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Effect of an Acid Antimicrobial on Turkey Breast Meat *Salmonella* and Total Aerobes

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Abstract: Acid solutions have been used as antimicrobials on meat and poultry. FreshFx is a commercially-available surface treatment for the reduction of pathogens and extension of shelf life of raw poultry. Two replicate trials were conducted to determine the effect of FreshFx on numbers of total aerobes and inoculated *Salmonella* on raw turkey breast meat during 14 d of refrigerated storage. In each trial, three turkey breast meat cutlets, obtained at retail, were inoculated with nalidixic acid-resistant *Salmonella* and cut in half. One half was dipped in FreshFx for 30 s and the other half dipped in water for 30 s as a control, then all cutlet halves were refrigerated at 4°C and sampled at 0, 1, 2, 7, 10 and 14 d for total aerobic bacteria and *Salmonella*. No reduction ($P>0.05$) in numbers of total aerobes or *Salmonella* was observed due to FreshFx. Mean log cfu for total aerobes was 7.1 on d 0 and 7.8 on d 14. Mean pooled *Salmonella* log cfu were 6.9 on d 0 and 5.1 on d 14. Total aerobe counts were artificially high on d 0 to 10 due to the large numbers of *Salmonella* initially inoculated onto the samples. The persistence of *Salmonella* on samples during refrigerated storage reinforces the concept of reducing or eliminating pathogens prior to packaging and cold storage. In this experiment FreshFx did not improve shelf life as measured by reduction of total aerobes during refrigerated storage, nor reduce numbers of inoculated *Salmonella* on raw turkey breast meat.

Key words: Turkey meat, *Salmonella*, shelf life, acid antimicrobial

INTRODUCTION

The growth of the turkey industry has been facilitated by the diversification of product form, from the traditional whole carcass to many types of raw and further processed items available at retail. The importance of food safety, in regard to numbers of both spoilage and pathogenic bacteria on product, has been studied as the industry expanded. Several experiments have been conducted on the effects of basic processing steps, from slaughter to chilling, on numbers or prevalence of *Salmonella* on turkey carcasses (Walker and Ayres, 1959; Campbell *et al.*, 1984; Clouser *et al.*, 1995). The effect of extended chilling and cold storage at various temperatures and times on psychrophilic organisms and pseudomonads has been reported for turkey carcasses (Barnes and Shrimpton, 1968). A review provided evidence that additional handling and processing past the whole carcass form resulted in higher *Salmonella* prevalence (Kraft, 1971). Processed turkey meat, including parts (e.g., drumsticks), deboned thigh meat and mechanically deboned meat were inoculated with *Salmonella* and held at 10° or 1°C (Denton and Gardner, 1988). *Salmonella* survived or increased in numbers by 13 d of storage at either temperature. *Salmonella* contamination of commercial turkey meat is likely, as previous reports show that commercial turkeys at slaughter had a prevalence of 33.3%, while post-chill swabs from baseline data collected during 1997-1998 were 19.6% positive (Rostagno *et al.*, 2006; Eblen *et al.*, 2005).

Methods or applications to reduce pathogens and improve shelf life of turkey products are needed. Some recent efforts have included processing, packaging or further processing changes. Steam or boiling water improved microbiology of turkey skin but is not commercially feasible (Avens and Morton, 1999). Modified atmosphere packaging increased the shelf life of turkey cutlets but not ground turkey meat (Fraqueza and Barreto, 2009; Saucier *et al.*, 2000). Marination of turkey meat with organic acids improved the shelf life and decreased *Listeria* on subsequently produced deli loaves (Carroll *et al.*, 2007; Lloyd *et al.*, 2009). Other reports utilizing chicken meat have shown an improvement in shelf life and reduction of *Salmonella* from applications of lactic acid (Gulmez *et al.*, 2006; Zhongping *et al.*, 1998; Xiong *et al.*, 1998). FreshFx is a commercially available acid solution approved for use in poultry processing as an antimicrobial. The objective of this experiment was to determine if FreshFx applied to raw turkey breast meat would initially reduce numbers of inoculated *Salmonella*, or control total aerobes during refrigerated storage.

MATERIALS AND METHODS

In each of two replicate trials, three packages of raw turkey breast meat cutlets were purchased from a local grocery store. One cutlet was removed from each package and inoculated with one mL of a mixture of nalidixic acid-resistant *Salmonella* serotypes; 0.5 mL

which was massaged into one side of the cutlet for 10 s with a sterile spreader, held for approximately 7.5 minutes, then the other side of the cutlet was similarly treated (0.5 mL massaged into fillet for 10 s and held for 7.5 min) to allow bacterial attachment to the cutlet. Each cutlet was then cut into approximately equal halves, with one half designated as Control and the other half designated as Treated, resulting in three Control and three Treated paired halves per trial.

The *Salmonella* inoculant mixture was prepared by placing each of four cultures (*S. Enteritidis*, *S. Heidelberg*, *S. Montevideo* and *S. Typhimurium*, all resistant to nalidixic acid), into 9 mL of Brain Heart Infusion broth (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 37°C for 18 h. The inoculant mixture was prepared by adding two mL from each broth culture to a sterile tube and mixing. The inoculant was serially diluted and plated onto Brilliant Green Agar (BGA) with sulfapyridine (Neogen Corp., Lansing, MI) with 200 ppm nalidixic acid (Fisher Scientific, Fair Lawn, NJ) and incubated at 37°C for 24 h to determine the approximate number of *Salmonella* bacteria added in each trial. The numbers of *Salmonella* inoculated onto the samples in Trial 1 were 9.3 log₁₀ cfu and 8.9 log₁₀ cfu in Trial 2.

The inoculated cutlet half designated as Control was dipped for 30 s into tap water while the other paired half, designated as Treated, was dipped for 30 s into an acidic antimicrobial solution, a 0.4% solution of FreshFx (Sterifx, Shreveport, LA). The solution was prepared by adding 4 mL of FreshFx to 1 liter of tap water (from the same source as that used for Control); the measured pH of the 0.4% solution was 1.8.

After dipping, each cutlet half was allowed to drip for 5 s and then placed into a plastic bag. A rinse solution, 50 mL of 1% buffered peptone water (BPW, Oxoid Ltd., Basingstoke, Hampshire, UK) was added to each bag which was then manually shaken for 1 min. Cutlet halves were transferred into new plastic bags after rinsing. The same three Control and three Treated cutlet halves that were sampled on day 0 were also sampled (by rinsing and re-bagging as previously described) on d 1, 2, 7, 10 and 14 in each trial and all samples were held in an incubator maintained at 4°C.

After each rinse sampling, one mL from each rinsate was serially diluted in BPW and plated onto duplicate plates of BGA with sulfapyridine with 200 ppm nalidixic acid and incubated at 37°C for 24 h. Serial dilutions were also plated on duplicate Standard Methods agar (Thermo Fisher Scientific, Lenexa, KS) and incubated at 35°C for 48 hours. After incubation, colonies indicative of *Salmonella* were counted, as were the total aerobes present on Standard Methods agar.

Bacterial counts were transformed to log₁₀ cfu per mL of rinsate. Data were analyzed using the SAS ANOVA procedure to determine main effects of Trial and Treatment; as no significant interactions (P<0.05) were

detected the means were pooled across Trial (SAS, 2004). SAS paired t test procedure was used to determine significant differences in bacterial numbers between Control and Treatment samples.

RESULTS AND DISCUSSION

There were no differences (P>0.05) in numbers of total aerobic bacteria between Control and Treated turkey meat samples (Table 1). The high number of total aerobes as seen on Days 0 through 7 may have been an artifact of the high inoculation level of *Salmonella* which grew on the Standard Methods media. The psychrophilic and psychotrophic organisms more representative of spoilage bacteria were evident by Days 10 and 14, as the total aerobe count appeared to increase, while numbers of *Salmonella* did not, as shown in Fig. 1.

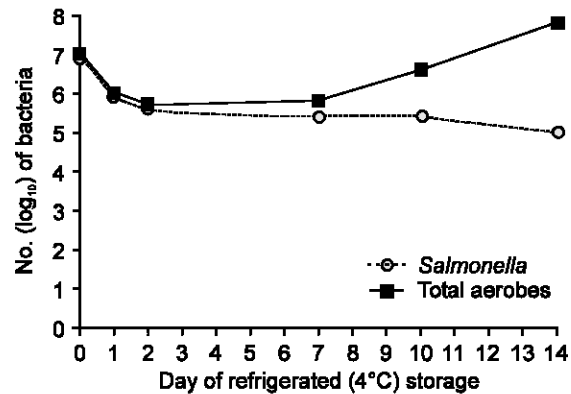


Fig. 1: Numbers (log₁₀) of total aerobes and *Salmonella* recovered from turkey breast meat cutlets inoculated with *Salmonella* after 0 to 14 days of storage at 4°C (n = 12)

There was no difference (P>0.05) in *Salmonella* counts between Control and Treated samples on any d of storage as shown in Table 1. Numbers of *Salmonella* decreased during storage but were still at log 5.1 on Day 14. The competition from increasing numbers of total aerobes may have contributed to the decrease in *Salmonella* numbers. Although numbers of *Salmonella* decreased by one log after one d of storage, an additional 13 d of refrigeration resulted in an additional reduction of only one log. This slow reduction shows the importance of reducing or eliminating *Salmonella* on turkey meat during initial processing, as it persists on the product for a number of days during refrigeration.

The growth of total aerobes and *Salmonella*, with combined Control and Treated samples over 14 d of refrigeration, are shown in Fig. 1. During sampling the bags were evaluated for odor and some off odor was detected at Day 10 when log counts were 6.6. At Day 14 an obvious spoilage odor was detected, along with

Table 1: Total aerobic bacteria and *Salmonella* counts (log₁₀ cfu/mL rinsate) of turkey breast meat cutlets dipped in water (Control) or a 0.4% vol/vol of Freshfx acid antimicrobial solution (Treated) and held at 4° C from 0 to 14 days (n = 6)

		Day of refrigerated storage (log ₁₀ cfu/mL)					
		0	1	2	7	10	14
Total aerobes	Control	6.9	5.9	5.7	5.6	6.7	7.8
	Treated	7.2	6.1	5.7	6.0	6.5	7.8
	Pooled SEM	0.09	0.06	0.03	0.10	0.15	0.10
	Probability	0.12	0.09	0.94	0.11	0.52	0.74
<i>Salmonella</i>	Control	6.7	5.8	5.5	5.3	5.3	5.0
	Treated	7.0	5.9	5.7	5.5	5.5	5.1
	Pooled SEM	0.10	0.04	0.05	0.06	0.08	0.07
	Probability	0.14	0.10	0.20	0.22	0.32	0.55

obvious slime when log counts reached 7.8. One previous report cited spoilage occurring between log 6.0 and 7.0, while another study reported spoilage occurring at log 8.0 (Thomson *et al.*, 1984; Mountney, 1966). Numbers of total aerobes in this study indicate spoilage may have occurred between log 6.6 and 7.8.

Research reports published more than 100 years ago showed that cold storage affects growth of bacteria and extends shelf life (Wiley *et al.*, 1908). In this study, although excessive numbers of mesophiles were inoculated onto the cutlets, refrigeration maintained apparent product integrity for at least seven days.

FreshFx ingredients include water, citric acid, phosphoric acid and hydrochloric acid. Previous reports have shown that organic acids applied as a marinade ingredient on turkey breast meat extended the shelf life of cooked deli loaves produced from that meat and kept in refrigerated storage. However, the exposure time of the meat to the acid was 1 h in the first report (Carroll *et al.*, 2007) and 2 h in the second report (Lloyd *et al.*, 2009) and obviously the post marinade treatment of the meat for both of those reports was much different than procedures outlined in this report. The repeated sampling of the same cutlets over several days using 1.0% BPW could have ameliorated the effect of FreshFx due to the buffering capacity of the rinse. However, the buffering capacity of the large surface area of the breast meat cutlet and leakage of sarcoplasmic proteins and fluid would likely exceed that of the BPW. A prior report showed that postrigor broiler breast had higher buffering capacity than other cuts of beef, pork, or leg or thigh broiler muscles (Puolanne and Kivikari, 2000). Also, the buffering capacity of BPW from Oxoid used in the present study has been shown to be lower than another brand of BPW (Baylis *et al.*, 2000). Potentially these larger portions of breast meat cutlets may not show a reduction in *Salmonella* numbers, since the application time was only 30 s and the buffering capacity of the meat itself may quickly negate the pH decrease typically provided by the FreshFx dip, more so than the rinse of BPW. For example, a single turkey breast cutlet was immersed in 50mL of prepared FreshFx solution for 30 s and the pH of the solution increased from 1.9 to 2.8.

FreshFx as applied in this experiment had no effect on reducing *Salmonella* contamination or extending shelf life. Although experimental conditions (high numbers of bacteria inoculated onto meat samples with ample attachment time) represented a considerable challenge, some reduction of *Salmonella* was expected. The high inoculation level of *Salmonella* and its subsequent survival, artificially inflated the total aerobic bacteria count in the first few days of the experiment. Further research is needed to determine effective methods of reducing or eliminating *Salmonella* on raw turkey meat.

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