

INFLUENCE OF STORAGE ON BACTERIAL NUMBERS DETECTED ON SPONGES FOLLOWING BEEF CARCASS TISSUE SAMPLING

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Summary

Uninoculated or inoculated (*Escherichia coli* - 4.3 and 7.4 log CFU/ml) beef brisket samples (10x10 cm) were sampled for microbiological analysis with the different sponge/buffer combinations. The sponges were analyzed immediately and after 24 hr at 4, 7, or 15°C, for aerobic plate counts (APC) on tryptic soy agar, and for total coliform counts (TCC) and *E. coli* (ECC) counts on 3M Petrifilm™ *E. coli* count plates (3M Health Care Products, St. Paul, MN). Neither type of sponge nor buffer affected ($P > 0.05$) bacterial counts. Factors having significant ($P < 0.01$) effects on bacterial counts were inoculum level and temperature of sponge storage. Storage at 15°C resulted in generally higher counts compared to those on sponges stored at 4 or 7°C for 24 h, which had somewhat reduced TCC and ECC. These results suggest that the commercially available sponges and buffers tested were similarly effective for use in beef carcass tissue sampling. Care should be taken, however, in controlling sponge temperature during shipment for subsequent analysis.

Introduction

According to United States Food Safety and Inspection Service (FSIS) regulations (Federal Register, 1996), sponging is an acceptable procedure for carcass sampling to determine *Escherichia coli* counts. However, concern has arisen regarding the effectiveness of sponging as a means of sampling for microbial testing. To comply with requirements for the Pathogen Reduction; HACCP Systems; Final Rule (Federal Register, 1996) FSIS allows companies to take samples for microbial monitoring by either the sponging or excising sampling methods. Research by Kotrola *et al.* (1997) indicated that exposure of *E. coli* cell suspensions to sampling sponges may reduce numbers of bacteria by as much as 99%. Gants (1997a) reported that this 99% reduction in bacterial recoverability could, perhaps, be attributed to the diluent used, to possible antimicrobial properties of the sponge, or to potential presence of chemical residues in the sampling bag. The findings of Kotrola *et al.* (1997) generated concern and prompted considerable discussion among scientists of FSIS, academia and industry regarding the most appropriate procedure for carcass tissue sampling (Gants, 1997b).

This study compared microbiological counts (aerobic plate counts – APC; total coliform counts – TCC; and generic *Escherichia coli* counts – ECC) recovered from beef brisket fat sampled by sponging vs. excising with each of two buffers (Butterfield's Phosphate – BP and Peptone Water – PW) and with each of two commercially available sampling sponges (A and B), immediately and after 24 h sponge storage at 4, 7 or 15°C.

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Materials and Methods

Experimental design

Two commercial brands of sampling sponges (A and B) and two hydrating buffers (Butterfield's Phosphate Buffer -- BP and 0.1% Peptone Water -- PW) were evaluated for their effectiveness as a means of sampling for microbiological analysis of beef carcass fat tissue that was uninoculated or inoculated with one of two inoculum levels of *Escherichia coli*. In addition, storage of sponges after sampling at three temperatures (4, 7, or 15°C) for 24 h to simulate sample shipment was evaluated. The complete factorial experiment (3 inoculum levels x 2 sponge types x 2 buffer types x 3 storage temperatures x 2 storage times) was replicated four times.

Tissue sampling

Beef brisket fat tissue samples (12 x 12 cm), collected during cattle slaughter, were tested uninoculated or after inoculation with 2 ml of an *E. coli* ATCC 25922 culture suspension at each of two levels (4.3 or 7.4 log CFU/ml). The suspension was spread on the tissue with a sterile, bent glass rod and the brisket samples were allowed to sit at ambient temperature for 0.5 h to allow for bacterial attachment prior to sampling. After 0.5 h, a 10 x 10 cm sterile template was used to identify the area to be sampled following the United States Food Safety and Inspection Service (FSIS) Meat and Poultry Inspection Regulation sponging procedure, (Federal Register, 1996). Sponges were also analyzed after 24 h storage at 4, 7, or 15°C to simulate shipment to a laboratory for analysis.

Microbial analyses

The sponges were analyzed (after 0.5 and 24 h) for aerobic plate counts (APC) on tryptic soy agar (Difco Laboratories, Detroit, MI) using the spiral plating method (Spiral Plater model D, Spiral Biotech, Bethesda, MD), and for total coliform (TCC) and *E. coli* (ECC) counts on 3M Petrifilm™ *E. coli* count plates (3M Health Care, St. Paul, MN).

Statistical analyses

The data were converted to log CFU/cm² and analyzed by the analysis of variance and general linear model procedures of SAS (1990) using the independent variables of sampling sponges, hydrating buffers, inoculum level, storage time, storage temperature, and all possible interactions. Means and standard deviations were calculated from the four replications (SAS, 1990) and, when F-values were significant ($P \leq 0.05$), means were separated by use of the least significant difference procedure (SAS, 1990).

Results and Discussion

Concern has been expressed regarding the effectiveness of carcass sponging and *E. coli* recovery using the sponging procedure specified by FSIS for carcass microbiological sampling. There has also been some concern as to the viability of *E. coli* during shipping and storage. This study evaluated factors (sponge type, buffer type, storage temperature, and inoculum level) that may affect resulting counts.

Variables and interactions (Table 1) having significant ($P \leq 0.05$) effects on bacterial counts recovered were inoculum level (APC, TCC, ECC); storage time (APC); storage temperature (APC, TCC, ECC); inoculum level x sponge type (ECC); inoculum level x buffer

type (ECC); inoculum level x time (TCC, ECC); sponge type x time (TCC, ECC); sponge type x temperature (TCC); time x temperature (APC, TCC, ECC); inoculum level x sponge type x buffer type (TCC); and inoculum level x buffer type x temperature (ECC), while the variables of sponge type and buffer type had no significant ($P > 0.05$) effects on APC, TCC or ECC.

Storage (24 h) at 15°C resulted in generally higher counts compared to those recovered before storage of sponges, while storage of sponges at 4°C or 7°C for 24 h resulted in reduced TCC and ECC (Table 2). It appears that controlling storage/shipping temperature is more critical than sponge or buffer type with respect to the viability and recovery of *E. coli*, as well as to the accuracy of the microbiological results.

References

- Federal Register. 1996. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule. 61(144): 38806-38989. Washington, DC.
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- Kotrola, N.A., J.S. Kolytols, T.K. Phebus, J.L. Marsden and C.L. Kastner. Evaluation of the USDA sponge sampling technique for beef carcasses for enumeration of *Escherichia coli*. Paper presented at 84th Annual meeting of International Association of Milk, Food and Environmental Sanitarians, Orlando, FL. July 6-9 1997. Abstract (T28) p. 60-61.
- SAS. 1990. Statistical Analysis System: User's guide, vol. 2 4th ed. SAS Institute Inc., Cary, NC.

Tables

Table 1: Analysis of variance of aerobic plate counts (APC), total coliform counts (TCC) and generic *E. coli* counts (ECC) showing the effect of all significant ($P \leq 0.05$) treatments and interactions.

Variable	DF	APC		TCC		ECC	
		Mean square	F-value	Mean square	F-value	Mean square	F-value
Inoculum level ¹	2	166.2	107.7**	132.6	225.4**	145.2	314.7**
Storage time ²	1	10.5	6.8**	2.0	3.3	1.3	2.7
Storage temperature ³	2	18.1	11.7**	7.2	12.2**	4.6	9.9**
Inoculum x Sponge ⁴	2	2.2	1.4	1.3	2.2	2.7	5.8**
Inoculum x Buffer ⁵	2	3.2	2.1	1.4	2.4	2.6	5.7**
Inoculum x Time	2	0.6	0.4	2.4	4.1*	2.1	4.5*
Sponge x Time	1	3.2	2.1	4.4	7.5**	2.5	5.4*
Sponge x Temp	2	3.7	2.4	2.9	4.9**	0.4	1.0
Time x Temp	2	12.7	8.3**	4.5	7.7**	4.1	8.8**
Inoculum x Sponge x Buffer	2	0.5	0.3	1.9	3.1*	1.2	2.6
Inoculum x Buffer x Temp	4	0.7	0.4	0.9	1.5	1.8	3.9**

¹Inoculum level = Uninoculated, Level 1 (4.3 log CFU/cm²), Level 2 (7.4 log CFU/cm²).

²Storage time = 0.5 h or 24 h; ³Storage temperature = 4, 7 or 15°C; ⁴Sponge type = A or B.

⁵Buffer type = Butterfield's phosphate buffer (BP) or 0.1% Peptone water (PW).

* $P \leq 0.05$; ** $P \leq 0.01$.

Table 2: Effect of sponge storage time and temperature (4, 7 or 15°C) and inoculum level on means (SD) (log CFU/cm²) of aerobic plate counts (APC), total coliform counts (TCC) and generic *E. coli* counts (ECC) recovered by sponging of beef carcass tissue

Inoculation level ¹	Storage		APC	TCC	ECC
	temperature (°C)	Storage time (h)			
Uninoculated	4	0.5	1.9 (0.9)	1.0 (0.8)	0.9 (0.7)
Uninoculated	4	24	1.6 (1.0)	0.8 (0.5)	0.8 (0.4)
Uninoculated	7	0.5	1.9 (0.9)	0.7 (0.3)	0.7 (0.3)
Uninoculated	7	24	1.6 (1.2)	0.8 (0.4)	0.8 (0.3)
Uninoculated	15	0.5	2.1 (1.4)	1.1 (0.7)	0.9 (0.7)
Uninoculated	15	24	3.3 (2.3)	1.3 (1.0)	1.1 (0.9)
Inoculum level 1	4	0.5	2.7 (0.8)	1.1 (0.5)	1.4 (0.5)
Inoculum level 1	4	24	2.9 (1.2)	0.9 (0.4)	1.7 (1.0)
Inoculum level 1	7	0.5	3.2 (0.8)	1.6 (0.5)	1.1 (0.5)
Inoculum level 1	7	24	3.3 (1.3)	1.5 (1.0)	0.8 (0.4)
Inoculum level 1	15	0.5	3.1 (1.1)	1.7 (0.9)	1.4 (0.5)
Inoculum level 1	15	24	4.3 (1.4)	2.0 (1.1)	1.4 (1.0)
Inoculum level 2	4	0.5	4.6 (1.1)	3.7 (0.5)	3.6 (0.6)
Inoculum level 2	4	24	4.4 (1.2)	2.5 (0.3)	2.5 (0.3)
Inoculum level 2	7	0.5	4.4 (1.0)	3.4 (0.9)	3.4 (0.9)
Inoculum level 2	7	24	4.5 (1.0)	2.6 (0.9)	2.6 (0.9)
Inoculum level 2	15	0.5	4.4 (1.2)	3.3 (0.9)	3.3 (0.9)
Inoculum level 2	15	24	5.7 (1.1)	3.7 (1.5)	3.7 (1.1)

N=32.

BP = Butterfield's phosphate buffer; PW = 0.1% Peptone water.

¹Uninoculated = normal flora of the beef tissue; Inoculum level 1 = 4.3 log CFU/cm²; Inoculum level 2 = 7.4 log CFU/cm².

