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Bacterial Cross-Contamination of Meat during Liquid Nitrogen Immersion Freezing†

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ABSTRACT

Prerigor beef carcass surface tissue (BCT) was used to simulate lamb carcasses on a processing line with a 15-min liquid nitrogen (LN) immersion freezing step, and the potential for the dissemination of bacteria during freezing was examined. Streptomycin-resistant strains of *Listeria innocua* and *Escherichia coli* O157:H7 spiked into a fecal slurry were inoculated onto BCT pieces that were introduced into the freezing process to represent contaminated carcasses. Following this introduction, subsequently frozen uninoculated BCT, LN, and LN containers were examined for the inoculated organisms. In the first study, BCT samples were inoculated with ca. 7 log CFU/cm^2 of both *L. innocua* and *E. coli* O157:H7, spray washed with water and frozen, distributed among uninoculated BCT, in LN for 15 min. In two separate trials, *L. innocua* was recovered by enrichment from all uninoculated BCT and LN samples. *E. coli* O157:H7 was also recovered from uninoculated BCT and LN, but this cross-contamination was more sporadic. Both species were recovered from the LN container following freezing. Attempts to enumerate cross-contaminating bacteria in the second trial indicated that contaminating levels were low (<1.0 CFU/cm^2 BCT). In a second study, a 2.0% lactic acid spray wash was used to reduce further the numbers of *L. innocua* introduced into the freezing system and resulted in fewer positive samples, although this organism was still recovered from many uninoculated BCT samples. When either bacterium was inoculated at lower initial levels (1.35 to 1.77 log CFU/cm^2) and BCT was water or 2.0% lactic acid spray washed prior to freezing, neither *L. innocua* nor *E. coli* O157:H7 was recoverable by enrichment from uninoculated BCT, LN, or from the freezing container. Results demonstrate that bacterial cross-contamination of meat during LN immersion freezing can occur but indicate that the use of good sanitation practices and product with low microbial numbers can limit this occurrence.

The callipyge phenotype, which causes heavy muscling in sheep, greatly improves feed efficiency *(10)*, dressing percentage, and carcass composition *(11, 12, 14)*, without any apparent effect on the incidence or severity of dystocia. These advantages present the lamb industry with an attractive method of efficiently producing lean meat. However, a major drawback of the callipyge condition is its negative effect on longissimus tenderness. Callipyge longissimus has low indices of meat tenderness and likely would not meet consumer expectations *(7, 12, 14, 16)*. Before the lamb industry can utilize callipyge lambs, processes would have to be implemented to mitigate and possibly eliminate the negative effect on longissimus tenderness. Koohmaraie et al. *(13)* reported that freezing callipyge carcasses in liquid nitrogen (LN) immediately after slaughter or postrigor injection with calcium chloride can effectively mitigate the negative effect of callipyge phenotype on longissimus tenderness. These researchers suggested a freezing system in which hanging lamb carcasses are carried through an LN vat in much the same manner as hanging pork carcasses are carried through hot water for dehairing *(13)*. In the course of this work, a question arose: can cross-contamination of foodborne pathogens between carcasses occur upon continued use of LN in a processing line? To our knowledge, there are no other studies that have investigated this possibility for meats or meat products nor for other products such as poultry and poultry products, seafood, fruits, or vegetables, for which LN is in common use for direct contact freezing, although it has been presumed that this possibility is unlikely *(2)*. The objective of the experiments reported in this manuscript was to examine the extent to which bacterial cross-contamination may occur between carcasses that are frozen in an LN vat.

MATERIALS AND METHODS

Organisms and inocula preparation. Streptomycin-resistant *Escherichia coli* O157:H7 MARCS-1 and *Listeria innocua* MARCS-1 have been described *(3)*. Cultures were inoculated from frozen stock into tryptic soy broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.5% yeast extract and containing 250 μg/ml (for *E. coli* O157:H7) or 500 μg/ml (for *L. innocua*) streptomycin, and incubated quiescently for 18 h at 37°C.

Fresh bovine feces were collected from three different cows fed a corn-silage ration. Ten grams of each fecal sample were pooled; then the entire mixture was diluted 1:10 using distilled water and autoclaved. The cultures were collected by centrifugation (1,250 × *g*) and washed twice in sterile 0.85% NaCl. To prepare the inoculum for study 1, cells from 20-ml cultures of *E. coli* O157:H7 and *L. innocua* were resuspended to one-half of their

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† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

original volumes in the sterile fecal slurry, and these two suspensions were mixed to yield an inoculum containing 10^9 cells/ml of each species. For study 2, *L. innocua* was resuspended in the fecal slurry to a concentration of 10^9 cells/ml. For study 3, *E. coli* O157:H7 and *L. innocua* were diluted $1:10^4$ in 0.85% NaCl and inoculated into 20 ml of the sterile feces preparation, to give a level of about 10^3 cells of each organism per ml.

Beef tissue inoculation, spray washing treatment, and LN freezing. Lean beef carcass surface tissue (BCT) was collected from prerigor carcasses shortly after slaughter at a local cow/bull processing facility. The samples were placed in an insulated container and transported immediately to the laboratory at the Roman L. Hruska U.S. Meat Animal Research Center for use in experiments. The samples were 10- × 20- × 1-cm pieces of BCT that were cut from larger pieces using sterile scalpel blades and forceps. Samples used to represent contaminated samples were inoculated with 2 ml of the appropriate inoculum, which was spread evenly over the entire 10- × 20-cm external BCT surface using a sterile spoon. The inoculated samples were allowed to stand 15 min prior to spray washing.

The model carcass washer used to apply the spray-washing treatments, modified for use in a biological safety hood, has been described *(3)*. Individual BCT samples were mounted on the surface of a stainless steel plate and, as appropriate to the study and treatment, were spray washed with 32°C water or 32°C 2.0% (vol/vol) lactic acid delivered at 80 psi for 12 s. To prevent cross-contamination prior to LN freezing, the uninoculated BCT samples were spray washed before the inoculated samples. Following spray washing, samples were subjected to LN freezing.

To simulate a processing line-type LN immersion freezing process, BCT samples were frozen in turn in LN contained in a 4-liter Dewar flask (Nalge Nunc International, Milwaukee, Wisc.). A 10-cm-long end of each tissue sample was clipped with two sterile alligator clips affixed 10 cm apart along a 27-cm-long metal rod. The BCT was lowered slowly into the LN, and the rod was placed across the 14.6-cm inner-diameter mouth of the flask, thus suspending the BCT in the LN. For all three studies, each sample was immersed for a total of 15 min *(13)*, and LN was replenished as necessary to maintain complete immersion of the sample. Following freezing, the samples were removed to sterile trays and allowed to thaw at room temperature prior to microbiological analyses.

Study 1. Pieces of BCT representing contaminated samples were inoculated with the fecal inoculum containing ca. 10^9 CFU/ml each of *E. coli* O157:H7 and *L. innocua* (target level: 7 log CFU/cm^2 of BCT of each organism). Both inoculated and uninoculated BCT samples were spray washed with water prior to LN freezing. Samples were frozen in turn for 15 min in LN, beginning with an inoculated sample followed by three uninoculated samples. This sequence was repeated twice. Following each third uninoculated sample, two volumes of LN were removed for analysis. The LN was sampled by lowering a premarked, sterile, plastic wide-mouth dilution bottle into the flask using tongs and removing ca. 100 ml of LN. The opened bottles were placed in a biological safety cabinet, and the LN was allowed to evaporate before capping the bottles and proceeding with the microbial analysis of the residue. Upon completion of the experiment, the Dewar flask was also placed under the hood. When the LN had evaporated, the inside of the flask was sponge-sampled by the method of Siragusa et al. *(17)* using 25 ml of 2% buffered peptone water containing 0.1% Tween 20 (BPW-T) as sponge solution.

All inoculated BCT were sampled to determine the numbers of *L. innocua* and *E. coli* O157:H7. In trial 1 of this study, frozen inoculated and uninoculated BCT, LN, and sponge samples were enriched as appropriate to determine the presence of these microorganisms. In trial 2, *L. innocua* and *E. coli* were both directly enumerated and assayed after enrichment of the samples.

Study 2. BCT samples representing contaminated samples were inoculated, as previously described, with fecal slurry containing 10^9 CFU/ml of *L. innocua*. Both inoculated and uninoculated BCT were spray washed with water or 2.0% lactic acid.

Two separate Dewar flasks were utilized in this study, one for water-washed samples and the other for lactic acid-washed samples. For both spray-wash treatments, an inoculated sample was frozen first, followed in turn by five uninoculated samples, and all were frozen for 15 min. Following freezing of the inoculated BCT and the final uninoculated BCT sample, 100-ml volumes of LN were removed for analysis as described above. In addition, ca. 1-ml volumes of LN were removed for bacterial enumeration using a sterile transfer pipet. These volumes of LN were placed immediately in 10 ml of BPW contained in a 50-ml conical test tube. The LN was allowed to evaporate prior to capping the tubes and proceeding with analyses. As with study 1, the interiors of the flasks were sponge-sampled after the LN had evaporated.

Inoculated and uninoculated BCT, LN samples, and sponge samples were both sampled to enumerate *L. innocua* as well as enriched to determine the presence of this microorganism.

Study 3. Samples were inoculated with fecal slurry containing 10^3 CFU/ml of *L. innocua* and *E. coli* O157:H7, yielding 1 log CFU/cm^2 of BCT of each organism. Both inoculated and uninoculated BCT samples were spray washed with either water or 2.0% lactic acid prior to LN freezing. As in study 2, individual Dewar flasks were used for freezing BCT receiving the different wash treatments. Also as in study 2, the BCT representing the contaminated sample was frozen first, followed by five uninoculated samples; LN and sponge samples were taken at the same intervals as study 2.

L. innocua and *E. coli* O157:H7 were enumerated on all inoculated samples, as well as uninoculated BCT, LN, and sponge samples. In addition, all samples were enriched for both microorganisms.

Microbiological analyses. Prewash and prefrozen BCT samples were cut in half using sterile forceps and scalpels, and one 10- × 10-cm piece was used for analysis. Each piece was placed in a filtered stomacher bag (Spiral Biotech, Bethesda, Md.), 100 ml of BPW-T was added, and the sample was stomached for 2 min in a model 400 Stomacher Lab blender (Tekmar, Inc., Cincinnati, Ohio). Filtered stomachate was diluted as necessary in BPW and spiral-plated in duplicate using a model D spiral plater (Spiral Systems Instruments, Bethesda, Md.) onto the appropriate agar medium. *L. innocua* was enumerated on Oxoid *Listeria* selective agar (Unipath Ltd., Basingstoke, Hampshire, UK) containing 500 μg/ml streptomycin (LSA). *E. coli* O157:H7 was enumerated on sorbitol MacConkey agar (Difco) containing 250 μg/ml streptomycin (SMAC). Bacteria on the low-inoculum postwash samples of study 3 were both enumerated and enriched as described below for frozen BCT samples.

Frozen BCT samples were allowed to thaw before they were divided in half, and one 10- × 10-cm piece was used for the analysis of each organism. To enrich for *L. innocua*, 100 ml of UVM modified *Listeria* enrichment broth (UVM; BBL, Cockeysville, Md.) was added to the sample in a stomacher bag, and the contents were stomached 2 min. Where enumeration of *L. innocua* was performed, 1 ml of this filtered stomachate was removed for plating. For inoculated BCT samples, each stomachate was diluted as necessary and spiral plated in duplicate onto LSA. For each uninoculated BCT sample, the 1-ml volume was divided evenly

between four LSA spread plates. The remaining UVM enrichment broth plus sample was incubated for 24 h at 30°C. Following this incubation, a 0.1-ml volume was transferred into 10 ml of Fraser broth (Bacto Fraser broth; Difco), which was incubated for 24 h at 35°C. LSA plates were streaked in duplicate with loopfuls of Fraser broth. For the enrichment of *E. coli* O157:H7, 100 ml of EC medium (International BioProducts Inc., Redmond, Wash.) containing 20 μg/ml novobicin (EC) was added to the second 10- × 10-cm BCT sample and the stomacher bag contents were stomached 2 min. For enumeration of *E. coli* O157:H7, a 1-ml volume of each stomachate was removed for plating on SMAC. The remaining sample plus enrichment was incubated with 100 rpm shaking at 37°C for 24 h. Following this incubation, the EC enrichment was streaked in duplicate onto SMAC.

Residues from 100-ml volumes of the LN were enriched for *L. innocua* and *E. coli* O157:H7 by adding 100 ml of UVM or EC to the bottles containing the residues. The bottles were capped and shaken vigorously. The broths were then poured into stomacher bags, and enrichments were incubated and examined as described for BCT samples. For enumeration of bacteria in LN in studies 2 and 3, 1-ml volumes of the 10 ml of BPW-containing residue from 1 ml of LN were divided evenly between four LSA or SMAC spread plates.

Following sponge sampling of each LN flask, the sponge was returned to the remaining sponge solution in the sample bag, and the bag contents were stomached 2 min. Five-milliliter volumes of the sponge solution were enriched in 100 ml UVM or EC as described. Where *L. innocua* and *E. coli* present on the container surface were enumerated, 1-ml volumes of the sponge solution were divided between four LSA or SMAC spread plates.

For all enumeration and enrichment isolations, LSA and SMAC plates were incubated for 24 h at 37°C before counting of or examination for typical *Listeria* or *E. coli* O157:H7 colonies, respectively. In addition, typical *E. coli* O157:H7 isolates were randomly selected and confirmed serologically (*E. coli* O157 test kit; Unipath).

RESULTS AND DISCUSSION

In this work, prerigor BCT was used to represent lamb carcasses on a processing line that included a 15-min LN freezing step. Streptomycin-resistant *L. innocua* and *E. coli* O157:H7 were utilized to facilitate the detection of potential bacterial cross-contamination during LN immersion freezing and were spiked into a sterile fecal slurry for use as a meat inoculant, since feces are a vehicle for the introduction of bacterial contamination to carcasses. To further simulate conditions that may normally be encountered in a slaughter process, BCT was spray washed with either water or 2.0% lactic acid prior to freezing, as both are common antimicrobial interventions used to reduce the numbers of bacteria on carcasses.

Study 1 was designed to enhance the detection of potential bacterial cross-contamination by accelerating the introduction of bacteria into the LN freezing process in the form of frequently occurring, highly contaminated "carcasses." The water spray wash reduced the initial populations of 7.0 log CFU/cm^2 of both *L. innocua* and *E. coli* to ca. 5.6 to 5.8 log CFU/cm^2 in the first trial and to ca. 6.7 log CFU/cm^2 in the second trial (Table 1). LN freezing reduced viable cell counts of both organisms, ranging over the two trials from log reductions of 0.33 to 1.77 log CFU/cm^2 for *L. innocua*, and 2.18 to 4.02 log CFU/cm^2 for *E. coli* O157:H7, as compared to the cell numbers remaining after spray washing. It is well established that freezing typically results in the immediate mortality of a proportion of a bacterial culture *(6, 8)*. The greater inactivation of *E. coli* seen in this study is likely a reflection of the generally greater susceptibility of gram-negative bacteria to rapid cooling, as compared to gram-positive bacteria *(9, 15)*. In addition, members of the genus *Listeria*, including *L. monocytogenes* and *L. innocua*, have been demonstrated to be tolerant to freezing *(4, 5)*. In both trials, *L. innocua* was recovered by enrichment from all uninoculated BCT, LN, and LN container samples. *E. coli* O157:H7 was detected on numerous uninoculated BCT samples, although its occurrence was more intermittent in comparison to that of *L. innocua*. In addition, *E. coli* was recovered by enrichment from both container samples but from only one of six LN samples.

In the second trial of this study, enumeration of cross-contaminating bacteria in uninoculated samples was attempted. *L. innocua* was recovered by direct plate count from four of the nine uninoculated BCT samples and the inside surface of the LN container, and *E. coli* was only recovered from the LN container. In all instances where either species was detected, the numbers on uninoculated samples were estimated to be less than 1 CFU/cm^2 of BCT or LN container surface. No bacteria were recovered by direct plate count of LN. Thus, despite the high bacterial counts on the inoculated meat and the common event of cross-contamination, the levels of this cross-contamination were quite low.

The greater occurrence of cross-contamination by *L. innocua*, as compared to *E. coli*, is likely a consequence of the greater freezing resistance of this microorganism, which resulted in greater numbers of surviving cells being available for dissemination to uninoculated BCT and surfaces. Thus, a lactic acid spray wash was employed in study 2 to determine if a greater reduction of *L. innocua* from inoculated BCT prior to freezing would affect the occurrence of cross-contamination. Compared to the water spray wash, the 2.0% lactic acid spray wash resulted in additional log reductions of 1.91 and 2.37 from inoculated BCT in trials 1 and 2, respectively (Table 2). As in study 1, there was little additional reduction of *L. innocua* due to freezing of inoculated BCT, regardless of the spray-wash treatment. Although *L. innocua* was recovered from several uninoculated samples in the lactic acid treatment, the use of 2.0% lactic acid, and subsequently lower levels of introduced *L. innocua*, did appear to have some impact on the frequency of detectable cross-contamination. In the first trial, *L. innocua* was recovered by enrichment from all uninoculated samples receiving the water spray-wash treatment and from all but the LN samples in the lactic acid spray-wash treatment. In the second trial, the organism was not recovered in LN sample enrichments from either treatment regime. In this trial, *L. innocua* was recovered by enrichment from all five uninoculated BCT that were water spray washed and from three of five uninoculated BCT that were lactic acid spray washed. In addition, *L. innocua* was detected by direct plate count on four of five uninoculated BCT in the water

TABLE 1. *Study 1—recovery of* Listeria innocua *and* Escherichia coli *O157:H7 from beef carcass tissue following liquid nitrogen immersion freezing—introduction of high numbers of* L. innocua *and* E. coli *O157:H7*

Sample order	Sample identification	Trial 1				Trial 2			
		Log_{10} CFU/cm² [a]		Enrichment		Log_{10} CFU/cm² [a]		Enrichment	
		L. innocua	*E. coli*	*L. innocua*	*E. coli*	*L. innocua*	*E. coli*	*L. innocua*	*E. coli*
	Before water wash and freezing:								
0	Inoculated BCT[b]	7.14	7.03	NT[c]	NT	6.94	7.06	NT	NT
	After water wash and before freezing:								
1	Inoculated BCT	5.81	5.63	NT	NT	6.68	6.71	NT	NT
	After water wash and freezing:								
2	Inoculated BCT	5.48	2.93	+[d]	+	5.11	2.69	+	+
3	BCT	NT	NT	+	−[d]	ND[e]	ND	+	−
4	BCT	NT	NT	+	+	ND	ND	+	−
5	BCT	NT	NT	+	+	ND	ND	+	−
6	Liquid nitrogen	NT	NT	+	−	ND	ND	+	−
7	Inoculated BCT	4.99	3.45	+	+	5.25	3.37	+	+
8	BCT	NT	NT	+	+	D[f]	ND	+	−
9	BCT	NT	NT	+	+	ND	ND	+	+
10	BCT	NT	NT	+	+	ND	ND	+	−
11	Liquid nitrogen	NT	NT	+	−	ND	ND	+	−
12	Inoculated BCT	5.26	3.20	+	+	4.91	3.19	+	+
13	BCT	NT	NT	+	+	D	ND	+	+
14	BCT	NT	NT	+	+	D	ND	+	+
15	BCT	NT	NT	+	+	D	ND	+	+
16	Liquid nitrogen	NT	NT	+	−	ND	ND	+	+
17	Container	NT	NT	+	+	D	D	+	+

[a] Values for inoculated samples are calculated for the 100-cm² of inoculated tissue surface; values for uninoculated samples are calculated for the entire 240-cm² suface of the tissue samples.

[b] BCT, beef carcass surface tissue.

[c] NT, not tested.

[d] + or − denote positive or negative recovery following enrichment for the indicated bacterial species.

[e] ND, not detected.

[f] D, detected at a cell level of <1.0 CFU/cm².

spray-wash treatment but was not detected by direct count on any uninoculated BCT in the lactic acid spray-wash treatment, indicating that the numbers of cross-contaminating bacteria were reduced due to the lactic acid spray wash. As seen in study 1, plate counts of all uninoculated samples indicated that the level of cross-contaminating *L. innocua* was less than 1 CFU/cm² of BCT.

Study 3 differed from study 2 in that *E. coli* was included with *L. innocua* in the fecal inoculum, and initial inoculation cell concentrations were lower than those used in either of the first two studies. These lower numbers were potentially more realistic with regard to levels likely to be encountered on commercial carcasses *(1)*. BCT was inoculated with ca. 1.70 log CFU/cm² of *L. innocua* and ca. 1.56 log CFU/cm² of *E. coli* and spray washed with either water or 2.0% lactic acid prior to freezing. At these low inoculation levels and for either spray-wash regime, neither species was recovered by plate count or by enrichment from uninoculated BCT, LN, or the LN containers following freezing (Table 3).

These experiments demonstrated that cross-contamination of bacteria between product can occur during freezing by immersion in LN. The U.S. Food Safety and Inspection Service recently has published a direct final rule allowing for the use of LN for contact freezing of meat and meat products *(2)*. It is hypothesized in this rule that cross-contamination of microorganisms is unlikely "because of the extremely fast chill, creating an immediate stabilization of the exterior surfaces upon contact." While the immediate freezing of the product surface is apparent, the vigorous boiling that occurs upon the initial immersion of warm product into LN may serve to dislodge some bacteria from the surface, making it available for contamination of subsequently frozen product. Thus, it is uncertain whether the LN dwell time used in the current work is important with respect to increasing the probability of bacterial cross-contamination. BCT was immersed in LN for 15 min, as this is the approximate time required for the interior of the longissimus muscle of a freshly slaughtered lamb carcass to fall to a temperature less than 0°C when immersed in LN *(13)* and is a longer time than would be usual for smaller, individual portions of meat or other product. BCT was used instead of lamb tissue primarily due to availability, and while we acknowledge that some difference in bacterial attachment to carcass surfaces of these species may exist, the impact of any differences on bacterial cross-contamination would be unlikely. The results of the current work imply that bacterial cross-contamination may be possible for any of a number of different types of

TABLE 2. *Study 2—effect of water vs. 2.0% lactic acid spray washing on recovery of* Listeria innocua *from beef carcass tissue following liquid nitrogen immersion freezing—introduction of high numbers of* Listeria innocua

Sample order	Sample identification	Trial 1		Trial 2	
		Water wash	Lactic acid wash	Water wash	Lactic acid wash
		Log_{10} CFU/cm^2 [a]/ enrichment	Log_{10} CFU/cm^2 [a]/ enrichment	Log_{10} CFU/cm^2 [a]/ enrichment	Log_{10} CFU/cm^2 [a]/ enrichment
	Before wash and freezing:				
0	Inoculated BCT[b]	7.17/NT[c]	7.17/NT	6.56/NT	6.56/NT
	After wash and before freezing:				
1	Inoculated BCT	5.94/NT	4.03/NT	5.95/NT	3.58/NT
	After wash and freezing:				
2	Inoculated BCT	5.82/+[d]	4.21/+	5.90/+	2.99/+
3	Liquid nitrogen	ND[e]/+	ND/−[d]	ND/−	ND/−
4	BCT	D[f]/+	D/+	D/+	ND/+
5	BCT	ND/+	ND/+	D/+	ND/−
6	BCT	ND/+	ND/+	D/+	ND/+
7	BCT	ND/+	ND/+	ND/+	ND/−
8	BCT	ND/+	ND/+	D/+	ND/+
9	Liquid nitrogen	ND/+	ND/−	ND/−	ND/−
10	Container	D/+	D/+	ND/+	ND/+

[a] Values for inoculated samples are calculated for the 100-cm^2 of inoculated tissue surface; values for uninoculated samples are calculated for the entire 240-cm^2 surface of the tissue samples.
[b] BCT, beef carcass surface tissue.
[c] NT, not tested.
[d] + or − denote positive or negative recovery following enrichment for the indicated bacterial species.
[e] ND, not detected.
[f] D, detected at cell level of <1.0 CFU/cm^2.

TABLE 3. *Study 3—effect of water vs. 2.0% lactic acid spray washing on recovery of* Listeria innocua *and* Escherichia coli *O157:H7 from beef carcass tissue following liquid nitrogen immersion freezing—introduction of low numbers of* L. innocua *and* E. coli *O157:H7*

Sample order	Sample identification	Trial 1 (log_{10} CFU/cm^2 [a]/enrichment)				Trial 2 (log_{10} CFU/cm^2 [a]/enrichment)			
		Water wash		Lactic acid wash		Water wash		Lactic acid wash	
		L. innocua	*E. coli*	*L. innocua*	*E. coli*	*L. innocua*	*E. coli*	*L. innocua*	*E. coli*
	Before wash and freezing:								
0	Inoculated BCT[b]	1.64/NT[c]	1.35/NT	1.64/NT	1.35/NT	1.75/NT	1.77/NT	1.75/NT	1.77/NT
	After wash and before freezing:								
1	Inoculated BCT	0.40/+[d]	0.40/+	ND[e]/+	0.48/+	0.95/+	ND/+	ND/+	ND/+
	After wash and freezing:								
2	Inoculated BCT	0.90/+	ND/+	ND/−[d]	ND/+	0.60/+	ND/+	ND/+	ND/−
3	Liquid nitrogen	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−
4	BCT	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−
5	BCT	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−
6	BCT	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−
7	BCT	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−
8	BCT	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−
9	Liquid nitrogen	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−
10	Container	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−	ND/−

[a] Values for inoculated samples are calculated for the 100-cm^2 of inoculated tissue surface; values for uninoculated samples are calculated for the entire 240-cm^2 surface of the tissue samples.
[b] BCT, beef carcass surface tissue.
[c] NT, not tested.
[d] + or − denote positive or negative recovery following enrichment for the indicated bacterial species.
[e] ND, not detected.

food products frozen in a continuous LN immersion freezing system.

In conclusion, the dissemination of bacterial contamination of meat during LN immersion freezing was demonstrated, which may be important to the microbial safety or spoilage of meat products, especially if thawed and stored under refrigeration for an extended period of time prior to further processing or heating. However, it was observed that the levels of cross-contaminating bacteria were low, even when high levels of bacteria were introduced into the freezing process on contaminated meat. When lower levels of bacteria were introduced, cross-contamination was not detected. These results indicate that proper sanitation practices and the use of quality product with low initial numbers of bacteria will serve to limit the occurrence of bacterial cross-contamination during LN immersion freezing, thus retaining the benefits of food preservation by this method.

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