

## Reduction of Bacterial Populations on Beef Heads and Tongues

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### SUMMARY

This study was conducted to determine the effectiveness of the CHAD washing and organic acid spray assemblies for beef heads and tongues in reducing bacterial contamination. Excision samples were collected, and samples were cultured and analyzed for Aerobic Plate Counts (APC) and generic *Escherichia coli* Counts (ECC). The wash cabinet assembly was effective in reducing APC and ECC on tongues but not on cheek meat. Including a lactic acid spray (2% solution) as the final step in the head and tongue wash cabinet assembly did not result in biologically significant reductions in bacterial contamination.

**Key Words:** beef variety meats, decontamination, spray-washing, lactic acid.

### INTRODUCTION

While reducing the bacterial contamination on the surface of beef carcasses has been a high priority for several years, the use of decontamination techniques in the production of beef variety meats is still largely in the development stage. Delmore *et al.* (2000) studied microbiological interventions for beef variety meats and determined that organic acid immersion and spraying (2% solution) were among the most effective treatments for reducing Aerobic Plate Counts (APC), Total Coliform Counts (TCC) and *Escherichia coli* Counts (ECC) on cheek meat, large intestine, lips, liver,

oxtail and tongue. Lactic acid (2%) spraying reduced APC on cheek meat, lips and tongues by 1.1, 1.5 and 1.7 log<sub>10</sub> CFU/gram, respectively (Delmore *et al.*, 2000).

The CHAD Company (Olathe, KS) has now developed cabinets that, (1) wash beef heads and tongues and, (2) spray beef heads and tongues with a solution containing organic acid. To determine the effectiveness of these procedures, a field study was conducted to quantify reductions in enumerated bacteria counts on beef heads and tongues.

### MATERIALS AND METHODS

To determine the effectiveness of the CHAD washing and organic acid assemblies for beef heads/tongues using a lactic acid (2%) solution, samples were obtained on two days in a commercial beef packing plant (Lexington, NE). On the first day of the study, the organic acid spraying portion of the head and tongue wash assembly was turned off (Day 1), and on the second day, the organic acid spraying portion of the head and tongue wash assembly operated normally (Day 2). On both Day 1 and Day 2, the warm water spray-wash portion of the head and tongue wash assembly was in operation. Temperature and pressure specifications for the head and tongue wash cabinet assembly are described in Table 1.

Test heads/tongues were selected and identified with rail tags as they were hung on the head/tongue chain immediately after being removed from the carcass. Excision samples of cheek meat and tongue were obtained for each test head at three points along the head/tongue chain. Site 1 was located in the area where heads, with the tongue still attached, were hung on the head/tongue rail chain, immediately prior to the first/second automatic head and tongue wash assembly. Tongues were removed from the heads at a break between the first and second head/tongue wash assemblies. Site 2 samples were

collected immediately after the heads and tongues emerged from the second head/tongue wash assembly and prior to USDA inspection. Site 3 samples were collected in the head work-up area immediately following the removal of the heads and tongues from the head/tongue rail chain.

At Site 4, in the cheek-meat boxing area, random samples of cheek meat were collected following their removal from the heads, and just prior to boxing.

Sample sizes were as follows: (a) cheek meat from the head (a piece 13 cm x 1 cm); (b) tongue from the whole tongue (a piece 13 cm x 1 cm from the upper side of the whole tongue); (c) lips in the head work-up area (a piece 13 cm x 1 cm); (d) cheek meat in the boxing area (a piece 13 cm x 1 cm).

Samples were collected using aseptic techniques. Researchers wearing sterile single-use latex gloves used sterile single-use scalpel blades and forceps (sanitized via immersion in 82° C water) to remove a strip of tissue approximately 13cm x 1cm in area. The tissue was then placed into sterile Whirl-Pak® (Nasco, Ft. Atkinson, WI) bags, cooled, and packaged for shipment. Samples were shipped via overnight carrier to Food Safety Net Services Laboratory in San Antonio, TX. During shipment, temperature was monitored using a TempTale (Sensitech Inc., Beverly, MA) temperature monitor so that temperature abused samples could be removed from the experiment. Samples were delivered on the days following collection at 8:50 am and 9:01 am, for Day 1 and Day 2 samples, respectively. All samples arrived at the laboratory at < 4° C and showed no evidence of temperature abuse.

### MICROBIOLOGICAL ANALYSIS

At the laboratory, sample weights were recorded for use in calculation of log<sub>10</sub> bacterial counts per gram of

product. A surface rinsing procedure was employed to dislodge bacteria from the irregular surface of each sample. A 100 ml quantity of Butterfield's phosphate buffer (Difco Laboratories, Detroit, MI) was added to each sample bag. The sample bags were sealed and the samples were rinsed using a rocking motion for 30 shakes (approximately one minute).

Appropriate decimal dilutions were plated on Petrifilm™ APC plates (3M™ Microbiology Products, St. Paul, MN) for determination of Aerobic Plate Counts (APC). Plates were incubated for 48 hr at 35° C, and the colonies were counted. One ml portions of appropriate dilutions also were deposited on Petrifilm™ *Escherichia coli* count plates (3M™ Microbiology Products, St. Paul, MN), incubated for 48 hr at 35° C and counted to obtain generic *E. coli* Counts (ECC). The threshold limit for detection of *E. coli* was ten (10) Colony Forming Units (CFU). In order to keep the analysis as conservative as possible, ECC for samples below this threshold for detection were estimated to be ten (10) CFU/ml of plated solution. For these samples, CFU/g was calculated using the following formula: (100 ml diluent / sample weight in grams) \* 10 CFU/ml.

All bacterial counts were reported as log CFU/g of wet tissue.

### STATISTICAL ANALYSIS

Day 1 (lactic acid component of head/tongue wash cabinet assembly turned OFF) served as the control, and thus Day 2 (lactic acid component of head/tongue wash cabinet assembly turned ON) results were evaluated in comparison to the results from Day 1. Initial, pre-intervention APC and ECC for tongue samples (adjusted for sample weight) at Site 1 differed ( $P < 0.05$ ) by day. Additionally, ECC for cheek meat differed by day ( $P < 0.1$ ). Thus, Site 2 and Site 3 data for both cheek meat and tongue samples were analyzed using analysis of covariance

(ANCOVA) procedures of SAS® with covariates including Site 1 APC (or Site 1 ECC) and day \* Site 1 APC (or Site 1 ECC) interactions.

### RESULTS AND DISCUSSION

The lowest mean APC ( $< 4$  log CFU/g) on each day for cheek meat occurred at Site 1 (Table 2), prior to any decontamination treatment. These low levels were not surprising since the cheek surface should be sterile until the hide is removed just minutes before the head reaches Site 1. Aerobic Plate Counts for cheek meat tended to increase as the heads moved from Site 1 to Site 3 (Table 2); however, variability tended to decrease.

At Site 3, APC for cheek meat were lower ( $P < 0.05$ ) on Day 2 (lactic acid rinse operating normally) than on Day 1 (lactic acid turned OFF); however, this difference was not biologically significant ( $< .5$  log CFU/g). Furthermore, researchers did not detect a difference in APC ( $P > 0.05$ ) between Day 1 and Day 2 for cheek meat at Site 4, the area where cheek meat was packaged (Table 2).

The highest mean APC ( $> 6$  log CFU/g) on each day for tongues occurred at Site 1, before the heads and tongues passed through the head/tongue wash cabinet assembly. As expected, visible ingesta were present on many of the tongues at this site. Samples from Site 2 indicated that the wash cabinet assembly effectively reduced mean APC by slightly more than 1 log CFU/g (1.26 and 1.02 log, respectively for Day 1 and Day 2). A further decrease in mean APC of approximately 1 log CFU/g (0.97 and 1.27 log, respectively for Day 1 and Day 2) was detected as tongues progressed from Site 2 to Site 3 (Table 2). No differences ( $P > 0.05$ ) in mean Aerobic Plate Counts for tongue samples were detected between treatments (Day 1 and Day 2) at Site 2 or Site 3. The reduction in APC occurring between Site 1 and Site 3

was not significantly different between Day 1 and Day 2 ( $P > 0.05$ ).

Between Site 2 and Site 3, the head/tongue chain transported the heads and tongues through USDA

inspection, above the carcass evisceration area, and finally to the head work-up area. During this phase between Site 1 and Site 3, mean APC for cheek meat increased by over 0.5 log CFU/g (0.58 and 0.68 log) on both Day 1 and Day 2, while mean APC for tongues decreased by more than 2 log CFU/g (2.23 and 2.29 log, respectively). This resulted in final Aerobic Plate Counts for both types of tissue equalizing at between 4 and 4.5 log CFU/g. These changes in APC occurring between Site 1 and Site 3 indicated that some contamination (via cross-contamination or airborne contamination) occurred. This plant has separated the hide-on portion of the slaughter floor from the hide-off area, and this strategy should reduce the potential for airborne bacteria to contaminate the heads and tongues. Cross-contamination may be occurring in the wash cabinet assembly. The wash cabinet assembly (with or without the lactic acid spray component) effectively reduced the APC on beef tongue by slightly more than 2 log CFU/g, however the data suggest that some of the contaminants being washed off of the tongues may be transferred to the heads.

Generic *E. coli* Counts were generally quite low for both cheek meat and tongue samples (Table 3). Except for tongues sampled at Site 1, half or less of the samples had detectable levels of *E. coli*. The head/tongue wash cabinet assembly was effective in reducing *E. coli* levels on tongues as the number of samples testing positive for *E. coli* (detection limit  $\leq 10$  colony forming units per 1ml of plated solution), was dramatically reduced from Site 1 to Site 2. As shown in Table 3, statistically significant differences ( $P < 0.05$ ) were detected between Day 1 and Day 2 for ECC for both cheek meat and tongue at Sites 2 and 3.

However, these differences are less than 0.5 log CFU/g and thus are not biologically significant. The data therefore show no beneficial effects on ECC due to adding the lactic acid rinse component to the head and tongue wash cabinet assembly.

As previous research has shown, it is often difficult to document consistent effects of decontamination strategies in reducing microbiological contamination on beef variety meats exposed to the environment of a commercial packing plant. Such difficulty was not unexpected and may occur because of several factors: (1) The bacterial contamination of beef variety meats is highly variable (not normally distributed) and often low (log CFU/g of 1 or 2). With low initial microbiological counts, application of a single treatment may only provide a small improvement in the microbial quality of those products because there is so little bacterial contamination to remove. (2) Commercial beef processing facilities may have environmental circumstances or situations, following treatment application, which may negatively affect the ability of a decontamination treatment to produce a significant and consistent reduction in bacterial counts. Such circumstances/ situations may include airflow, overall sanitation, employee handling practices, harvest facility design, chilling methods, and processing floor temperatures (Delmore *et al.*, 1999). Decisions on which decontamination strategies to implement should be made within existing prerequisite GMP and SOP programs, and within the framework of the Hazard Analysis and Critical Control Points system.

Any decontamination strategy is only as strong as the weakest link in the system. During this study, the researchers were somewhat concerned that the head work-up area may be a potential weak link in the microbiological control system. Although hot water sanitizers were available, workers rarely used them to

clean their knives and equipment. Also, the work areas did not appear to be cleaned regularly throughout the day, creating opportunity for cross-contamination.

### CONCLUSIONS

The CHAD washing and organic acid spray assemblies for beef heads and tongues significantly improved the microbiological quality of beef tongues, which had relatively high initial Aerobic Plate Counts. However, APC for cheek meat increased as the heads moved through the processing system. Spray washing may be causing some degree of cross-contamination by transferring contaminants from tongues to the heads while these items are traveling through the head and tongue wash cabinet.

Previous studies have shown a lactic acid spray to be an effective component of a multiple hurdle decontamination strategy for beef carcasses (Graves-Delmore *et al.*, 1998; Castillo *et al.*, 1999). Furthermore, Delmore *et al.* (2000) showed that lactic acid spraying (2%) effected reductions in APC on beef cheek meat, lips, and tongue. Data from this study indicated that adding a lactic acid spray (2% solution) component to the head/tongue wash assembly resulted in no additional reduction in bacterial contamination on beef heads and tongues.

### LITERATURE CITED

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**Table 1. Temperature and pressure measurements for head/tongue wash cabinet assembly.**

Measurement:	Day 1	Day 2
Estimated water PSI (at cabinet)	250	250
Water PSI (gauge on wall)	290	290
Water temperature	29.4° C	29.4° C
Head chain speed	4' in 9 sec.	4' in 9 sec.
Lactic acid solution concentration	-	1.9 - 2.0%
Lactic acid solution temperature (at tank)	-	47.8° C
Lactic acid PSI (gauge above cabinet)	-	20

**Table 2. Effect of using a lactic acid rinse component with the head/tongue wash cabinet assembly for reducing Aerobic Plate Count (APC) on beef cheek meat, tongues, and lips.**

Variety meat	Day 1			Day 2		
	x <sup>a</sup>	se <sup>b</sup>	n <sup>c</sup>	x <sup>a</sup>	se <sup>b</sup>	n <sup>c</sup>
Cheek meat						
Site 1 <sup>d</sup>	3.84 <sup>x</sup>	0.25	18	3.40 <sup>x</sup>	0.25	18
Site 2 <sup>e</sup>	3.85 <sup>x</sup>	0.14	18	3.71 <sup>x</sup>	0.14	18
Site 3 <sup>e</sup>	4.42 <sup>x</sup>	0.08	18	4.08 <sup>y</sup>	0.08	18
Site 4 <sup>d</sup>	4.23 <sup>x</sup>	0.16	10	4.25 <sup>x</sup>	0.16	10
Tongue						
Site 1 <sup>d</sup>	6.73 <sup>x</sup>	0.15	18	6.29 <sup>y</sup>	0.15	18
Site 2 <sup>e</sup>	5.47 <sup>x</sup>	0.20	18	5.27 <sup>x</sup>	0.20	18
Site 3 <sup>e</sup>	4.50 <sup>x</sup>	0.28	18	4.00 <sup>x</sup>	0.28	18

<sup>a</sup>Least squares means of Aerobic Plate Counts (log<sub>10</sub> CFU/g).<sup>b</sup>Standard error of the mean.<sup>c</sup>Number of samples per treatment.<sup>d</sup>Arithmetic means.<sup>e</sup>Least squares means adjusted for Site 1 APC, and day \* Site 1 APC interaction.<sup>x,y</sup>Within a row, means without a common superscript letter differ (P < 0.05).**Table 3. Effect of using a lactic acid rinse component with the head/tongue wash cabinet assembly for reducing generic *Escherichia coli* Count (ECC) on beef cheek meat, tongues, and lips.**

Variety meat	Day 1				Day 2			
	x <sup>a</sup>	se <sup>b</sup>	n <sup>c</sup>	n <sup>*d</sup>	x <sup>a</sup>	se <sup>b</sup>	n <sup>c</sup>	n <sup>*d</sup>
Cheek meat								
Site 1 <sup>e</sup>	1.94 <sup>x</sup>	0.05	18	16	1.82 <sup>x</sup>	0.05	18	14
Site 2 <sup>f</sup>	2.16 <sup>x</sup>	0.06	18	17	1.90 <sup>y</sup>	0.06	18	15
Site 3 <sup>f</sup>	1.98 <sup>x</sup>	0.03	18	17	1.86 <sup>y</sup>	0.02	18	17
Site 4 <sup>e</sup>	1.69 <sup>x</sup>	0.11	10	5	1.61 <sup>x</sup>	0.11	10	7
Tongue								
Site 1 <sup>e</sup>	2.78 <sup>x</sup>	0.11	18	1	2.14 <sup>y</sup>	0.11	18	5
Site 2 <sup>f</sup>	2.15 <sup>x</sup>	0.04	18	18	1.83 <sup>y</sup>	0.04	18	18
Site 3 <sup>e</sup>	1.86 <sup>x</sup>	0.03	18	17	1.72 <sup>y</sup>	0.03	18	17

<sup>a</sup>Least squares means of *Escherichia coli* Counts (log<sub>10</sub> CFU/g).<sup>b</sup>Standard error of the mean.<sup>c</sup>Number of samples per treatment.<sup>d</sup>Number of samples in which *Escherichia coli* Counts (ECC) were not detected at a detection limit of ≤ 10 colonies/1 ml plated solution.<sup>e</sup>Arithmetic means.<sup>f</sup>Least squares means adjusted for day, Site 1 ECC, and day \* Site 1 ECC interaction.<sup>x,y</sup>Within a row, means without a common superscript letter differ (P < 0.05).