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Comparing the Recovery of Indicator Microorganisms from Beef Trimmings Using Swabbing, Rinsing, and Grinding Methodologies

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Abstract: Different bacterial sampling methods that are destructive or nondestructive in nature have been developed to evaluate the microbial quality of meat and determine if it fulfills criteria for distribution to the public. The purpose of this study was to evaluate the effectiveness of swabbing, rinsing, and grinding as sampling methodologies for recovery of indicator microorganisms on beef trimmings. A total of fifteen samples ($n = 15$) of beef trimmings were collected using the N60 trim sampling technique adopted by the USDA Food Safety and Inspection Service. Each of the 15 samples were divided into 3 parts, with each part assigned to a methodology (swabbing, rinsing, or grinding), and subjected to multiple sequential sampling for a total of three times. A comparison was made between the first sampling and the total bacteria recovered by each methodology. For total aerobic counts (TAC) obtained by the rinse and the grinding methodology, the first sampling as well as the total resulted in aerobic bacteria counts that were not significantly different ($P > 0.05$); however, swabbing yielded counts that were significantly lower ($P < 0.05$) than both rinsing and grinding. For total coliforms, rinsing was not significantly different ($P < 0.05$) from either swabbing or grinding, yet swabbing recovered the least number of bacteria. Within each sampling methodology, the decline in aerobic bacteria counts due to multiple sequential samplings was calculated. Rinsing and grinding resulted in significantly lower counts ($P < 0.05$) after the initial samplings, whereas swabbing did not ($P > 0.05$). Linear models showed strong relationships, with a coefficient of determination (r^2) of 0.81 (swabbing vs. grinding), 0.67 (swabbing vs. rinsing), and 0.70 (rinsing vs. grinding). Although the sampling methodologies investigated are reliable to determine true bacterial counts of tested samples, consistency and uniformity of sampling is required for the proper interpretation of the acquired results.

Introduction

Spoilage and pathogenic microorganisms can be introduced into red meat from improper carcass dressing procedures that may lead to the transference of fecal materials, intestinal content, and cross-contamination

from hides. In addition, cross-contamination can also occur from processing equipment, human contact, the structural components of the facility, and from carcass-to-carcass transfer (Capita et al., 2004; Huffman, 2002). The hygienic condition of a meat processing facility can be improved by developing and implementing a Hazard Analysis Critical Control Point (HACCP) plan with sound validation methods. Therefore, microbiological analysis of carcasses becomes important for validation of a HACCP plan (Capita et al., 2004).

Evaluating the hygienic performance of carcasses harvest processes differs among regulatory agencies throughout the world. The European Union Decision *Escherichia coli*/471/2001 is a legislation that requires

the implementation of HACCP on meat and poultry slaughter and dressing operations (Commission of the European Communities, 2001), stating that hygienic performance shall be evaluated by enumeration of indicator microorganisms (aerobic count and *Enterobacteriaceae* counts) on the carcass at the end of the process (Capita et al., 2004). The standards used to assess the hygienic performance should be completely based on the results acquired, in this case, from destructive sampling methods (Capita et al., 2004) such as excision techniques. In contrast, the USDA Food Safety and Inspection Service (FSIS) published its final rule in 1996 for pathogen reduction; HACCP Systems(FSIS, 1996). The final rule requires the implementation of HACCP plans as a part of a company's control process and is mandatory in all inspected meat and poultry facilities in the United States (FSIS, 1996). However, unlike the European Union standard, it requires a nondestructive method, a swab sample in this case, to be taken from carcass surfaces for the enumeration of microbial counts.

Many sampling methods can be used to evaluate wholesomeness. These methods can be categorized into 2 groups: destructive and nondestructive (Lee and Fung, 1986), with each method having its own set of pros and cons. Excision, being destructive, provides more reliable results due to the efficient recovery of strongly attached bacteria. However, only a limited area can be sampled, processing is time-consuming, and skilled workers are required to properly collect the sample (Capita et al., 2004). In contrast, nondestructive sampling methods, such as swabbing, causes minor or no damage to the surface being tested, allows for larger areas to be sampled, and bacteria with uneven distribution and low colony-forming units can be recovered. The disadvantage of the latter method is that the results vary because only loosely attached bacteria are recovered (Capita et al., 2004).

Previous studies have been conducted to evaluate the efficiency of different sampling methods and enumeration of microorganisms. For instance, Anderson *et al* (1987) evaluated the effectiveness of swabbing and excision sampling method for the recovery of aerobic bacteria, *Enterobactericaceae*, and *E. coli*. The study indicated that there was a higher recovery via excision than swabbing, where swabbing recovered an average of 6 to 16% of aerobic counts compared to the amounts recovered by excision. Similarly, the recovery of Generic *Escherichia coli* ranged from 5 to 12% of the amount recovered by excision. In agreement with Anderson et al. (1987), additional studies have reported that excision to be a more effective and accurate method compared to swabbing in the recovery of indicator bacteria

(Fliss et al., 1991; Martínez et al., 2010; Gallina et al., 2015). However, the altreration of swab materials can enhance its recovery. Gill and Jones (2000) reported the total aerobic bacteria recovered from carcass sides of pork and beef in packing plants were similar whether recovered by excision or swabbing with sponge or gauze. In addition, they reported the more abrasive the swab, the more bacteria would be recovered (Gill and Jones, 2000). While there have been studies to determine differences in sampling methods on carcasses, little information exists to determine the efficacy of various methods in sampling beef trimmings.

The objective of this study was, therefore, to evaluate the recovery of indicator (aerobic bacteria, coliforms, and *Escherichia coli* biotype I) microorganisms on beef trimmings using 3 different methods: 1) swabbing, 2) rinsing, and 3) grinding.

Materials and Methods

Sample collection

Meat manufacturing trimmings with varying surface fat contents (5 to 30%) were acquired from a commercial facility and transported to the Texas Tech University Gordon W. Davis Meat Laboratory. Over 3 independent replications, 5 samples of beef trimmings were collected using the N60 technique, with individual pieces measuring approximately 3 in long by 1 in wide and 1/8 inch thick following the USDA-FSIS methodology (FSIS 2014, 2015, 2016). For each replication, each of the 5 N60 samples obtained for the microbial analysis represented originally a single lot (2000 lb combo) at the commercial facility. The samples collected by the N60 method were then transferred to the food safety laboratory located in the Experimental Sciences building at Texas Tech University under refrigerated conditions and processed no later than 24 h after collection as required by the FSIS protocol.

Swab sampling

Each package of the beef trimmings obtained by the N60 sampling was aseptically opened using a sterile scissor, which was sanitized using 95% ethanol and flaming technique. The samples were placed on trays that were covered with labeled aluminum foil. A 100 cm^2 template was placed on top of each sample. Sterile EZ Reach sponges pre-hydrated with 25 mL Buffered peptone water (BPW) were used for swabbing. The sponge was

squeezed inside its bag before swabbing the area to remove the excess liquid. The delimited area was swabbed in horizontal and vertical motion from left to right and from top to bottom. The sponge was then put back in its respective bag, with the swabs being then stomached at 230 rpm for 30 s (Stomacher 400 circulator). Serial 10-fold dilutions were performed in BPW 9 mL tubes for each swab. Appropriate dilutions were plated on 3M Petrifilm Aerobic Plate Count Plates (APC) and 3M Petrifilm *E. coli*/Coliform Count Plates (*E. coli*/Coliform Petrifilm) and incubated at 36°C ± 1°C. The APC petrifilms were counted at 48 h, whereas *E. coli*/coliform petrifilms were counted twice at 24 and 48 h procedure was repeated three times in the same manner.

Rinse sampling

In a separate location from the 100 cm^2 area that had been swabbed, 25 g from each of the N60 sample were acquired using sanitized forceps and scissors, and placed in a 55oz Whirl pack bags (Nasco, Fort Atkinson, WI). A volume of 225 mL of BPW was added to each sample. The samples were stomached at 230 rpm for 2 min, and serial 10-fold dilutions in BPW 9 mL tubes were performed. Then, the enrichments were drained out of Whirl pack bags, and the meat pieces were transferred aseptically using forceps to another set of Whirl pack bags, and again 225 mL of BPW was added to each sample. The samples then underwent stomaching at 230 rpm for 2 min, and serial dilutions were performed. This procedure was repeated for a total of three times. Appropriate dilutions were plated onto APC and *E. coli*/coliform petrifilms and incubated at 36°C ± 1°C. APC petrifilms were counted at 48 h, whereas *E. coli*/coliform petrifilms were counted twice at 24 and 48 h.

Grind sampling

For each N60 sample, 100 g of the trimmings were collected aseptically separate from the 100 cm^2 that had been previously swabbed, using sanitized forceps into Cabela's Deluxe meat grinder (Cabela's, model 541091, China) to be ground. Between each grinding, the grinder was disassembled, cleaned, and sanitized with bleach to avoid cross contamination. Then, parts were rinsed with potable water to eliminate sanitizer residue. A total of 25 g from each of the ground samples were placed into a Whirl pack bags. The samples then were diluted with 225ml BPW, stomached at 230 rpm for 2 min, and serial 10- fold dilutions were performed. Then, the enrichments were drained out of

Whirl pack bags, and the ground meat was transferred aseptically using a spatula to another set of Whirl pack bags, and again 225 mL of BPW was added to each sample. Subsequently, samples were stomached at 230 rpm for 2 min, and serial dilutions were performed. This procedure was repeated for a total of three times. Appropriate dilutions were plated onto APC Petrifilm and *E. coli*/coliform and incubated at 36°C ± 1°C. APC petrifilms were counted after 48 h, whereas *E. coli*/coliform petrifilms were counted two times after 24 and 48 h.

Scanning electron microscopy

Scanning electron microscope (SEM) was used to evaluate and show bacterial retention on the sponge after multiple dilutions and stomaching. To achieve this, a sample was taken by swabbing the shoulder portion of a beef carcass with a sponge in 25 mL of BPW (pre-hydrated EZ Reach sterile sponges). The swab was stomached for 30 s at 230 rpm. Excess liquid was squeezed off the sponge before cutting. On both sides of the swab, a 1cm × 1cm piece was cut using a sterile surgical blade and forceps. Each piece was cut horizontally in 2 to obtain subsamples from the exterior and interior of the swab. Subsamples were placed in 6-well plate, one in each well. The swab was transferred to a sterile stomacher bag and weighed. The BPW was added into the bag to obtain 1:10 dilution and stomached. Liquid was squeezed off the swab and 1cm × 1cm subsamples from the exterior and interior ("first rinse") were obtained. The same procedures were performed to get subsamples after the second rinse.

Four mL of 2% glutaraldehyde (Sigma, USA) in phosphate buffered saline, PBS (Sigma-Aldrich, Saint Louis, MO) was added into the wells containing the subsamples. The latter were incubated for 24 h at 4°C. After fixation, the solution was removed from each well using a disposable transfer pipette. The subsamples were washed with PBS twice, followed by dehydration in ascending concentrations of ethanol (25, 70, 95, and 100%), for 10 min at each concentration. Each dehydration was done once except the 95% and 100% which were done 2 times and 4 times, respectively. Critical point drying was performed using the manufacturer's protocol (BAL-TEC, Balzers, Liechtenstein), where the critical temperature of CO_2 was 31.1 C° and the critical pressure of CO_2 was 73.8 bar (1073 psi). The subsamples were mounted on aluminum stubs, sputter-coated with a thin layer of gold, and examined under a Hitachi S-4300SE/N SEM.

Statistical analysis

Experiments were performed in triplicate. For each of the samples analyzed, duplicate plates were obtained for each dilution and averaged prior to analysis. For each of the tested methodologies, microbial counts were collected and transformed to either log10 counts per 100 cm^2 (swabbing) or log10 counts per g (rinsing and grinding) prior to analysis to allow control and stabilization of statistical variance and fulfillment of the requirements for normality prior to the analysis. Log counts were considered a dependent variable of interest. Analysis of variance was performed using RStudio (version 1.0.44). Each sampling method was analyzed individually from the others, but within each methodology, comparisons of means were obtained between the first, second, and third consecutive collection. Pearson product-moment correlation coefficients were calculated to identify the relationship between the sampling techniques implemented using RStudio (version 1.0.44). In addition, simple linear regressions were computed and graphed using Microsoft Excel (2016). In all tests, the significance level was set at α ≤ 0.05.

Results

The first enumerations obtained from the beef trimmings using swabbing, rinsing, and grinding are presented in (Table 1). There was no significant difference ($P > 0.05$) in the numbers of aerobic bacteria recovered when comparing the rinsing to grinding methods. However, aerobic bacteria recovered by swabbing was significantly lower ($P < 0.05$) than both rinsing of the whole sample or grinding followe by rinsing.

The decline in the bacterial numbers recovered as a result of subjecting each sample to multiple sequential samplings is presented in (Table 2). The sequential sampling using rinsing and grinding techniques resulted in a significant decline ($P < 0.05$) in the number of bacteria recovered. However, the bacterial recovery when using the swabbing technique was not significantly different ($P > 0.05$) for each of the 3 sequential samplings. Therefore, the decrease in bacterial number was not significant ($P > 0.05$) indicating much bacteria remained on the surface of the sample.

By adding all bacterial counts together from all 3 sequential samplings obtained by each of the methods (swabbing, rinsing, and grinding) a comprehensive enumeration of total aerobic bacteria and coliforms present in the beef trimmings was obtained (Table 3). When observing the aerobic bacteria counts, there was no significant difference ($P > 0.05$) between rinsing of the whole

Table 1. Aerobic bacteria counts obtained from the first enumeration from N60 samples (n = 15) using swabbing, rinsing, and grinding

Sampling method	Aerobic bacteria	
	Log10 CFU	SEM[1]
Swabbing, 100 cm^2	1.9[a]	0.18
Rinsing, g	3.0[b]	0.21
Grinding, g	3.0[b]	0.16

[a,b]Different superscripts within the column denote statistical differences ($P < 0.05$).

[1]Standard Error of the Mean.

Table 2. Aerobic bacteria plate count obtained from N60 beef trimmings (n = 15) as a result of repeated sampling using swabbing, rinsing and grinding

Collection frequency	Sampling method	Mean of log counts	SEM[1]
1st	Swabbing, 100 cm^2	1.9[a]	0.19
2nd	Swabbing, 100 cm^2	1.7[a]	0.16
3rd	Swabbing, 100 cm^2	1.7[a]	0.24
1st	Rinsing, g	3.0[a]	0.22
2nd	Rinsing, g	2.2[ab]	0.23
3rd	Rinsing, g	1.7[bc]	0.20
1st	Grinding, g	3.0[a]	0.17
2nd	Grinding, g	2.3[bc]	0.17
3rd	Grinding, g	1.8[c]	0.20

[a–c]Different superscripts within column for each of the sampling methods denote statistical differences ($P < 0.05$). No statistical comparisons were conducted between the methods.

[1]Standard Error of the Mean.

Table 3. Comprehensive counts of total aerobic bacteria and coliforms recovered from set of N60 (n = 15) samples as a result of repeated sampling using swabbing, rinsing, and grinding techniques consecutively for 3 times. Comprehensive counts were determined by adding the total number of bacteria recovered in each collection frequency (first, second, and third) together[1]

Sampling method	Aerobic bacteria		Coliform	
	Log$_{10}$ CFU	SEM^2	Log$_{10}$ CFU	SEM^2
Swabbing, 100 cm^2	2.3[a]	0.18	0.1[a]	0.09
Rinsing, g	3.1[b]	0.22	0.4[ab]	0.20
Grinding, g	3.1[b]	0.17	0.9[b]	0.19

[a,b]Different superscripts within the column denote statistical differences ($P < 0.05$).

[1]Generic E.coli counts were below the detection limits (< 10 CFU/ml).

[2]Standard Error of the Mean.

piece and grinding followed by rinsing. Nevertheless, counts obtained by swabbing were significantly lower ($P < 0.05$) than rinsing and grinding as it was observed when

comparing just the initial samples. For total coliform counts, rinsing was not significantly different ($P > 0.05$) from either swabbing or grinding, yet swabbing yielded significantly lower counts ($P < 0.05$) than grinding.

The correlation coefficient was calculated to measure the strength and the direction of a linear relationship among the 3 methods. The correlations were 0.90, 0.82, and 0.83 for swab vs. grind, swab vs. rinse, and rinse vs. grind, respectively (Fig. 1, 2, and 3). Simple linear regression was performed to examine the relationship between the first recovery of all possible pairs of the 3 sampling methods, the results of which are illustrated in (Table 4). First, a linear model was computed to examine the relationship between swabbing and grinding and resulted in an r^2 of 0.81. The samples subjected to grinding and then rinsing had approximately 1.47 log more bacteria than the samples that were swabbed. The predicted recovery of grinding was 1.47 + 0.8X where X is the bacterial recovery obtained by swabbing (Fig. 1). Second, a linear model was performed to evaluate the relationship between swabbing and rinsing and resulted in r^2 of 0.67 indicating the percentage of variation of the response variable (Rinsing Log CFU/g) explained by our model. Rinsing of the whole piece had approximately 1.16 log more bacteria than swabbing of

the whole piece. The predicted recovery of rinsing was 1.16 + 0.93X where X is the number of aerobic bacteria obtained by swabbing (Fig. 2). Finally, the linear model between rinsing and grinding recovery resulted in an $r2$ of 0.70, indicating that 70% of the response variation is explained by the linear model. The predicted recovery or grinding was 1.07+ 0.66X where X is the number of bacteria recovered by rinsing (Fig. 3).

Scanning electron microscopy (SEM) pictures of swabs were obtained to examine its bacterial retention, as it was hypothesized that grinding the sample would yield higher bacterial counts because a larger surface area would be exposed in comparison with rinsing and swabbing. It was thought that subjecting the trimmings to multiple samplings would result in a decline in the number of bacteria until it becomes undetectable. The SEM pictures should hypothetically show bacteria attached to the swab on the interior and exterior surfaces since the sponge might retain part of the bacteria recovered from the sample. Results obtained from the SEM pictures revealed that some bacterial cells remained on the interior and exterior layers of the sponge. There was not a specific pattern in how bacteria appeared on the sponge, as they could be seen as a single cell or clusters and were distributed over the entire area.

Table 4. The relationship between the first recovery of all pairs of sampling methods (swabbing, rinsing, and grinding)

| Sampling methods	r	r^2	β_1[1]	CI	P-value	β_0[2]	CI	P-value
Swabbing vs. Grinding	0.90	0.81	0.80	0.58 - 1.03	< 0.05	1.47	1.00 - 1.94	< 0.05
Swabbing vs. Rinsing	0.82	0.67	0.93	0.55 - 1.32	< 0.05	1.16	0.36 - 1.95	< 0.05
Rinsing vs. Grinding	0.83	0.70	0.66	0.40 - 0.91	< 0.05	1.07	0.28 - 1.87	< 0.05

[1]$\beta1$ indicates the slope.

[2]$\beta0$ Indicates the intercept.
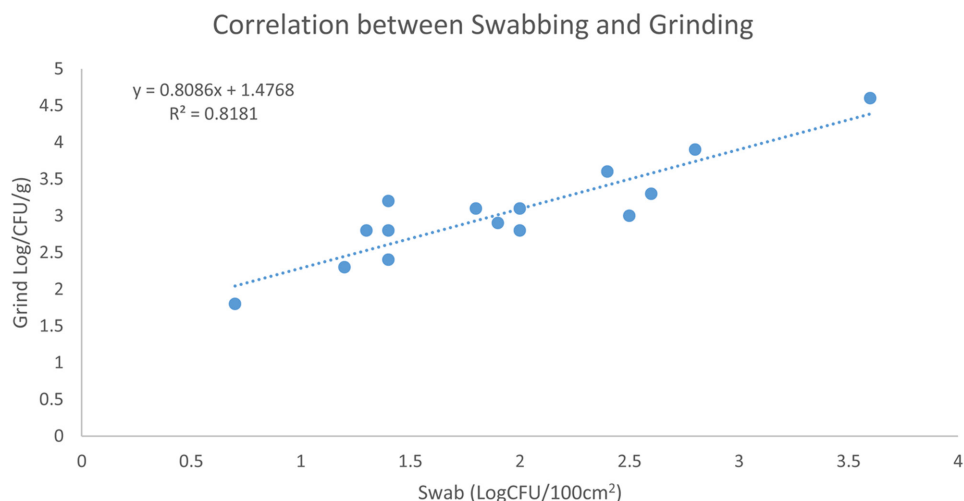
Figure 1. Correlation between swabbing and grinding methods for aerobic bacteria counts recovered from N60 beef trimmings.

Correlation between Swabbing and Rinsing

$y = 0.9363x + 1.1631$
$R^2 = 0.6797$
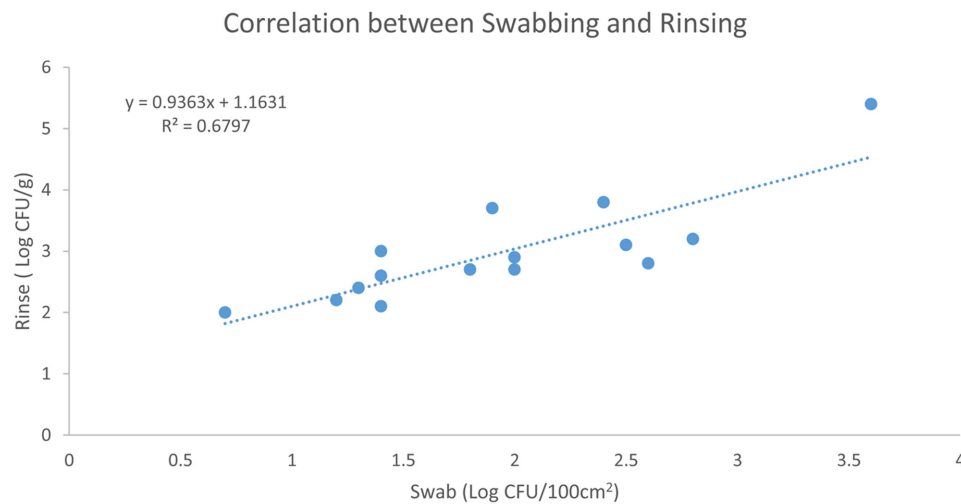
Figure 2. Correlation between swabbing and rinsing methods for aerobic bacteria counts recovered from N60 beef trimming.

Correlation between Grinding and Rinsing

$y = 0.6601x + 1.0774$
$R^2 = 0.7032$
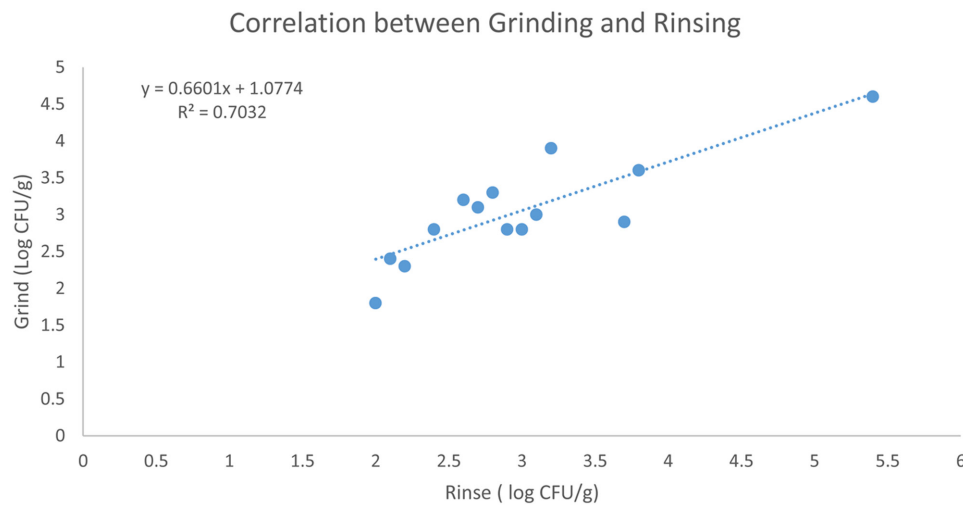
Figure 3. Correlation between grinding and rinsing methods for aerobic bacteria counts recovered from N60 beef trimming.

Discussion

This study indicated that there is noteworthy variation among sampling methods to recover indicator bacteria from beef trimmings. Swabbing is the least effective means to recover bacteria from trim samples, and if the surface is swabbed consecutive times in the same area, there is a possibility that the same amount of bacteria will be recovered the second and third time. Rinsing of either the whole piece of the sample or grinding and then rinsing the grind resulted in improved bacterial recovery with no differences between the 2 methods. The effectiveness of recovering aerobic bacteria and coliforms was highest when using the grinding method, followed by rinsing and least by swabbing. Swabbing yielded lower bacterial counts than any type of method used in this study, likely because swabbing recovers only part of the surface micro-

flora (Anderson et al., 1987). Reid et al. (2002) stated that the nature of the swabbing technique supports the 2-way transfer of bacteria from hide to swab and from swab to hide which can occur simultaneously during swabbing. This also could have occurred during the swabbing of the beef trimmings in this study. Furthermore, the SEM pictures confirmed what was stated by Reid et al. (2002), and showed that the swabs retained bacteria as can be seen in Fig. 4 and 5. Additionally, the fat present in beef trim may fill the pores of the swab as the sample is collected, which then could result in lowering the bacterial recovery (Seager et al., 2010). Many studies that compared swabbing with other sampling techniques such as rinsing and excision found swabbing to be the least effective method (Anderson et al., 1987; Dorsa et al., 1996; Gill and Jones, 2000). Grinding of beef trimmings increases the surface area exposed to the diluent, which could have been the
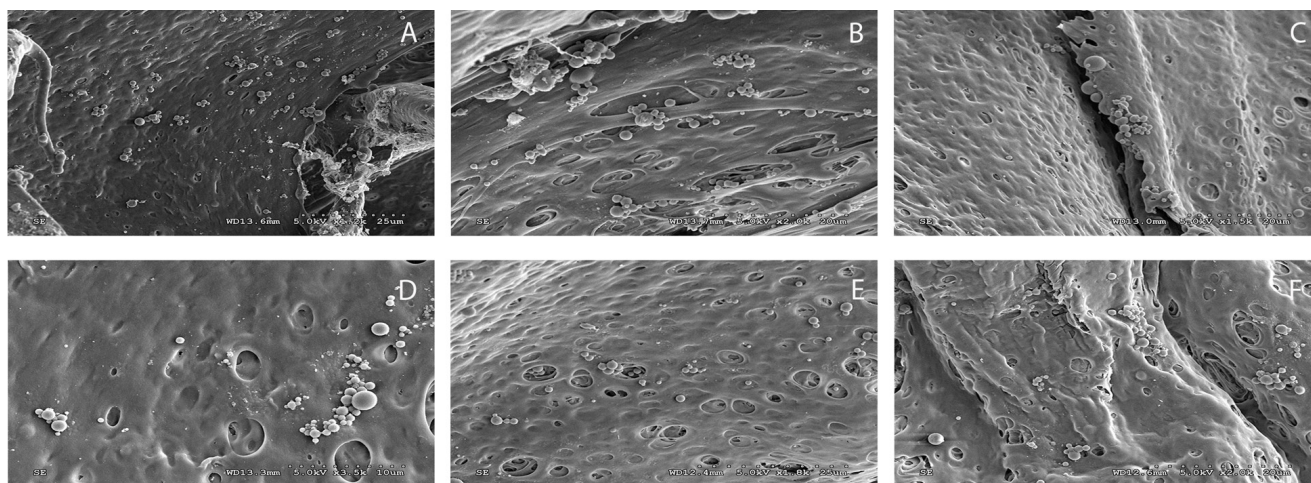
Figure 4. Scan electron microscopy (SEM) pictures from the exterior surface of sponge show bacterial cells attached to the swab: (A&B) Exterior surface without rinse, (C&D) Exterior surface after First rinse, and (E&F) Exterior surface after 2 rinses.
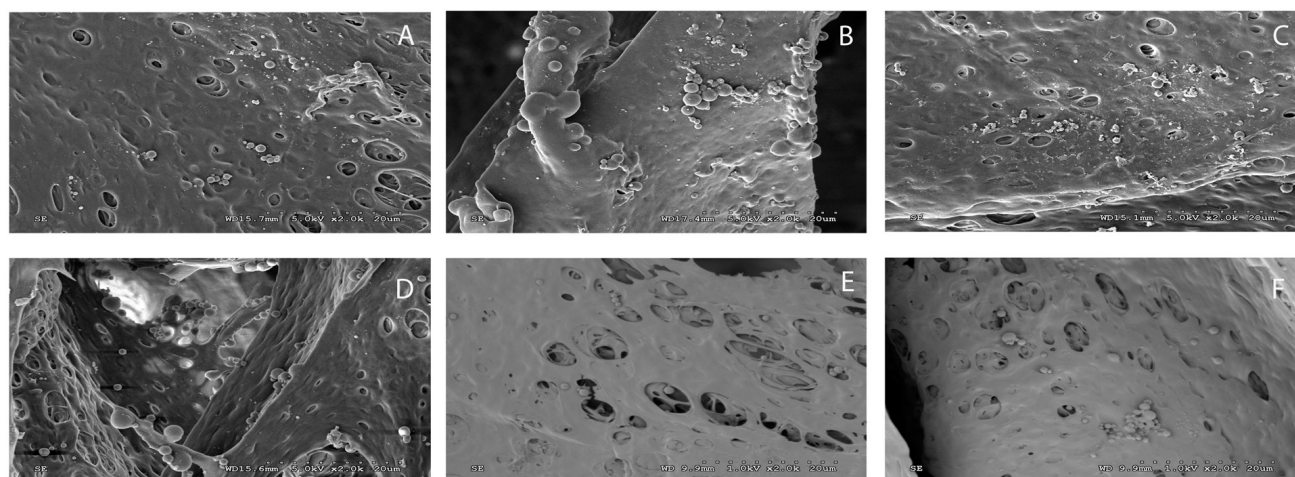
Figure 5. Scan electron microscopy (SEM) pictures the interior surface of the sponge show bacterial cells attached to the swab: (A&B) Exterior surface without rinse, (C&D) Exterior surface after First rinse, and (E&F) Exterior surface after 2 rinses.

reason this sampling method showed superior bacterial recovery in beef trimmings. However, rinsing resulted in counts that were not significantly different than grinding, probably because most of the bacteria are located on the exterior surface of the trimmings especially when it is still intact, like the beef trims.

As expected, the first sampling recovered the highest number of bacteria in all three sampling methods used in this study. This could be because most of the bacteria that are found on the external surface of the trimmings are not necessarily firmly attached. Then, after sampling is performed multiple times, the recovery decreases either because bacteria have already been removed, bacterial attachment is stronger, or there is difficulty reaching deep areas of the meat as in the case of swabbing.

Despite the variation of the effectiveness in each sampling method, the ability to implement any of these meth-

ods commercially would be a crucial factor to determine which one should be used. In this study, swabbing was less time-consuming and easier to perform, however, our results indicated that swabbing recovered around one-tenth of what rinsing or grinding followed by rinsing recovered. In addition, grinding the sample, as the results show, did not add much to the recovery of indicators. The time needed for the grinder to be cleaned and sanitized for each sample to be processed was also far greater, making this method more intricate and time consuming. Rinsing the whole pieces of beef trimmings would be the ideal method among those studied when assessing the microbiological condition of beef trims as it recovers more bacteria than swabbing and requires less time to perform in comparison to grinding, yielding basically the same results.

While swabbing recovered fewer bacterial cells, it still plays an important role in process control because

it is a nondestructive and non-invasive method. For any given agency or industry conducting microbial data collection, sampling consistency is the key for proper interpretation of results. It is very important that the samples collected and reviewed over time are compared to results from samples collected in the same manner to make informed decisions about process control. It is also critical that each laboratory has written guidelines for sample collection that are followed to achieve consistency from day to day, which allows for a proper verification of the microbial loads present on beef trimmings.

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