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A Representative Microbial Sampling Method for Large Commercial Containers of Raw Beef Based on Purge†

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ABSTRACT

The purge from beef combos (a boxed collection of beef trimmings) was tested as a means of representatively sampling the microbial content of this raw product. In the first experiment, purge was sampled from model beef combos that had been inoculated with bovine feces. Data from this experiment indicated a strong correlation ($r = 0.94$) between the total aerobic bacteria counts derived from the purge samples of a model beef combo and the total aerobic bacteria present in a rinse sample of the entire model beef combo. In a second experiment, two 500-g meat pieces were inoculated with an antibiotic-resistant *Escherichia coli* O157:H7 and placed at various levels within a 75-cm meat column. The marked bacteria were retrievable from the purge of the meat column after 24 h, showing that bacteria are carried downward into the purge. During the third part of the study, 90 beef combos (~900 kg beef/combo) were randomly selected at the receiving dock of a commercial grinding facility and sampled using both purge and concurrently used 11-g core samples. Purge samples from these combos recovered significantly greater numbers of mesophilic and psychrotrophic aerobic bacteria, coliforms, and *E. coli* than core samples from the same combos. Additionally, coliforms and *E. coli* were recoverable from 100% and 80%, respectively, of the purge samples taken, whereas core samples were only able to recover 60% and 40%, respectively, from the same combos. These findings indicate that a purge sample from a beef combo is a more efficacious sampling method for determining the general bacterial profile and identifying the presence of coliforms and *E. coli* than randomly taken core samples.

In recent years, ground beef has been implicated in several human disease outbreaks. As a result, the U.S. Department of Agriculture (USDA)–Food Safety and Inspection Service (FSIS) has declared *Escherichia coli* O157:H7 an adulterant in raw ground beef as defined by the Federal Meat Inspection Act (*2*). Consequently, the FSIS initiated a national survey testing program for the presence of *E. coli* O157:H7 in ground beef and is in the process of implementing hazard analysis and critical control point (HACCP)–based food safety regulations (*3*). These events have elevated the need for more reliable and representative microbial sampling methods to a high priority. This is especially true with regard to ground beef production because there is no standard sampling method for beef combos that is representative or is sufficiently rapid for HACCP monitoring.

It is commonly accepted that any sample from a given food used to determine the microbial profile must be both adequate and representative. This becomes even more critical when assaying for pathogens that are sparsely distributed within a large area, as is the case in a 900-kg raw beef combo. The typical commercial combo is a large 3-ft^3 plastic-lined cardboard container of cut beef pieces supported by a wooden pallet. These combos produce a reservoir of meat juices (purge) at their base. A few studies have successfully used purge from vacuum-packaged beef to assay for specific bacteria (*5, 6, 8*). The use of purge for sampling microorganisms from packaged meat has also been mentioned in at least one sampling review (*7*). The current study examined the efficacy of purge samples to adequately represent the overall microbial profile of commercial beef combos.

MATERIALS AND METHODS

Sample preparation. Phase 1. Lean beef tissue was collected from beef carcasses less than 15 min after slaughter from a local cow/bull processing facility. Beef tissue was placed in a plastic bag and then into an insulated container to maintain carcass temperature and immediately transported to the laboratory at the USDA Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE (MARC). Transportation time was approximately 30 min. Tissue was then removed from the plastic bags, individually placed onto a sterile cutting board, and cut to a size of 1,000 g (~30 × 30 × 1 cm).

Phase 2. Lamb meat was collected by deboning 35 lambs slaughtered and chilled for 24 h in the USDA-inspected abattoir located at MARC. Lamb meat was used for this phase of the study because it was determined to be a good model for red meat, easy to obtain in the quantities necessary, and inexpensive enough to be destroyed after being inoculated with a pathogen. To mimic a commercially produced meat combo, the deboned meat pieces were of various sizes and shapes. After deboning, meat pieces were immediately transported to a laboratory housed in the same building.

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† Mention of a trade name, proprietary product, or specific equipment is necessary to report factually on available data; however, the 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.

Phase 3. Raw beef combos (ca. 900 kg) from eight different suppliers were randomly sampled, using methods described in the experimental design section, at the refrigerated receiving dock of a commercial grinding facility. The suppliers were either slaughter or deboning facilities, and ranged in distance from less than 1 mile to 1,000 miles. Consequently, individual combos were held under refrigeration 24 to 120 h before sampling.

Experimental design. Phase 1. On a plastic tray that had been ultraviolet (UV) treated for 20 min, the 1,000-g pieces were spray inoculated with either (i) sterile distilled water, (ii) 1:10 feces/sterile distilled water slurry, (iii) 1:1 feces/sterile distilled water slurry, or (iv) 1:1 feces/sterile distilled water that had been incubated at 37°C overnight. These procedures were replicated on seven separate sampling periods. Feces used in inoculations were collected immediately after defecation from cattle that were maintained on a corn-silage diet and were held in confined areas at MARC.

After overnight incubation at 5°C in a walk-in cooler, each of the 1,000-g samples was cut into approximately 60 pieces, placed into a UV-treated 25 × 45-cm, 1.5-mil clear poly bag (Associated Bag Co., Milwaukee, WI) into which an inverted 12.5 × 12.5 × 7 cm sterile Nalgene Unwire test tube rack (Nalge Nunc Inter., Milwaukee, WI) had been placed. The test tube rack was used to create a space under the meat from which purge was collected. The entire bag and rack assembly was contained inside a snugly fitted, bottomless corrugated cardboard box with inside dimensions of 12.8 × 12.8 × 35 cm. After the meat was placed into the "mini-model combo" assembly (MinC), the plastic liner bag was folded over the top of the meat to eliminate any air circulation, a 900-g plastic weight was placed on top of the meat, and the MinC was placed into the 5°C walk-in cooler. After a 24-h incubation period, a sterile syringe was used to puncture the poly bag at the bottom of a MinC and extract all available purge (1–5 ml).

Phase 2. The meat combos constructed for this study were enlarged versions of the MinC. A sterile, plastic-covered, autoclaved 33 × 27 × 9 cm metal rack (Nasco, Fort Atkinson, WI) was placed inverted into a 50.5 × 30 × 122 cm 3-mil poly bag (Silver State Plastics, Loveland, CO). The bag/rack assemblies were placed into 35 × 27.5 × 97.5 cm reinforced-corrugated-cardboard bottomless boxes. Lamb meat pieces were randomly placed into these "maxi-model combos" (MaxC) until they filled the MaxCs to one of three levels: (i) 25 cm, (ii) 50 cm, and (iii) 75 cm. Three replications were performed for each depth. Two 500-g inoculated meat pieces were then placed in the center of the combo so as not to touch the poly bag liner, and the remaining space was filled to a total depth of 75 cm with uninoculated meat pieces. As a control, one MaxC in each replication day was completely filled with uninoculated meat. Approximately 59 kg of meat was required to fill a MaxC to a final depth of 75 cm. The poly bag was folded over the top of the meat, and the MaxCs were placed into a sterile plastic pan, then moved into a 5°C walk-in cooler and refrigerated for 24 h. After the 24-h refrigeration period, a sterile syringe was used to puncture the poly bag at the bottom of a MaxC and extract a purge sample (ca. 10 ml).

The 500-g meat pieces were inoculated by completely submerging them for 15 min into a 4-liter beaker containing 1 liter of physiological saline containing approximately 7 $\log_{10}$ CFU/ml of a streptomycin-resistant *E. coli* O157:H7 (*4*). After the inoculation period, the pieces were removed and allowed to drip excess liquid for 30 sec. A preliminary study using beef determined that a 5 × 5 × 1 cm (60-cm^2 surface area) piece of meat inoculated in the manner described above resulted in a per square centimeter surface inoculation approximately 1 $\log_{10}$ lower than the CFU/ml contained in the inoculation dip liquid. It was also determined that a 500-g piece of beef would retain approximately 175 ml of the dip liquid after inoculation (data not shown).

Phase 3. Over a 6-day period, 15 commercial beef combos were randomly selected daily and sampled (n = 90). Purge samples from the bottom of the combos were taken by cutting the cardboard outer container away from the inner plastic lining, spraying the lining with isopropyl alcohol, then puncturing the bag with a 16-g hypodermic needle and extracting approximately 70 ml of purge into a syringe. Corresponding samples (ca. 100 g) were taken from the same combos by plant quality control personnel in the manner normally used by the grinding company. These samples were taken using a sterile knife to cut small sample pieces from the upper layers of the combo (cores) and placing the sample into a sterile Whirlpak® bag (Nasco). All samples were transported to an on-site laboratory for analysis.

Sample enumeration. Phase 1. After extracting the purge sample, the entire 1,000-g meat sample from the MinC was transferred to a sterile, filtered stomacher bag and 150 ml of 2% buffered peptone water (BPW; BBL, Cockeysville, MD) with 0.1% Tween 20 added. The stomacher bag was closed and hand shaken for 1 min. The resulting rinse was used for aerobic plate count determination of the 1,000-g meat sample. Both the purge samples and the rinse samples from the MinCs were serially diluted as required in BPW and spiral plated on trypticase soy agar (BBL) using a Model D Spiral Plater (Spiral Systems Instruments, Bethesda, MD). Plates were incubated aerobically at 35°C for 36 h and enumerated using a CASBA IV optical colony-counting system (Spiral Biotech, Inc., Bethesda, MD).

Phase 2. Purge samples were spread plated onto sorbitol MacConkey agar with 250 μg/ml streptomycin (Sigma Chemical Co., St. Louis, MO). Plates were incubated for 18 to 24 h aerobically at 37°C. Typical *E. coli* O157:H7 isolates present on the plates were randomly selected and confirmed serologically (*E. coli* O157 Test kit, Unipath Ltd., Basingstoke, Hampshire, UK).

Phase 3. Purge samples were plated after making suitable dilutions in BPW on appropriate 3M Petrifilm and enumerated according to the manufacturer's instructions (3M, Inc., St. Paul, MN) for mesophilic aerobic, coliform, and *E. coli* counts. The same samples were also enumerated using impedance for mesophilic and psychrotrophic aerobes and coliforms using a Bactometer according to the manufacturer's instructions (bioMerieux Vitek, Inc., Hazelwood, MO).

Sample enumeration: commercial grinders' protocol. Phase 3. Eleven grams of each corresponding core sample was randomly cut from the original 100-g sample and placed into a sterile stomacher bag with 99 ml of BPW and pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, OH). Samples were plated in duplicate on the appropriate 3M Petrifilm® and enumerated using impedance as described for the purge samples above.

Data analysis. Aerobic plate count, coliform, and *E. coli* data were converted to $\log_{10}$ CFU/ml, and the correlation (r) between sampling methods was calculated using the statistical package in Microsoft Excel. To facilitate statistical analysis of the counts in logarithms during phase 3, any plate count or Bactometer result of 0 was assigned a value of 0.5, a value below the lowest limit of detection (1 and 10 CFU/ml, respectively) for the two methods. Least squares means were analyzed using the general linear model procedure of SAS (SAS Institute, Cary, NC) with a probability level of 0.05 unless otherwise stated.

RESULTS AND DISCUSSION

The typical raw beef used to produce ground beef consists of deboned pieces of varying sizes, from a large number of individual animals, packed in plastic-lined bulk containers holding on the average 900 kg of product. A commonly used sampling method for microbial profiling of these combos is to take a meat core sample of 25 g (*1*). This sampling method attempts to ascertain the microbial profile of the entire 900 kg of beef present using a sample that is only approximately 0.00002% of the total combo weight. Although this method is commonly used in the industry, it is clear that it is neither adequate nor representative and is likely to produce misleading results.

Data from phase 1 of the current study determined that there is a strong correlation ($r = 0.94$) between the total mesophilic aerobic bacteria counts derived from the purge samples of a beef combo and the total mesophilic aerobic bacteria present in a rinse sample of the entire contents of that combo (Figure 1).

In phase 2, the marked *E. coli* O157:H7 was retrievable from the purge after 24 h of refrigerated storage regardless of the location or depth of the inoculated pieces of meat in the 75-cm meat column. The means of recoverable *E. coli* O157:H7 originating from inoculated meat placed at 25, 50, and 75 cm in the meat column were 5.5, 4.5, and 3.8 $\log_{10}$ CFU/ml, respectively. These data show that bacteria are carried downward into the purge of beef combos. Collectively, these observations indicate that the use of a purge sampling method to determine the overall bacterial constituency of a beef combo is representative when they are present at the levels examined. Additionally, it would appear that if a single piece of beef present in a 900-kg combo was contaminated with *E. coli* O157:H7, a sample of purge from the bottom of a combo would be more likely to recover the organism than a randomly taken core sample.

Purge samples were successfully taken from beef combos at the receiving dock of a commercial grinding operation during the third phase of this study. Future modifications of the methods used during the current study to capture purge samples from beef combos might make sampling greater numbers of combos more feasible. Purge samples were capable of recovering *E. coli*, coliform, psychortrophic, and mesophilic aerobic bacteria at significantly greater levels ($P < 0.0001$) than core samples (Table 1), regardless of the enumeration method used. The percentage of beef combos that exhibited recoverable levels of specific bacterial groups was also substantially greater when purge sampling results were compared with core sampling results from the same combos (Table 1). For example, *E. coli* was recovered from 80% of the combos sampled by the purge method, while being recovered in only 40% of the same combos sampled by the core method.

These results indicate that purge samples are more representative of the microbial profile of a beef combo than an 11-g core sample. However, elevated numbers observed in purge samples might be the result of a concentration effect of beef surface bacteria. Therefore, some caution should be exercised when attempting to compare purge counts with those from cores that have experienced a dilution effect resulting from the addition of a stomaching buffer to both surface and aseptically exposed interior tissue.

In addition to being more representative, purge sampling offers several logistic advantages over the core sampling method. Unlike the core sampling method, purge sampling is nondestructive and rapid. Additionally, purge samples can be easily pooled from combos originating from a single lot or source and tested for selected bacteria of interest, making screening more efficient and cost-effective. Finally, purge sampling is ideally suited for incorporation into a HACCP plan at the critical control point for monitoring the raw product of a beef grinding operation. The potential for incorporating the purge sampling method into more rapid microbial analytical systems, such as microbial adenosine triphosphate bioluminescence (*9*,) could further exploit the utility of this sampling method.

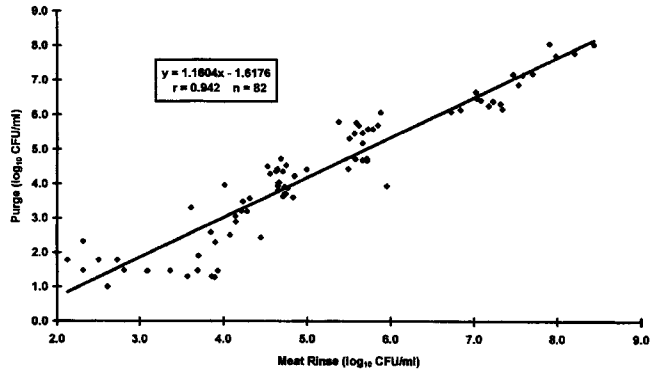

FIGURE 1. *Scatterplot of the total aerobic bacteria of 82 separate 1,000-g beef "mini" combos as determined by rinsing the entire 1,000-g beef sample in 150 ml of buffered peptone water and determined from the same beef combos using a purge sampling method. The solid line is the regression of all data points.*

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TABLE 1. *Least squares means of recoverable bacteria ($\log_{10}$ CFU/ml) from the purge and core samples collected from 900 kg (2,000 lb) beef combos at a commercial grinding facility (n = 90)*

	Purge samples		Grab samples	
	Bactometer	Petri-film	Bactometer	Petri-film[a]
APC	3.3 (98.9)[b]	3.3 (100)	2.2 (85.6)	1.8 (100)
Coliforms	2.4 (94.4)	2.2 (100)	0.7 (44.4)	0.3 (60.0)
E. coli	ND[c]	1.3 (80.0)	ND	0.2 (40.0)
Psychrotrophs	3.2 (96.7)	ND	2.4 (86.7)	ND

[a] *Core samples Petri-film means are from the last 45 samples taken.*
[b] *Percentage of samples in which at least 1 CFU/ml was detected.*
[c] *ND, counts were not determined.*

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