Fate of Listeria monocytogenes and Salmonella in Flaked Unwashed and Color-Modified Turkey

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SUMMARY:

The growth/survival of L. monocytogenes and Salmonella spp. was evaluated in two thermally-processed turkey raw materials. Boneless, skinless thighs were processed to produce flaked unwashed turkey (FUT), and a portion was washed with 0.03 M sodium phosphate buffers to prepare color-modified turkey (CMT). Raw materials were cooked and inoculated with L. monocytogenes and Salmonella to simulate post-processing contamination conditions. Inoculated samples held at 4 and 20°C were analyzed at selected time intervals. Salmonella numbers declined in cooked samples stored at 4°C for 21 days, but increased approximately 6 logs by 2 days in FUT and CMT held at 20°C. L. monocytogenes increased approximately 5 logs in samples held 14 days at 4°C. By 2 days at 20°C, L. monocytogenes increased more than 5 logs in both materials. Generally, the growth/survival of these pathogens was not found to differ between FUT and CMT.

INTRODUCTION:

Flaking and color modification procedures can increase the utilization of poultry dark muscle tissue in further processed products. Flaking is a particle reduction method that has been shown to enhance texture, flavor, tenderness, and binding properties of meat. It may be employed as an alternative technique to chopping, grinding, slicing, and mechanical deboning. Color modification (washing) can be used to lighten the color of dark poultry tissue by removing myoglobin and hemoglobin. Color modification produces a raw material that resembles breast meat in appearance and is lower in fat content. The combination of flaking and color modification methodologies yields a raw material that may be potentially incorporated into value-added turkey products.

Functional (Elkhalifa et al., 1988), compositional (Elkhalifa et al., 1988; Phelps et al., 1990), nutritional (Phelps et al., 1990), and microbial quality characteristics (Pruett et al., unpublished data) have been described for flaked unwashed turkey (FUT) and color-modified turkey (CMT). The increased tissue surface area created by flaking and the changes in tissue chemistry produced by washing appear to alter thigh meat relative to its potential as a microbial growth medium. Therefore, information is needed regarding the activities of foodborne pathogens in these raw materials, especially when inherent microbial flora levels are reduced. The objective of this research was to determine the fate of L. monocytogenes and Salmonella in cooked FUT and CMT. Furthermore, a comparison of the numbers of pathogens between FUT and CMT was conducted throughout time intervals of each study. This was performed to indicate whether washing of thigh tissue changed the properties of this substrate relative to the growth/survival abilities of these pathogens.

MATERIALS AND METHODS:

Two-strain composites of both Salmonella and L. monocytogenes were used for inoculation. Strains employed in this study were nalidixic acid-resistant mutants of Salmonella typhimurium and Salmonella enteritidis, L. monocytogenes strain Scott A, and a L. monocytogenes meat isolate.

In preparation for inoculation, the two strains of Salmonella were inoculated into individual tubes of trypticase soy broth (TSB; Difco) and incubated for 24 hr at 37°C. From trypticase soy broth cultures of each Salmonella strain, 8 mL of broth were transferred to a 15 mL centrifuge tube, and cells were harvested according to methods suggested by Schnaitman (1981). Inoculum solutions were spectrophotometrically adjusted and pooled to obtain a 1:1 strain ratio. Inoculum preparation for L. monocytogenes strains that were cultured in TSB with 0.6% yeast extract and incubated for 24 hr at 37°C was similar to that described for Salmonella.

Thigh handling, flaking, and color modification was accomplished using the method outlined by Elkhalifa et al. (1988) and modified by Phelps et al. (1990). Portions of CMT and FUT (200g) were aseptically weighed into 250 mL sterile metal beakers, covered with aluminum foil, and placed in a 93.3°C water bath. The tissues were cooked for approximately 25 min or until an internal temperature of 71.1°C was achieved. After cooking, CMT and FUT portions were allowed to cool and then were aseptically divided into 11 g quantities. Eleven-gram samples in sterile petri dishes were spiked with 0.11 mL of inoculum from either of the two-strain composites formulated for each pathogen. The resulting inoculum level was approximately 3.5 log CFU/g for Salmonella-inoculated samples and 3.0 log CFU/g for meat spiked with L. monocytogenes. To determine the extent of the initial competing microbial flora, aerobic bacterial numbers were determined in cooked tissue portions (11 g) on the day of heat processing.

Following inoculation, the meat samples were transferred from petri dishes to sterile Stomacher bags and hand kneaded for 15 sec to disperse the inoculum. Samples were incubated at 4°C (typical refrigeration temperature) or 20°C (abuse temperature). Uninoculated controls of CMT and FUT were stored with L. monocytogenes-inoculated samples to ensure that L. monocytogenes did not survive cooking. This was not necessary for the inoculation studies with Salmonella since only nalidixic acid mutants of this pathogen should be recovered on the selective medium.

Inoculated tissues containing Salmonella strains and incubated at 20°C were analyzed at 0, 1, 2, 3, 4, and 5 days. Samples inoculated with L. monocytogenes and held at 20°C were tested at 0, 12, 24, 36, and 48 hr. Portions spiked with either Salmonella or L. monocytogenes and stored at 4°C were evaluated at days 0, 1, 4, 7, 14, and 21.

At selected time intervals, inoculated samples were homogenized with 99 mL of 0.1% peptone water to obtain 1:10 dilutions. Salmonella numbers were determined by spread-plating 0.1 mL portions onto Brilliant Green (Difco; Detroit, Mich.) agar plates supplemented with 100 ppm nalidixic acid (Sigma; St. Louis, Mo). Plates for the recovery of salmonellae were incubated at 37°C for 24 hr. L. monocytogenes counts were estimated by spread-plating 0.1 mL portions onto Modified Vogel Johnson (Difco) agar plates and incubating at 37°C for 48 hr. Isolates of pathogens from selective media were confirmed using appropriate biochemical and serological tests.

Moisture determinations were made on cooked CMT and FUT samples following AOAC (1984) methods. pH values were obtained from the 1:10 dilutions used for plating at sampling intervals. This was done to monitor the effect that pathogenic bacterial growth had on muscle pH. Microbial counts were converted to logarithms and analyzed by the General Linear Models procedure of the Statistical Analysis System (SAS, 1984) using a split-plot design of ANOVA.

RESULTS AND DISCUSSION:

The mean moisture content for cooked FUT was 70.1% and that for cooked CMT was 77.3%. Since the moisture content of cooked tissues was at or above that of fresh poultry, which has a water activity of .98 to .99 (Bryan, 1980), it was not considered a factor limiting microbial proliferation in these tissues. pH values of inoculated FUT and CMT samples remained around neutrality. Therefore, the change in muscle pH was not regarded as a parameter substantially affecting microbial growth.

Aerobic counts were <2.0 log CFU/g in cooked FUT and CMT following thermal processing. Initial inoculum levels of pathogens were higher than those of the inherent microbial flora. The reduction in the initial microbial flora could have enhanced the competitive ability of Salmonella and L. monocytogenes in cooked tissues.

Salmonella counts declined gradually in inoculated FUT and CMT held at 4°C (Table 1). Decreases ($p<0.05$) in salmonellae were seen in FUT by day 21 and in CMT by day 14. Concentrations in both meat types were

similar until after day 7 when counts in CMT began to fall more rapidly than those in FUT (Table 1). Nevertheless, there was no difference ($p>0.05$) in Salmonella numbers between the two refrigerated tissues at any sampling interval. The combination of refrigerated storage and color modification may have had a

Table 1. Salmonella numbers estimated in inoculated FUT and CMT stored at 4 and 20°C.

Time (days)	Log CFU/g ± S.D. FUT	CMT
	4°C	
0	3.57 ± .12[a]	3.56 ± .10[a]
1	3.53 ± .17[ab]	3.51 + .14[a]
4	3.53 ± .12[ab]	3.50 ± .07[a]
7	3.40 ± .07[ab]	3.45 ± .10[a]
14	3.37 ± .12[ab]	3.21 ± .34[ab]
21	3.31 ± .35[b]	3.02 ± .28[b]
	20°C	
0	3.57 ± .12[a]	3.56 ± .10[a]
1	7.71 ± .23[b]	7.65 ± .46[b]
2	9.62 ± .28[c]	9.26 ± .17[c]
3	9.93 ± .07[cd]	9.78 ± .08[d]
4	9.89 ± .14[cd]	9.71 ± .19[d]
5	9.96 ± .06[d]	9.83 ± .07[d]

[a,b,c,d]Values in the same column with identical letters are not different ($p>0.05$).

Table 2. L. monocytogenes numbers estimated in inoculated FUT and CMT stored at 4 and 20°C.

Time (days)	Log CFU/g ± S.D. FUT	CMT
	4°C	
0	3.00 ± .06[aA]	3.06 ± .13[aA]
1	3.11 ± .03[aA]	3.14 ± .11[aA]
4	3.97 ± .12[bA]	4.13 ± .19[bA]
7	5.01 ± .67[cA]	5.73 ± .77[cB]
14	8.19 ± .29[dA]	7.94 ± .41[dA]
21	8.68 ± .08[eA]	8.27 ± .28[eA]
	20°C	
0	3.06 ± .13[a]	3.01 ± .05[a]
12	4.10 ± .05[b]	4.37 ± .10[b]
24	6.97 ± .73[c]	7.06 ± .44[c]
36	8.14 ± .14[d]	7.79 ± .10[d]
48	8.68 ± .47[e]	8.29 ± .90[e]

[a,b,c,d,e]Values in the same column with identical letters are not different ($p>0.05$).

[A,B]Values in the same row with identical letters are not different ($p>0.05$).

more adverse effect on the survival of salmonellae in tissues as opposed to cold storage at 4°C alone. Storage beyond 21 days may have yielded a difference ($p<0.05$) between CMT and FUT Salmonella numbers.

A possible underestimation of Salmonella numbers in tissues held at 4°C may have occurred as a result of cell injury. Direct plating on the Salmonella-selective medium (Brilliant Green agar with nalidixic acid) could have prevented the recovery of cells potentially injured by refrigeration.

Both tissues at 20°C were excellent growth media for salmonellae. Numbers in FUT and CMT increased rapidly (approximately 6 logs) from 0 to 2 days (Table 1). Counts peaked at approximately 10 log CFU/g by day 3 and remained at that level until day 5. Exhaustion of nutrients and/or the buildup of waste products may explain the leveling of counts after 3 days of storage. After 1 day at 20°C, counts tended to be slightly lower in CMT than in FUT throughout the storage period. However, there was no difference ($p>0.05$) in Salmonella numbers between FUT and CMT at any sampling interval.

Our work is supported by previous researchers who have demonstrated the effect of temperature on proliferation of salmonellae in poultry. Morad et al. (1982) reported that counts of Salmonella typhimurium in inoculated cooked turkey held at 4°C remained near 5.0 log CFU/g throughout 8 days of storage. After 6 days storage at 15°C, Salmonella counts increased approximately 5 logs in vacuum-packaged turkey slices and breasts (To and Robach, 1980).

Increases ($p<0.05$) in L. monocytogenes occurred by day 4 in FUT and CMT samples held at 4°C (Table 2). Concentrations of listeriae increased over 5 logs in both tissues by day 21. On day 7, numbers in CMT were higher ($p<0.05$) than those in FUT. At other sampling intervals, no differences ($p>0.05$) in L. monocytogenes counts occurred between the two tissues. Nevertheless, after 7 days, numbers in FUT tended to be higher than those in CMT. Multiplication of L. monocytogenes at 4°C was not surprising since prior research has shown this organism to grow at a minimum temperature of 1°C (Seelinger and Jones, 1986).

L. monocytogenes proliferated at virtually the same rate in both tissues stored at 20°C, with increases ($p<0.05$) in numbers occurring by 12 hr (Table 2). Levels in both tissues increased to above 8.0 log CFU/g by 48 hr from an initial inoculum of around 3.0 log CFU/g. A near 4-log increase was seen from 0 to 24 hr in both tissues, before growth slowed from 24 to 48 hr. Counts of listeriae did not differ between FUT and CMT ($p > 0.05$) at any sampling time.

Carpenter and Harrison (1989) and Harrison and Carpenter (1989) demonstrated that L. monocytogenes populations that survived cooking in chicken breasts could proliferate at a typical refrigeration temperature (4°C) and a slightly abusive temperature (10°C). However, a slower growth rate would be expected for cells injured by thermal processing. As a result, comparing the growth characteristics of L. monocytogenes in the above studies (pre-processing contamination) to those of our study (post-processing contamination) would be difficult. Nevertheless, L. monocytogenes was shown to grow in cooked poultry at 4°C in the current and cited studies.

CONCLUSIONS:

In all studies, pH and moisture were not considered to be major factors influencing microbial growth. Salmonella concentrations declined gradually in cooked raw materials held at 4°C. Salmonella proliferated essentially at the same rate in inoculated, cooked samples of FUT and CMT held at 20°C. Counts in both tissues increased approximately 6 logs after 2 days of storage.

L. monocytogenes grew readily in cooked tissues held at 4 and 20°C. Counts increased over 5 logs in samples held at 4°C for 21 days. By 48 hrs at 20°C, numbers of listeriae in CMT and FUT increased more than 5 logs. A 4 log increase was seen from 0 to 24 hrs in both tissues followed by a reduced growth rate. Except for one sampling interval, (day 7 tissues inoculated with L. monocytogenes), counts of pathogens did not differ ($p > 0.05$) between FUT and CMT.

It was concluded that neither FUT nor CMT was a unique environment for the growth/survival of either Salmonella or L. monocytogenes. Also, it was found that washing thigh tissue generally does not ($p>0.05$) make this material a more or less favorable growth medium for either of these pathogens.

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