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

Mi-Hwa Oh¹, Beom-Young Park¹, Hyunji Jo, Soomin Lee, Heeyoung Lee, Kyoung-Hee Choi², and Yohan Yoon*

Department of Food and Nutrition, Sookmyung Women's University, Seoul 140-742, Korea
¹National Institute of Animal Science, RDA, Suwon 441-706, Korea
²Department of Oral Microbiology, College of Dentistry, Wonkwang University, Iksan 570-749, Korea

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 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 lecinthinases, 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...
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. 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 KH₂PO₄, 1.5 g of Na₂HPO₄·7H₂O, 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 (Accumet®, 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 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-
increased 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 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).

A study by Boutefroy *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.

Acknowledgements

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ009237)” Rural Development Administration, Republic of Korea.

References

1. Allende, A., Mcevoy, J. Tao, Y., and Luo, Y. (2009) Antimicrobial effect of acidified sodium chloride, sodium chloride, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro. *Food Control* 20, 230-234.

2. Arnaut-Rollier, I., Vauterin, L., De V os, P., Massart, D. L., Deveriese, L. A., De Zutter, L., and Van Hoof, J. (1999) A numerical taxonomic study of the *Pseudomonas* flora isolated from poultry meat. *J. Appl. Microbiol.* 87, 15-28.

3. Bajpai, V. K., Rahman, A., Dung, N. T., Huh, M. K., and Kang, S. C. (2008) *In vitro* inhibition of food spoilage and food-borne pathogenic bacteria by essential oil and leaf extracts of *Magnolia liliflora*. *Desr. J. Food Sci.* 73, M314-M320.

4. Bouttefroy, A., Mansour, M., Linder, M., and Milliere, J. B. (2000) Inhibitory conditions of nisin, sodium chloride, and pH on *Listeria monocytogenes* ATCC15313 in broth by an experimental design approach. *Int. J. Food Microbiol.* 54, 109-115.

5. Champagne, C. P., Laing, R. R., Roy, D., and Mafu, A. A. (1994) Psychrotrophs in dairy products: Their effects and their control. *Crit. Rev. Food Sci. Nutr.* 34, 1-30.

6. Chirife, J. and Fontan, C. F. (1980) Prediction of water activity of aqueous solutions in connection with intermediate moisture foods: experimental investigation of the aₜₙ lowering behavior of sodium lactate and some related compounds. *J. Food Sci.* 45, 802-804.

7. Dogan, B. and Boor, K. J. (2003) Genetic diversity and spoilage potentials among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. *Appl. Environ. Microbiol.* 69, 130-138.

8. Geornaras, I., Skandamis, P. N., Belk, K. E., Scanga, J. A., Kendall, P. A., Smith, G. C., and Sofos, J. N. (2006) Postprocess control of *Listeria monocytogenes* on commercial frankfurters formulated with and without antimicrobials and stored at 10°C. *J. Food Prot.* 69, 53-61.

9. Jay, J. M. (2000) Taxonomy, role, and significance of microorganisms in foods. In: *Modern food microbiology*. Apac Publishers Services, Singapore, pp. 13-34.

10. Jung, H. M. and Cho, K. P. (1991) Microbial distribution in refrigerated beef. *Korean J. Microbiol.* 29, 195-198.

11. Kwon, Y. I., Apostolidis, E., Labbe, R. G., and Shetty, K. (2007) Inhibition of *Staphylococcus aureus* by phenolic phytochemicals of selected clonal herb species of *Lamiaceae* family and likely mode of action through proline oxidation. *Food Biotechnol.* 21, 71-89.

12. Lin, Y. T., Kwon, Y. I., Labbe, R. G., and Shetty, K. (2005) Inhibition of *Helicobacter pylori* and associated urease by oregano and cranberry phytochemical synergies. *Appl. Environ. Microbiol.* 71, 8558-8564.

13. Patel, J. R., Sanglay, G. C., Sharma, M., and Solomon, M. B. (2006) Combining antimicrobials and hydrodynamic pressure processing for control of *Listeria monocytogenes* in frankfurters. *J. Muscle Foods.* 18, 1-18.

14. Shetty, K. and Wahlqvist, M. L. (2004) A model for the role of proline-linked pentosephosphate pathway in phenolic phytochemical biosynthesis and mechanism of action for human health and environmental applications. *Asia. Pac. J. Clin. Nutr.* 13, 1-24.

15. Skandamis, P. N., Stopforth, J. D., Yoon, Y., Kendall, P. A., and Sofos, J. N. (2007) Modelling the effect of storage atmosphere on growth-no growth interface of *Listeria monocytogenes* as a function of temperature, sodium lactate, sodium diacetate, and NaCl. *J. Food Prot.* 70, 2329-2338.

16. So, Y. J., Kim, S., Lee, J. H., Park, E. Y., Kim, H. J., Kim, J. S., and Kim J. W. (2013) A survey on the perception of consumer organizations to promote risk communication for food additives. *Korean J. Food Cookery Sci.* 29, 105-113

17. Sorhaug, T. and Stepaniak, L. (1997) Psychrotrophs and their control. *Crit. Rev. Food Sci. Nutr.* 37, 802-804.

18. USDA-FSIS (U.S. Department of Agriculture-Food Safety and Inspection Service) (2000) FSIS to increase permissible levels of food ingredients used as antimicrobials and flavoring agents. *Fed. Regist.* 65, 3121-3123.

19. USDA-FSIS (U.S. Department of Agriculture-Food Safety and Inspection Service) (2003) Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; final rule. *Fed. Regist.* 68, 34208-34254.

20. Vasseur, C., Baverel, L., Hebraud, M., and Labadie, J. (1999)
Effect of osmotic, alkaline, acid, or thermal stresses on the growth and inhibition of *Listeria monocytogenes*. *J. Appl. Microbiol.* **86**, 469-479.

23. Yoon, Y., Kendall, P. A., Belk, K. E., Scanga, J. A., Smith, G. C., and Sofos, J. N. (2009) Modeling the growth/no-growth boundaries of postprocessing *Listeria monocytogenes* contamination on frankfurters and bologna treated with lactic acid. *Appl. Environ. Microbiol.* **75**, 353-358.

(Received 2014.3.24/Revised 2014.7.25/Accepted 2014.7.25)