C32

REDUCTION OF LISTERIA MONOCYTOGENES, ESCHERICHIA COLI 0157: H7 AND SALMONELLA TYPHIMURIUM DURING STORAGE ON BEEF SANITIZED WITH FUMARIC, ACETIC AND LACTIC ACIDS.

R.K. PODOLAK[1], J.F. ZAYAS[1], C.L. KASTNER[2], and D.Y.C. FUNG[2], Dept. Foods and Nutrition[1] and Dept. of Animal Sciences and Industry[2], Kansas State University, Manhattan, KS 66506.

KEYWORDS: fumaric, acetic, lactic acid, E. coli 0157:H7, L. monocytogenes, S. typhimurium, beef.

BACKGROUND

Although the U.S. food supply is generally considered to be safest in the world, a recent outbreak of Escherichia coli 0157: H7 and an increasing incidence of salmonellosis are signals for concern (Doyle, 1993). Achieving a product of zero tolerance of food products for food-borne pathogens even under good manufacturing practices is not possible. The development of effective chemical methods of food preservation has become critically important. The number of antimicrobial chemical preservatives approved for use is limited. Studies were reported on the effectiveness of various organic acids or chemical additives to reduce microbial surface counts (Netten and Veld, 1994; Dickson and Siragusa, 1994). Among the chemicals used in long-term preservation of food, fumaric acid possesses certain characteristics that provide distinct and unique advantages. Fumaric acid is one of the most widely distributed acids in nature and very cost effective compared to other acidulants. Fumaric acid does not absorb moisture, which degrades flavor ingredients in many dry-mix products, and does not contribute to flavor defects. Fumaric acid occurs in many foods through fermentation of glucose or molasses. For this reason, it is considered nontoxic. Fumaric acid was naturally present in fresh meat at concentrations of 0.14-0.20%. The low solubility of fumaric acid makes it less appropriate for certain food uses. To overcome this problem, fumaric acid is mixed with 0.1-0.2% diocetylo-sodium-sulfosuccinate (DOSS). The Food and Drug Administration (FDA) has approved the use of fumaric acid and its salts for human consumption at a level not to exceed the amount required to accomplish the intended effects (21 CFR 172.350). Fumaric acid is designated as Generally Recognized As Safe (GRAS) by the FDA. Information related to the effect of fumaric acid on the storage stability, shelf life, and safety of meat and meat products is very limited.

THE OBJECTIVES of this study were: 1) to assess the efficacy of fumaric acid in reducing populations of Listeria monocytogenes, E. coli 0157: H7, and Salmonella typhimurium on lean beef evaluated before (0 day) and after 5, 7, and 14 days of vacuum storage; 2) to compare the effectiveness of fumaric, lactic, and acetic acids in eliminating spoilage bacteria from vacuum-packaged beef; and 3) to evaluate effects of different sanitizing times on numbers of selected types of bacteria, remaining on meat surfaces after acid treatments.

MATERIALS AND METHODS

A model meat system was used to assess the effect of fumaric, acetic and lactic acid treatments on reductions of L. monocytogenes, E. coli 0157:H7 and S. typhimurium as compared to a control water treatments at 55°C at 0, 3, 7 and 14 days of storage at 4°C. **Bacterial cultures** were maintained in tryptic soy broth (TSB) at 4°C and propagated in TSB to provide approximately 10^9 CFU/mL. **Organic acids.** Solutions of fumaric acid at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5%; acetic acid at 1.0%; and lactic acid at 1.0% were prepared in distilled water. All solutions of acids were stored at ambient temperature until used. **Inoculation and acid treatment of meat core samples**. Samples of lean beef were obtained from semitendinosus muscles. Beef core samples (4.3 cm dia. x 0.6 cm height, surface area of 37.5 cm^2) were obtained aseptically. Meat cores were dipped in the inoculum of E. coli 0157:H7, L. monocytogenes or S. typhimurium for 10 min and air dried for 10 min. Meat cores were then sanitized by dipping in the appropriate acid solutions at 55° C for 15 or 30 sec. Control samples were dipped into distilled water at 55°C. Samples then were vacuum packaged and stored at 4°C for up to 14 days. **Bacterial enumeration**. At 3, 7, and 14 days of storage, cores of meat from all treatments were removed aseptically from the bags. Each sample was placed in a stomacher bag with 50 mL of 0.1% peptone water and stomached for 2 min before plating of the appropriate dilutions using the spiral system. The plating media were Listeria selective agar base (MOX) for L. monocytogenes, MacConkey Sorbitol Agar for enumeration of E. coli 0157: H7; and Bismuth Sulfite Agar for enumeration of S. typhimurium. Plates were incubated at 37°C for 24 hr, then counted. **Statistical analysis**. Data were analyzed by using a completely randomized design. Differences between untreated and treated samples were calculated as log reduction (log_{10} CFU/cm^2). Analysis of variance (ANOVA) was performed to obtain least significant differences (LSD). Experiments were replicated three times.

RESULTS AND DISCUSSION

The antimicrobial effects of organic acids have been related to: 1) undissociated acid molecules that interfere with cellular metabolism; 2) intracellular pH changes causing a decrease in biological activity, and 3) the inhibitory effect of low molecular weight carboxylic acids on glycolysis of cells. The average reductions in counts of L. monocytogenes on lean beef dipped for 15 sec in fumaric acid at concentrations of 1.0 and 1.5%, 1.0% acetic acid, and 1.0% lactic acid at 0, 3, 7, and 14 days of storage are presented in Fig.1. Fumaric acid treatment at concentrations of 1.0 and 1.5%, generally resulted in a significantly greater ($P<0.05$) reductions in counts of L. monocytogenes throughout the storage period when compared to acetic or lactic acid treatments. At 3 and 7 days of storage, significant differences were observed between 1.0 and 1.5% fumaric acid and the rest of the tested acids. Fumaric acid at a concentration of 1.5% was the most effective in reducing the growth of the pathogen. After 7 days of storage, fumaric acid at a concentration of 1.5% completely inhibited the growth of L. monocytogenes, resulting in a 2.44 log_{10} reduction.

Samples of lean beef treated with 1.0 and 1.5% fumaric acid for 15 sec exhibited significantly greater ($P<0.05$) reductions of E. coli 0157: H7 than those treated with acetic and lactic acids (Fig.2). No significant differences ($P>0.05$) were observed between 1.0% acetic and 1.0% lactic acid treatments. Fumaric acid at a concentrations of 1.0 and 1.5% was the most effective treatment in reducing the growth of the pathogen. The effect of this treatment was maximal at 3 days and approached a 1.96 log_{10} reduction. Reductions of E. coli populations were lower than those of L. monocytogenes or S. typhimurium. Other researchers have documented that various strains of E. coli 0157:H7 are more resistant to organic acids than the pathogens, S. typhimurium and L. monocytogenes (Greer and Dilts, 1992).

Reductions of S. typhimurium population of samples treated with tested acids for 15 sec are presented in Fig.3. At 0 day of storage, 1.5% fumaric acid promoted a significant reduction ($P<0.05$) of 1.51 log_{10} units. After 14 days of storage, reductions of S. typhimurium populations were greater with 1.0% fumaric and 1.0% acetic acid treatments than those after 7 days. Significant differences ($P<0.05$) were observed between 1.0 and 1.5% fumaric acid, except after 14 days of storage. Treatment with 1.0% lactic acid reduced the populations by 0.2 to 0.8 log_{10} units. Anderson and Marshall (1990) reported a similar reduction in the population of S. typhimurium on lean beef muscle dipped for 15 sec in 1.0% lactic acid at 55°C.

Average reductions in counts of L. monocytogenes on lean beef dipped for 30 sec in fumaric, lactic, and acetic acids are presented in

Fig. 4. Fumaric acid at concentrations of 1.5 and 1.0% completely inactivated the growth of L. monocytogenes after 7 and 14 days of storage, respectively. This effect resulted in a 3.02 $\log_{10}$ unit reduction in the pathogen. No significant differences ($P>0.05$) were observed between treatments with lactic and acetic acids after 3, 7, and 14 days of storage.

Samples of lean beef treated with 1.0 or 1.5% fumaric acid for 30 sec exhibited significantly greater ($P<0.05$) reduction of E. coli 0157: H7 than those treated with acetic or lactic acids (Fig. 5). This effect may have been related to a higher proportion of fumaric acid molecules that were undissociated and, thus, increased its effectiveness as an antimicrobial agent. Also, glycolysis is much more sensitive to dicarboxylic acids, such as fumaric acid, than monocarboxylic acids like acetic and lactic acids. Fumaric acid at 1.5% was the most effective in reducing the growth of population of E. coli 0157:H7 throughout the entire storage periods, but reached a maximum of a 1.98 $\log_{10}$ reduction before vacuum storage (0 day).

Reductions in S. typhimurium on beef samples dipped for 30 sec in fumaric, lactic, and acetic acids are shown in Fig. 6. Reductions in counts of S. typhimurium were significantly ($P<0.05$) greater with 1.5% than 1.0% fumaric acid. At 0 and 3 days of storage, the 1.5% fumaric acid treatment reduced the pathogen to levels 2.54 and 2.33 $\log_{10}$ units lower, respectively, than those of the controls. Comparisons of single acids showed that lactic and acetic acids gave much less inhibitory effect than fumaric acid. The inhibitory effect of 1.5% fumaric acid treatment was at least 5 times higher than that of acetic or lactic acid treatment.

CONCLUSIONS

Experimental data showed that bacteria were increasingly inactivated by tested acids as time of sample sanitizing was increased from 15 to 30 sec, except for E. coli 0157:H7. That pathogen was comparatively resistant to increase time of dipping samples in acid solutions. Thus, E. coli 0157: H7 should be a good test organism for future sanitizing studies. Inhibition of the pathogens was affected by the type and concentration of organic acids. A concentration of 1.5% fumaric acid applied for 15 sec caused complete inhibition of L. monocytogenes after 7 days of storage. Samples treated with 1.0% and 1.5% fumaric acid solutions for 30 sec showed 2.54 and 1.51 $\log_{10}$ reduction in S. typhimurium population compared to nontreated samples at 0 days. In reduction of E.coli 0157:H7, 1.5% fumaric acid treatment for 30 sec exerted the maximum inhibitory effect. Although all acids exhibited antimicrobial activity, fumaric acid was most effective and had the longest residual effect.

REFERENCES

ANDERSON, M. and MARSHALL, R.T. 1990. Reducing microbial population on beef tissues: concentration and temperature of lactic acid. J. Food Safety 10:181-189.

DICKSON, J.S. and SIRAGUSA, G.R. 1994. Survival of Salmonella typhimurium, Escherichia coli 0157:H7 and Listeria monocytogenes during storage on beef sanitized with organic acids. J. Food Safety 14: 313-327.

DOYLE, P.M. 1993. Food safety in the 21st century. J. Dairy Food and Environmental Sanitation. 13(7):377-440.

GREER, G.G., and DILTS, B.D. 1992. Factors affecting the susceptibility of meat borne pathogens and spoilage bacteria to organic acids. Food Res. Inter.25:355-364.

NETTEN, P. and VELD, J.H. The effect of lactic acid decontamination on the microflora on meat. 1994. J. Food Safety 4:243-257.

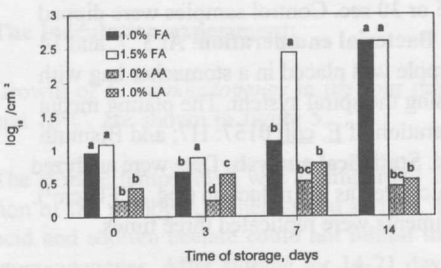

Fig. 1- Reductions in counts of E. coli 0157:H7 on lean beef muscle dipped for 15 sec in acid solutions before (0 day) and after 3, 7, and 14 days of vacuum-storage at 4 °C. Bars in Figs 1 to 6 with different letters (within day) are significantly different ($P<0.05$)

FA-fumaric acid LA-acetic acid AA-acetic acid

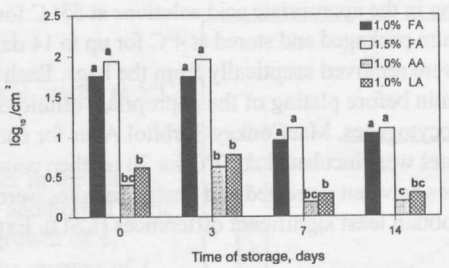

Fig. 2- Reductions in counts of E. coli 0157:H7 on lean beef muscle dipped for 15 sec in acid solutions before (0 day) and after 3, 7, and 14 days of vacuum-storage at 4 °C.

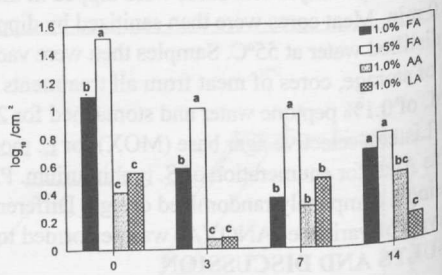

Fig. 3- Reductions in counts of S. typhimurium on lean beef muscle dipped for 15 sec in acid solutions before (0 day) and after 3, 7, and 14 days of vacuum-storage at 4 °C.

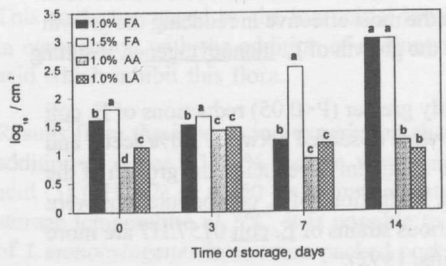

Fig. 4- Reductions in counts of E. coli 0157:H7 on lean beef muscle dipped for 30 sec in acid solutions before (0 day) and after 3, 7, and 14 days of vacuum-storage at 4 °C.

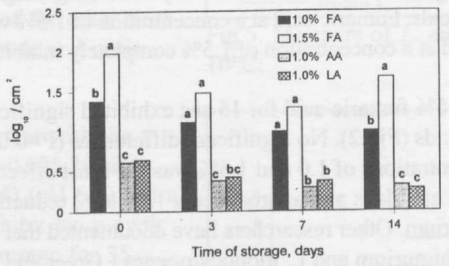

Fig. 5- Reductions in counts of E. coli 0157:H7 on lean beef muscle dipped for 30 sec in acid solutions before (0 day) and after 3, 7, and 14 days of vacuum-storage at 4 °C.

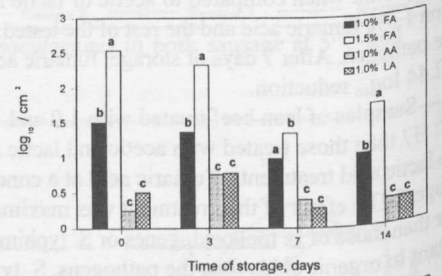

Fig. 6- Reductions in counts of S. typhimurium on lean beef muscle dipped for 30 sec in acid solutions before (0 day) and after 3, 7, and 14 days of vacuum-storage at 4 °C.