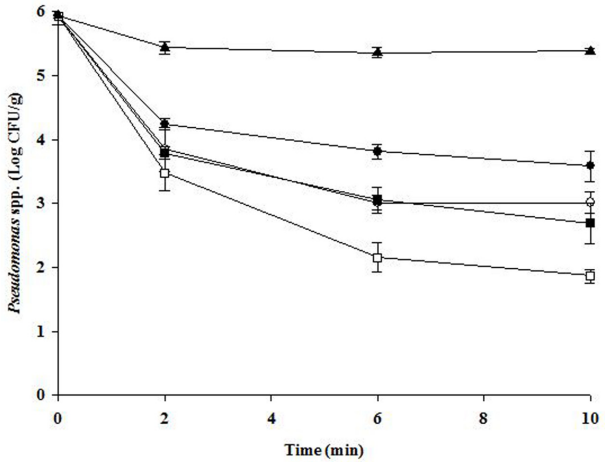

Fig. 3. Survivals of *Pseudomonas* spp. on frankfurters during dipping into acidified antimicrobial food additive solutions for 10 min; ● : Acidified 5% sodium diacetate, ○ : Acidified 10% sodium diacetate, ■ : Acidified 5% sodium lactate, □ : Acidified 10% sodium lactate, ▲ : Acidified sterile distilled water.

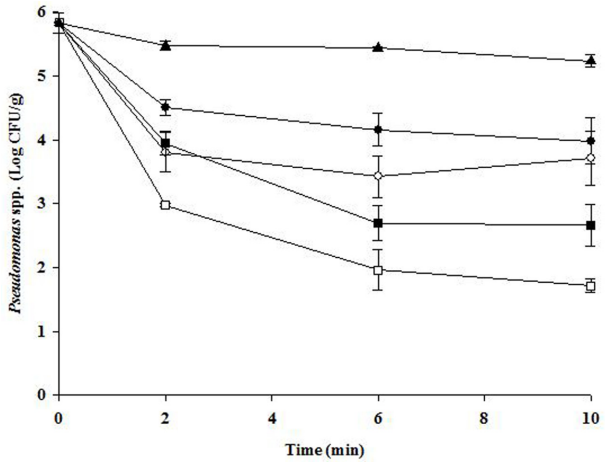

Fig. 4. Survivals of *Pseudomonas* spp. on ham during dipping into acidified antimicrobial food additive solutions for 10 min; ● : Acidified 5% sodium diacetate, ○ : Acidified 10% sodium diacetate, ■: Acidified 5% sodium lactate, □: Acidified 10% sodium lactate, ▲ : Acidified sterile distilled water.

A study by Bouttefroy *et al.* (2000) showed that nisin had an immediate pH-dependent bactericidal effect on *L. monocytogenes*. Allende *et al.* (2009) presented increased antimicrobial effects of sodium chloride by acidification. However, dipping RTE meats into acidified antimicrobial solutions can cause acidic taste, but this can be fixed by additional washing with water after additive solution

treatment. This result indicates that acidified sodium diacetate and sodium lactate solutions can be used to reduce the number of *Pseudomonas* spp. cells on frankfurters without changing the pH of food products.

In conclusion, dipping solutions with a combination of 10% sodium diacetate and 10% sodium lactate, and acidified antimicrobial food additive solutions should be useful in controlling *Pseudomonas* spp. contamination in frankfurters and ham. In addition, this method may reduce consumer concerns related to food additives because these additives can be washed off post treatment removing the acidic flavor caused by dipping RTE meats in antimicrobial additive solutions.

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