

INFLUENCE OF SAMPLING PROCEDURE, HANDLING AND STORAGE ON THE MICROBIOLOGICAL STATUS OF FRESH BEEF

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Summary

Sponging and excising were evaluated as sampling procedures for microbiological analysis of 192 beef carcasses in a commercial plant. In addition, subprimals (46 clods and 96 top butts) were sampled by sponging in the plant and when they reached their destination at the retail level, and retail cuts (64 steaks or roasts) were sampled by sponging at 0 h of cutting and following 48 h of display at 5°C. The samples were analyzed for aerobic plate counts (APC/25°C and APC/35°C), total coliform counts (TCC), and *Escherichia coli* counts (ECC), and for presence of *Salmonella* spp., *Staphylococcus aureus*, *Listeria* spp. and *Listeria monocytogenes*. Samples obtained by carcass sponging had higher APC/35°C (4.4 log CFU/300 cm²) than excised samples (3.9 log CFU/300 cm²); APC/25°C carcass counts were similar between sponged and excised samples. Excision of samples from carcasses yielded 2.5 and 2.5 log CFU/300 cm² of TCC and ECC, respectively, while sponging of carcasses yielded 1.7 and 1.6 log CFU/300 cm², respectively. There were no significant ($P > 0.05$) differences in bacterial counts recovered from the different subprimals in the plant or at the retail level. In addition, the retail cuts derived from the subprimals indicated no significant ($P > 0.05$) differences among types of cuts, but there was a significant ($P \leq 0.05$) increase in counts when the cuts were held for 48 h at 5°C compared to sampling immediately after cutting. Key words: sponging, excising, beef, bacteria

Introduction

In 1992-1993, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) determined microbiological baselines for beef carcasses (FSIS-USDA, 1994). Contamination increases following slaughtering and during fabricating and transporting of beef. The speed and conditions of the beef chilling process determine the rate at which bacteria proliferate. Rey *et al.* (1970) showed that bacteria counts did not increase during 2°C chilling unless carcasses were held for extended periods of time (e.g., to help induce the aging process). Increases in bacterial counts on beef carcasses are dependent on the type of microorganisms present; competitive inhibition by the most-favored bacteria can cause many strains of bacteria to grow slowly or fail to initiate growth (Gill and Newton, 1980).

Process controls such as implementation of Hazard Analysis and Critical Control Point (HACCP) Systems and transportation under refrigeration are designed to reduce or prevent the proliferation of bacteria on beef. Verification that process controls (e.g., HACCP systems) are working is performed by taking samples by use of either the sponging or excising method and analyzing them for *Escherichia coli* and *Salmonella* following the new government regulation (FSIS-USDA, 1996). The excising method has

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been shown to be the better of the two sampling procedures in terms of bacteria recovery from fresh beef carcass tissue (Anderson *et al.*, 1987; Dorsa *et al.*, 1996, 1997). Although the excising method usually recovers a higher number of bacteria than the sponging method, it may not be the most appropriate method for sampling products such as subprimals and steaks, because it is more difficult to apply and damages the physical integrity of the product.

This study determined microbiological counts on: (a) beef carcasses sampled after 24-48 h chiller storage by sponging and excising, (b) wholesale cuts, sampled by sponging, prior to vacuum packaging at the packing plant and after arrival at the retail store and (c) retail cuts, sampled by sponging, prior to being placed in the retail case and after 48 h of retail storage (5°C).

Materials and Methods

Sampling procedures

Carcass sampling was conducted according to the United States Meat and Poultry Inspection Regulations (FSIS-USDA, 1996) for each of three carcass sites (brisket, flank, round). Carcasses were sampled either by excising or sponging (Whirl-Pak™, Nasco, Modesto, CA). Forty-eight beef sides were sampled, on each of four days following 48 h of chiller storage at a commercial packing plant. Randomly selected carcasses were sampled at three sites--the round, flank and brisket--with the same sponge or cutting tool for each side. Samples were analyzed for aerobic plate counts (APC/25°C and APC/35°C), total coliform counts (TCC), and *Escherichia coli* counts (ECC), and for the presence of *Salmonella*, *Listeria* spp. and *Listeria monocytogenes*.

Two types of subprimals (clods and top butts) were evaluated at the beef packing plant immediately prior to packaging and at the retail store prior to fabricating into retail cuts. The subprimals were sponged on 100 cm² areas of the fat and lean, separately, while samples of trimmings were also taken for microbiological analyses. The sponging method was performed using procedures outlined in the U.S. Meat and Poultry Inspection Regulation (FSIS-USDA, 1996). There were a total of 96 samples (24 clod/fat, 24 top butt/fat, 12 clod/lean, 12 top butt/lean, 12 clod/trimmings and 12 top butt/trimmings) taken during the first replication. Samples were analyzed for APC/25°C, TCC, LAB and ECC, plus determinations of presence of *Salmonella*, *Listeria* spp. and *L. monocytogenes*. There was a total of 96 samples (24 clod/fat, 24 top butt/fat, 24 clod/lean and 24 top butt/lean) taken in the second replication for an overall total of 192 subprimal samples taken at the stage-1 sampling site (beef packing plant).

Stage-2 sampling of the subprimals was at the retail store, approximately two weeks after sampling was done at the packing plant where they were sponged on 100 cm² areas of the fat and lean, separately. A total of 46 subprimals (16 clod/fat, 16 clod/lean, 7 top butt/fat and 7 top butt/lean) were evaluated. Samples were analyzed for APC/25°C, TCC and ECC, and for the presence of *Salmonella*, *Listeria* spp. and *L. monocytogenes*.

Retail cuts were sampled in the form of steaks or roasts at the retail store. The samples were taken prior to the cuts being placed in the retail case and after 48 h storage (5°C) in the retail case. All retail cuts were sponged on 100 cm² areas of the lean for microbiological analysis. A total of 64 retail cuts (18 clod/0 h, 18 clod/48 h, 14 top butt/0 h and 14 top butt/48 h) were evaluated. Samples were analyzed for APC/25°C, TCC and ECC, and for the presence of *Salmonella*, *Listeria* spp., *Listeria monocytogenes* and *Staphylococcus aureus*.

The data were analyzed using the general linear model procedure of SAS and sample means were separated with the least square mean test (SAS, 1985).

Results and Discussion

Carcass sampling procedure (sponging or excising) had a significant ($P \leq 0.001$) effect on APC/35°C, TCC and ECC (Tables 1 and 2), with the excision sampling procedure recovering significantly higher LAB, TCC and ECC (data not shown). The biggest difference in counts between the two sampling methods was for the carcass ECC, where excising recovered 2.5 log CFU/300 cm² and sponging recovered 1.6 log CFU/300 cm². However, the two sampling procedures showed no significant ($P > 0.05$) differences in the APC/25°C, with excising recovering 4.3 log CFU/300 cm² and sponging recovering 4.3 log CFU/300 cm². The sponging procedure recovered significantly ($P \leq 0.05$) higher APC/35°C than did the excising method (4.4 log CFU/300 cm² vs. 3.9 log CFU/300 cm²). Overall, however, the effectiveness of recovering bacterial counts with the excising method exceeded that of the sponging method. Results obtained by use of each method of sampling should be compared with baseline results determined by the corresponding method.

There were no significant ($P > 0.05$) differences between the two subprimal fat samples in ECC (clod = 2.8 log CFU/300 cm² and top butt = 2.7 log CFU/300 cm²), and APC/25°C (clod = 5.0 log CFU/300 cm² and top butt = 4.9 log CFU/300 cm²) (data not shown). Clod fat samples had higher ($P \leq 0.05$) APC/35°C (6.0 log CFU/300 cm²) and LAB (4.2 log CFU/300 cm²) compared to top butt fat samples (APC/35°C = 4.5 log CFU/300 cm² and LAB = 3.4 log CFU/300 cm²). However, the top butt fat surface had higher ($P \leq 0.05$) TCC (3.8 log CFU/300 cm²) than the clod (2.1 log CFU/300 cm²). There was also a significant ($P \leq 0.05$) difference in bacterial counts recovered from the lean samples of the clod vs. top butt with means of 3.8 log CFU/300 cm² vs. 4.5 log CFU/300 cm² (APC/25°C), 2.3 log CFU/300 cm² vs. 3.5 log CFU/300 cm² (TCC), and 1.9 log CFU/300 cm² vs. 2.8 log CFU/300 cm² (ECC), respectively. Bacterial counts recovered from trimmings of subprimals were higher for those from the clod than for those from the top butt (data not shown).

There were no major differences in APC/25°C, TCC or ECC recovered from the fat vs. lean surfaces of the top butt samples at the beef packing plant, but for the clod samples, fat and lean were significantly different in APC/25°C, TCC and ECC (data not shown). The fat surface of the clod had higher ($P \leq 0.05$) APC/25°C and ECC than did the lean surface. Subprimals were also evaluated for bacterial counts when they reached the retail level (Tables 3 and 4). The top butt surface samples showed significant ($P \leq 0.05$) differences in TCC (lean = 3.2 log CFU/300 cm² and fat = 2.6 log CFU/300 cm²) and ECC (lean = 2.6 log CFU/300 cm² and fat = 2.1 log CFU/300 cm²) but not APC/25°C (lean = 5.0 log CFU/300 cm² and fat = 5.2 log CFU/300 cm²), whereas the fat vs. lean surfaces of clods showed no significant ($P > 0.05$) differences for APC/25°C, TCC or ECC (data not presented in tabular form).

After 48 h, the top butt retail cuts had significantly ($P \leq 0.05$) higher APC/25°C (8.3 log CFU/300 cm²), TCC (3.4 log CFU/300 cm²) and ECC (3.1 log CFU/300 cm²) than the top butt retail cuts sampled at 0 h (APC/25°C = 4.0 log CFU/300 cm², TCC = 2.0 log CFU/300 cm² and ECC = 2.0 log CFU/300 cm²). The clod retail cuts, sampled after 48 h of display, had higher APC/25°C (8.2 log CFU/300 cm²), TCC (3.4 log CFU/300 cm²) and ECC (3.6 log CFU/300 cm²) than the clod retail cuts sampled at 0 h (APC/25°C

= 4.9 log CFU/300 cm², TCC = 2.7 log CFU/300 cm² and ECC = 2.5 log CFU/300 cm²) (data not shown).

Salmonella spp., *S. aureus*, and *L. monocytogenes* were not detected in either the sponged or excised carcass samples. However, the sponging procedure detected a higher incidence of *Listeria* spp. (8.3%) than did the excising procedure (2.1%) for carcasses (Table 5). Clod fat samples showed no presence of *L. monocytogenes*, but did show a 20.8% incidence of *Listeria* spp. (Table 5). Clod lean samples had an 8.3% incidence of both *Listeria* spp. and *L. monocytogenes*. Clod samples had a higher incidence of *Listeria* than top butt samples. Top butt fat samples had an incidence of *Listeria* spp. of 2% and *L. monocytogenes* of 2%. Top butt lean samples had an incidence of *Listeria* spp. of 8.3% and of *L. monocytogenes* of 8.3%. Subprimal trimming samples had an incidence of *Listeria* spp. of 16.7%, but no incidence of *L. monocytogenes*.

Clod fat or lean samples had no incidence of *Listeria* spp. or *L. monocytogenes* (Table 6). Top butt fat samples had an incidence of *Listeria* spp. of 28.6% and of *L. monocytogenes* of 28.6% and an incidence in the lean samples of *Listeria* spp. of 57.1% and *L. monocytogenes* at 28.6%. Incidence of *Listeria* spp. was 0% and 5.6% for the clod at 0 and 48 h respectively, and 14.3% and 7.1% for top butt retail cuts at 0 and 48 h, respectively (Table 6).

Conclusions

Sampling of beef carcasses by using the excising procedure recovered, overall, higher (difference of <1 log CFU/300 cm²) bacteria counts (with the exception of APC) than by sponging. In addition, use of the excising procedure resulted in less variation in counts recovered, making the excising method superior in bacterial recovery to the sponge method. However, excising is more destructive to the product thereby making it less economically feasible to use. There was no significant increase in bacterial counts between the subprimal samples taken at the beef packing plant and those taken from the subprimals at the retail store. This verifies the cleanliness of the processes at the packing and retail facilities and the proper transportation procedures. Differences in bacterial counts between fat vs. lean surfaces of subprimals were minor. The major increase in bacterial counts on steaks and roasts between 0 h and 48 h of retail display (5°C) can be minimized by maintaining proper retail display case temperatures below 5°C. Improved sanitation procedures should reduce incidence of pathogenic bacteria, such as *Listeria monocytogenes*.

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Tables

Table 1. Analysis of variance of aerobic plate counts at 25°C and 35°C (APC/ 25°C, APC/ 35°C) recovered from beef carcasses and subprimal (clod and top butt) cuts in a packing plant.

Variable	DF	APC/25°C			APC/35°C		
		Mean Square	F-value	Pr > F	Mean Square	F-value	Pr > F
Carcass sampling procedure ¹	1	0.14	0.48	0.4908	5.34	6.99	0.0001*
Subprimals ²	1	0.70	0.92	0.3380	41.63	149.79	0.0001*
Surface type ³	1	13.44	17.83	0.0001*	0.02	0.05	0.8170
Subprimal * Surface type	1	8.78	11.65	0.0001*	0.09	0.34	0.5616

¹Sampling procedure = sponging (Whirl-Pak™ sponges, Nasco, Modesto, CA) and excising (only for carcasses).

²Subprimals = clod and top butt.

³Surface type = fat and lean.

Table 2. Analysis of variance of total coliform counts (TCC) and *E. coli* counts (ECC) recovered from beef carcasses and subprimal (clod and top butt) cuts in the packing plant.

Variable	DF	TCC			ECC		
		Mean Square	F-value	Pr > F	Mean Square	F-value	Pr > F
Carcass sampling procedure ¹	1	14.28	119.22	0.0001*	37.16	219.07	0.0001*
Subprimals ²	1	50.48	15.98	0.0001*	0.09	0.15	0.7032
Surface type ³	1	0.17	0.49	0.4836	8.36	13.59	0.0001*
Subprimal * Surface type	1	1.02	3.07	0.0833	7.68	12.48	0.0001*

¹Sampling procedure = sponging (Whirl-Pak™ sponges, Nasco, Modesto, CA) and excising (only for carcasses).

²Subprimals = clod and top butt.

³Surface type = fat and lean.

Table 3: Analysis of variance of aerobic plate counts at 25°C (APC/25°C) recovered from subprimals (clod and top butt) and retail cuts (steaks or roasts) taken at the retail store.

Variable	DF	APC/25°C		
		Mean Square	F-value	Pr > F
Subprimals ²	1	1.53	1.60	0.2128
Retail cuts ¹	1	75.62	124.48	0.0001*
Surface type ³	1	0.47	0.49	0.4894
Subprimal * Surface type	1	0.34	0.35	0.5575

¹Retail cut = steaks or roasts cut from either the clod or top butt sampled at 0 h and 48 h at 5°C.

²Subprimals = clod and top butt.

³Surface type = fat and lean.

Table 4: Analysis of variance of total coliform counts (TCC) and *E. coli* counts (ECC) recovered from subprimals (clod and top butt) and retail cuts (steaks or roasts) taken at the retail store.

Variable	DF	TCC			ECC		
		Mean Square	F-value	Pr > F	Mean Square	F-value	Pr > F
Subprimals ²	1	0.70	3.68	0.0620	0.57	2.83	0.1000
Retail cuts ¹	1	6.19	8.13	0.0001*	2.84	3.93	0.0127*
Surface type ³	1	0.28	1.46	0.2344	0.87	4.33	0.0436*
Subprimal * Surface type	1	0.91	4.78	0.0345*	0.57	2.83	0.1000

¹Retail cut = steaks or roasts cut from either the clod or top butt sampled at 0 h and 48 h at 5°C.

²Subprimals = clod and top butt.

³Surface type = fat and lean.

Table 5: Incidence (%) of *Listeria* spp. and *Listeria monocytogenes* on subprimals (clod and top butt) samples in a packing plant.

Sampling method	Sampling type	Sampling surface	N	<i>Listeria</i> spp.	<i>L. monocytogenes</i>
Sponging	Carcass	—	96	8.3	0
Excising	Carcass	—	96	2.1	0
Sponging	Clod	Fat	48	20.8	0
		Lean	24	8.3	8.3
	Top butt	Fat	48	2.0	2.0
		Lean	24	8.3	8.3
Trimmings	Clod	—	12	0	0
	Top butt	—	12	33.3	0

Table 6: Incidence (%) of *Listeria* spp. and *Listeria monocytogenes* on samples recovered from subprimals (clod and top butt) and retail cuts (steaks or roasts) at a retail store.

Sampling type	Form	Surface type	Storage time ¹	N	<i>Listeria</i> spp.	<i>L. monocytogenes</i>
Clod	Subprimal	Fat	—	16	0	0
Clod		Lean	—	16	0	0
Top butt		Fat	—	7	28.6	28.6
Top butt		Lean	—	7	57.1	28.6
Clod	Steaks/roasts		0	18	0	0
Clod			48	18	5.6	0
Top butt			0	14	14.3	0
Top butt			48	14	7.1	0

Sampling procedure = sponging (Whirl-Pak™ sponges, Nasco, Modesto, CA)

¹Storage time = measured in h and products held at 5°C.

