

Research Note

Impact of pH Enhancement on Populations of *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in Boneless Lean Beef Trimmings[†]

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ABSTRACT

Boneless lean beef trimmings were inoculated with multiple strains of salmonellae, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 at levels of ca. $6 \log_{10}$ CFU/g. pH enhancement with ammonia gas was then used to increase the pH of the trimmings to ca. 9.6. The product was then frozen, chipped, and compressed into blocks. pH enhancement reduced the populations of salmonellae, *L. monocytogenes*, and *E. coli* O157:H7 by approximately 4, 3, and $1 \log_{10}$ cycles, respectively. After the product had been frozen and compressed into blocks, no salmonellae or *E. coli* O157:H7 were detectable by enumeration or after enrichment and isolation. The final populations of *L. monocytogenes* were reduced by ca. $3 \log_{10}$ cycles relative to the initial populations. When uninoculated pH-enhanced lean boneless trimmings were blended with inoculated ground beef to a final concentration of 15% (wt/wt), pathogen populations in the ground beef were reduced by approximately $0.2 \log_{10}$ cycles.

Escherichia coli O157:H7 is a significant human pathogen and has been linked to several foodborne-disease outbreaks involving ground beef. It is estimated that approximately 73,000 cases of illness are caused by this pathogen annually in the United States and that approximately 85% of these cases result from foodborne transmission (7). A recent ground beef–related disease outbreak highlights the need for additional research on the control of this *E. coli* O157:H7 (2).

Lean boneless trim (LBT), also referred to as lean finely textured beef, is produced from beef trimmings. The beef trimmings used to produce LBT are warmed to approximately 42°C and processed through a centrifugal separator to produce two output streams: an edible beef fat stream and a lean (<5% ± 2% fat) beef trimmings stream (the LBT stream). The edible beef fat stream is further processed. The LBT stream is rapidly frozen on a drum freezer, and the frozen product is broken into chips (ca. 1 by 1 by 0.3 cm). The chips are then compressed into blocks at up to 4,000 lb/in² and packed into boxes. The entire process, from the warming of the beef trimmings to the final boxing, takes less than 20 min (10). The resulting frozen product is then used as an ingredient in formulated meat products and ground beef.

Because of concerns about microbial contamination, an additional step has been developed as an antimicrobial in-

tervention in the overall process. This step involves rapidly raising the pH of the LBT to approximately 9.5 through the injection of ammonia gas into the product (pH enhancement (10)). The objective of this study was to evaluate the antimicrobial effects of pH enhancement on populations of bacteria that may occur in ground beef, as well as the public health significance of this treatment. An additional study was conducted to evaluate how microbial populations are affected by the blending of the pH-enhanced product with ground beef.

MATERIALS AND METHODS

Bacterial cultures. Five-strain mixtures of *E. coli* O157:H7 and *Listeria monocytogenes* and three strains of salmonellae were prepared by growing each of the individual cultures in tryptic soy broth or (for *L. monocytogenes*) in tryptic soy broth containing 0.6% yeast extract at 37°C for 18 h and then mixing 1-ml portions of each of the separate cultures into a homogenous mixture. The cultures were selected to represent both human clinical isolates and strains that are commonly associated with beef. The *L. monocytogenes* strains also included strains isolated from ready-to-eat meat products implicated in a large multistate outbreak of listeriosis. The individual strains of each of the bacteria are listed in Table 1.

pH-enhanced LBT. Normal processing procedures were used to prepare regular LBTs. The meat was inoculated with a mixture of the three different bacteria to a final population of approximately 2×10^6 CFU/g (1×10^5 CFU/g for *L. monocytogenes*) by adding 500 ml of the mixed cultures to ca. 100 kg of the meat and mixing the cultures and meat in a commercial paddle-type mixer for 10 min. pH enhancement of the meat was carried out by injecting gaseous ammonia and mixing until the pH

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TABLE 1. Bacterial strains used for inoculation purposes

Bacterium	Strains ^a
<i>Escherichia coli</i> O157:H7	FSRL-Y24, ^b FSRL-Y25, ^b ATCC 43895, ^c ATCC 43894, ^c ATCC 35150 ^c
<i>Listeria monocytogenes</i>	H7762 (4b), ^d H7769 (4b), ^d 1/2a FSIS, ^d Scott a (4b), ^c H7764 (1/2a) ^d
<i>Salmonella enterica</i>	Dublin (FSRL-G10), ^b Typhimurium (FSRL-G68), ^b Typhimurium (FSRL-G32) ^b

^a FSRL, Food Safety Research Laboratory, Iowa State University.

^b Veterinary clinical isolate (cattle).

^c Human clinical isolate.

^d Food product isolate.

had been raised to ca. 9.6. To simulate the remaining LBT production process, the product was then frozen on a drum freezer and broken into chips. The chips were then compressed under pressure to form a block. Approximately half of the pH-enhanced meat was removed from the mixer for freezing, and then pelleted CO₂ was added to lower the pH by ca. 1 pH unit (LBT-CO₂). The resulting product was then further processed as described above.

Blended ground beef. Since federal regulations allow the blending of LBT with ground beef, experiments were conducted to determine how microbial populations are affected by the blending of pH-enhanced LBT with ground beef. Approximately 50 kg of ground beef (90% lean) was inoculated with the mixed bacterial culture as previously described. Uninoculated pH-enhanced LBT was added to the ground beef (15%, wt/wt), and the LBT and beef were blended in a commercial mixer for 10 min.

Experimental design: pH-enhanced LBT. Samples were obtained for analysis after inoculation and mixing, after pH enhancement, and after frozen-block formation. Ten independent samples were analyzed at each sampling point and held at 4°C (after mixing) or -20°C (for chips and blocks) pending analysis.

Experimental design: blended ground beef. Samples were obtained for analysis after inoculation (ground beef) and after mixing with LBT (blended ground beef). Additional samples of the blended ground beef were taken and stored at 4°C for up to 48 h. Five independent samples were analyzed at each sampling point. All samples were analyzed within 24 h of processing except for the blended ground beef samples, which were intentionally stored for an additional 24 or 48 h.

Microbiological analysis. Samples (25 g each) were homogenized (1:10) in sterile buffered peptone water with a Tekmar Stomacher 400 Mark II (Tekmar, Cincinnati, Ohio). Bacterial populations were enumerated with the use of a Whitley Automated Spiral Plater (Microbiology International, Frederick, Md.). Salmonellae were enumerated on xylose lysine desoxycholate agar, *E. coli* O157:H7 strains were enumerated on sorbitol MacConkey agar, and *L. monocytogenes* strains were enumerated on modified Oxford medium. Plates were incubated for 24 h (48 h for *L. monocytogenes*) at 37°C, and bacteria were enumerated with a ProtoCOL plate counter (Microbiology International). Bacterial counts were transformed to log₁₀ values and analyzed with Proc GLM of SAS (SAS Institute, Inc., Cary, N.C.). A 25-g portion of each sample for which there were no observable colonies on the selective media after incubation was enriched (1:10) in buffered

TABLE 2. pHs of pH-enhanced LBT with and without added CO₂

Sampling point	pH of:	
	pH-enhanced LBT	pH-enhanced LBT with CO ₂
After inoculation	6.04	6.04
After pH enhancement	9.63	9.63
After addition of CO ₂		8.76
Frozen-chip stage		8.68
Frozen-block stage	8.72	8.54

peptone water for 24 h at 37°C and then streaked on the media for isolation to determine whether there were survivors present at levels below the assay's limits of detection. All culture media were obtained from Difco, Becton, Dickinson and Company (Sparks, Md.).

pH analysis. A subsample was taken from each sample prior to homogenization for pH analysis. Each 10-g subsample was thoroughly mixed with 90 ml of degassed distilled water, and its pH was determined with a pH meter (Fisher Scientific).

RESULTS

pH-enhanced LBT. The pH enhancement (ammoniation) of LBT resulted in an immediate pH increase of ca. 3.5 pH units (Table 2). Ammoniation reduced populations of *E. coli* O157:H7 by ca. 3.0 log₁₀ CFU/g, reduced populations of salmonellae by ca. 4.5 log₁₀ CFU/g, and reduced populations of *L. monocytogenes* by ca. 0.5 log₁₀ CFU/g (Fig. 1). After the product had been frozen and formed into blocks, the populations of *E. coli* O157:H7 and salmonellae were reduced to below the detection limits of the assay. The freezing and compression of non-pH-enhanced LBT was found to reduce populations of the enteric bacteria by <1 log₁₀ cycle (data not shown). Subsequent enrichment of the frozen-block samples demonstrated that the samples contained no recoverable *E. coli* O157:H7 or salmonellae. *L. monocytogenes* survived in the frozen blocks at ca. 1.5 log₁₀ CFU/g.

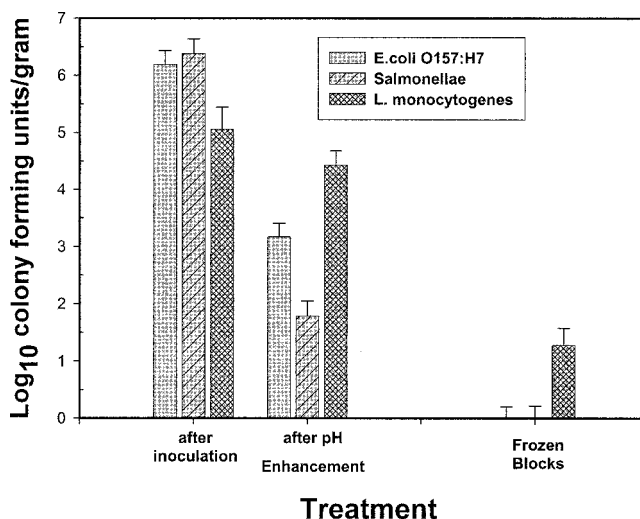
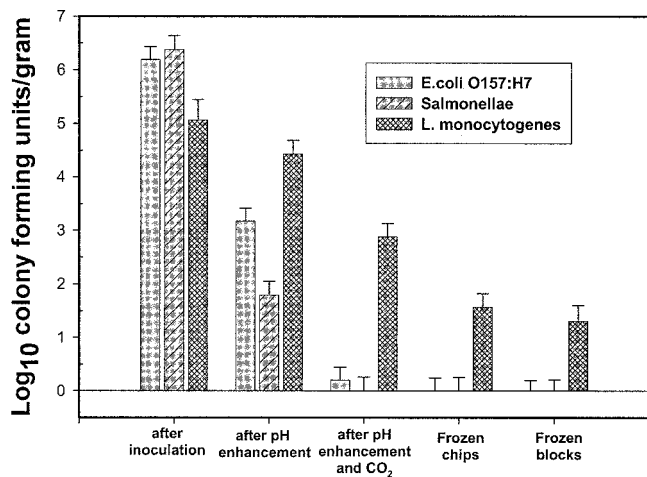


FIGURE 1. Populations of *E. coli* O157:H7, salmonellae, and *L. monocytogenes* in inoculated pH-enhanced LBT.



Treatment

FIGURE 2. Populations of *E. coli* O157:H7, salmonellae, and *L. monocytogenes* in inoculated pH-enhanced LBT-CO₂.

Addition of CO₂ to pH-enhanced LBT. The addition of CO₂ to the LTB immediately lowered the pH by approximately 1 pH unit (Table 2), reduced populations of salmonellae to below the detection limits of the enumeration assay, and significantly reduced populations of *E. coli* O157:H7 to levels below those observed with pH enhancement (Fig. 2). The reduction in pH did result in the recovery of viable *E. coli* O157:H7 and salmonellae from the LTB-CO₂, however (Table 3). Although all 10 samples at each sampling point (after the addition of CO₂, at the frozen-chip stage, and at the frozen-block stage) were below the detection limits of the assay, viable bacteria were recovered after enrichment. *L. monocytogenes* survived in the frozen blocks at ca. 1.3 log₁₀ CFU/g. The presence of viable *E. coli* O157:H7 and salmonellae in the samples after the addition of CO₂ suggests that the lowering of the pH allowed some of these bacteria to survive, but the populations were clearly quite small.

Blended ground beef. The blending of pH-enhanced LBT with inoculated ground beef raised the pH from 6.43 to 6.68. Populations of *E. coli* O157:H7 and salmonellae were reduced by ca. 0.2 log₁₀ cycles, although this reduction was not statistically significant ($P > 0.10$; Fig. 3).

DISCUSSION

Boneless beef trim, the material used to produce LBT, consists of irregularly shaped high-fat pieces of beef and typically includes most of the material from the outer surfaces of the carcass. While this material is a product of the processing of federally inspected and passed cattle, it does contain larger microbiological populations than most intact muscle cuts. Deep muscle tissue from healthy animals is presumed to be sterile (1), but microbiological contamination arising from processing may occur on the outer surfaces of animal carcasses. For this reason, it is reasonable to examine potential microbiological intervention strategies for the production of LBT.

There exists a considerable body of research on the use

TABLE 3. Numbers of LTB-CO₂ samples that tested positive after enrichment in buffered peptone water

Sampling point	No. of samples positive for bacterium after enrichment ^a	
	<i>E. coli</i> O157:H7	Salmonellae
After inoculation	NA	NA
After pH enhancement	NA	NA
After addition of CO ₂	4 (10)	10 (10)
Frozen-chip stage	5 (10)	10 (10)
Frozen-block stage	6 (10)	9 (10)

^a The number in parentheses is the number of samples ($n = 10$) for which population levels were below the detection limit of the enumeration assay. NA, not applicable (samples contained levels of bacteria that were sufficient for enumeration).

of organic acids for microbial interventions (4). However, relatively few data on the use of alkaline agents with meat products exist (3, 5, 6). Gram-negative bacteria are thought to be more susceptible to the effects of high-pH environments because their cell walls are thinner than those of gram-positive bacteria (8). The results of the experiments presented here are in agreement with this theory, with reductions in the populations of the gram-negative bacteria always being larger than reductions in the populations of the gram-positive bacteria.

The results of the initial studies involving pH-enhanced LBT demonstrate that raising the pH of the meat to >pH 9.0 had an immediate bactericidal effect on the bacteria. When pH enhancement was combined with compression and freezing, populations of *E. coli* O157:H7 and salmonellae were reduced to levels that were not detectable even after enrichment. Freezing and compression would reduce bacterial populations somewhat, but the addition of pH enhancement to the treatment process resulted in a significantly larger reduction in these populations. The pH enhancement process effectively reduced populations of both *E. coli* O157:H7 and salmonellae by 6 log₁₀ CFU/g and effectively reduced populations of *L. monocytogenes* by 3.5

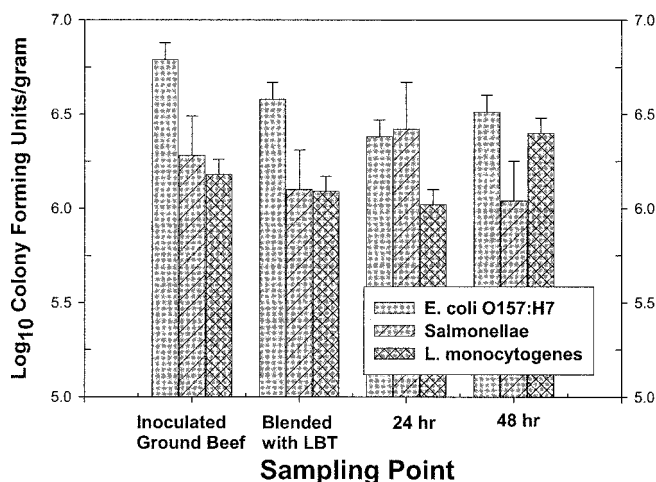


FIGURE 3. Populations of *E. coli* O157:H7, salmonellae, and *L. monocytogenes* in inoculated ground beef blended with pH-enhanced LBT.

\log_{10} CFU/g and represents a significant intervention for LBT.

Because of potential issues surrounding the palatability of a pH-9.5 product, an effort was made to reduce the pH of the product with dry ice after the initial pH enhancement. The lowering of the pH allows the survival of a portion of the bacterial population, although the overall reductions in bacterial populations were still highly significant and would provide a significant margin of safety for the product.

The blending of LBT with ground beef is allowed under federal regulations. When pH-enhanced LBT was blended into inoculated ground beef, little if any visible change in the ground beef was observed. The high-pH LBT did slightly raise the overall pH of the blend, but this outcome is unlikely to have any practical effect on the product. The bacterial populations in the blended ground beef did exhibit a slight, although not statistically significant, decrease as a result of blending. This finding was consistent with the results of an earlier study (data not shown), in which a similar ($\sim 0.2\text{-}\log_{10}$) reduction in the population of *E. coli* O157:H7 in 18% (wt/wt) blended ground beef was found when the initial *E. coli* level was ca. $3.0 \log_{10}$ CFU/g. This finding may suggest an inhibitory effect of the pH-enhanced product, since the observed reduction was consistently larger than the reduction that would be expected merely from the dilution effect arising from the addition of uninoculated LBT to inoculated ground beef. Further studies will be carried out to determine the effect of the addition of pH-enhanced LBT to ground beef inoculated with very low levels of pathogenic bacteria. The reported populations of *E. coli* O157:H7 in meat linked to foodborne disease outbreaks are generally small (typically <50 CFU/g (9, 11)). The pathogenic bacterial population reductions reported in this study would provide a significant margin of safety for LBT and may be applicable to other fresh meat products.

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