Reduction of Microbial Counts at a Commercial Beef Koshering Facility

M. N. Hajmeer, J. L. Marsden, B. A. Crozier-Dodson, I. A. Basheer, and J. J. Higgins

ABSTRACT

Effectiveness of a water soaking, salting, and water rinsing (koshering) process in reducing microbial counts was examined. Beef briskets (30) were sampled at four stages in the plant, viz. prewashing, postwashing, after 24 h chilling, and after koshering. Quantitative aerobic plate, coliforms, and *Escherichia coli* counts, and qualitative analysis for *Salmonella* were determined. PROC MIXED and GLM procedures were applied to determine statistical differences among least square means (LSM) at p≤0.05. Microbial counts did not always decrease from prewashing to postwashing, after 24 h chilling, or after koshering but koshering reduced APC, coliforms, and *E. coli* from initial counts. *Salmonella* were also reduced.

Key Words: beef, decontamination, salt wash, koshering

INTRODUCTION

THE BEEF INDUSTRY REDUCES THE LIKELIHOOD OF PATHOGENS using decontamination methods including knife trimming (Gorman et al., 1995; Hardin et al., 1995; Federal Register, 1996); carcass washing (Barkate et al., 1993; Hardin et al., 1995; Prasai et al., 1995); steam pasteurization (Phebus et al., 1997; Nutsch et al., 1998); and/or use of antimicrobials such as organic acids (Prasai et al., 1991; Podolak, 1996). Available decontamination techniques are not totally effective on their own in eliminating microbes on carcasses. Commonly, several methods are used at different steps in the process line to achieve the desired control.

The kosher beef industries apply in their slaughter houses water soaking, salting, and water rinsing protocols on beef trimmings to provide adequately cleaned meat for consumption (Regenstein and Regenstein, 1988). The term "koshering of meat" usually refers to blessing of an animal at slaughter time and inspection of a carcass by a rabbi (religious man) for abnormalities such as lung adhesions after carcass splitting. If a carcass passes inspection it will then be certified as "kosher," and meat trimmings from certified carcasses are subjected to water soaking, salting, and water rinsing. We refer to the term koshering only to indicate collectively the steps of water soaking, salting, and water rinsing of beef trimmings. Salt exerts a drying effect on both food and microorganisms, thus leading to food preservation (Jay, 1992). Salt application during koshering of meat (e.g., briskets) might reduce or inhibit microbial growth including pathogens due to associated changes in water activity and ionic strength. Our objective was to examine the commercial performance of a beef koshering facility, and the effectiveness of koshering trimmings in reduction of microbial flora.

MATERIALS & METHODS

BEEF CARCASSES (THIRTY) WERE RANDOMLY SAMPLED (10 carcasses, 5 rabbi-certified and 5 rabbi-noncertified, per day for 3 consecutive days) at a commercial kosher beef processing facility. Samples were collected from the brisket area only and at four stages of the slaughtering process: (i) prewashing (PR-W); (ii) postwashing (PS-W) with water prior to carcass chilling; (iii) 24 h after chilling at <2.3°C; and (iv) after koshering (i.e., water soaking, salting, and water rinsing of briskets). Of the 15 rabbi-certified carcasses, 5 were inadvertently shipped after 24 h of chilling. Therefore, no "after koshering" samples were collected from the briskets of those five carcasses (i.e., n=10 samples were collected after koshering).

The briskets, from rabbi-certified and noncertified carcasses, were sampled for the PR-W, PS-W and chilling stages directly from the carcasses. Certified briskets were removed from the carcasses, trimmed, deveined, koshered, and sampled again (Fig. 1). Both rabbi, certified and noncertified carcasses were spray-washed with water (after evisceration) at 11 MPa of pressure and a temperature of 11.1°C prior to railing carcasses into the cooler. After 24 h chilling, non-certified carcasses were fabricated and packaged without further treatments. Certified carcasses were koshered by soaking, fully submerged, for 30 min in a tub filled with tap water. After soaking, water was drained from the briskets, they were removed from the tub, and salted with Morton® *Top Flake* extra coarse salt (Morton Salt, Chicago, IL). Salting was performed by hand sprinkling of salt onto each brisket until the entire surface was covered. After salting, briskets were placed in a tank and held in salt for 1 h. Then, the briskets were rinsed on a conveyor line to remove salt. The conveyor line had a stainless steel tank with two 284 L dip tanks for rinsing trimmings. Salted briskets were placed on the conveyor belt, and advanced under two filtered fresh water spray nozzles and into and through each of two 284 L dip tanks and then to a drip station.

To trace the decontaminating effectiveness of washing, chilling, and eventually koshering on the carcasses, four 5 cm × 5 cm areas were marked on briskets using a sterile two-scaple exciser (developed at Kansas State University, KSU, Manhattan, KS). The exciser was disinfected using a hypochlorite solution (500–1000 ppm) before and after use, and sterile gloves were used throughout sampling. The two-scaple exciser had two sharp blades, 5 cm apart. The brisket was marked by making a superficial cut onto the meat surface horizontally and vertically to provide four 25 cm^2 areas (Fig. 1) which were distinguishable after treatments. The first 25 cm^2 area was excised prior to

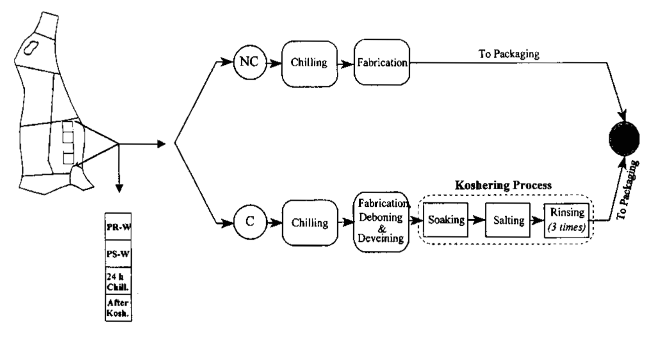

Fig. 1—Process flow at the commercial facility after 24 h of carcass chilling. C = Certified carcasses and NC = Noncertified carcasses.

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carcass washing (PR-W stage), the second immediately after washing (PS-W stage), the third after 24 h chilling, and the fourth after koshering of briskets. Although the carcasses were randomly selected, the assigned areas to be excised at each stage were not randomly selected to avoid mistakes in sample collection down the line at different stages of the process.

Using sanitized knives and forceps, the 25 cm^2 samples (0.5 cm thick) were aseptically excised at each stage from each brisket, and were placed in a sterile filter stomacher bag (Spiral Biotech, Bethesda, MD). Immediately after collection, samples were shipped refrigerated overnight express to our laboratory at KSU for microbial analyses. When samples reached the laboratory, peptone water + Tween 80 (25 mL PW-T; 10° dilution) was added to each bag. The bag was stomached for 2 min with a Stomacher Lab 400 (Tekmar Company, Cincinnati, OH). Serial dilutions (10^{-1} to 10^{-4}) were prepared with PW-T from each stomached bag. Aerobic plate counts were determined by inoculating 3M Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN) in duplicate using 1.0 mL of the initial and all subsequent dilutions. The Petrifilms™ were incubated at 35°C for 48±2 h. Coliforms and *Escherichia coli* were determined in a similar manner; however, 3M Petrifilm™ *E. coli* Count Plates (3M, St. Paul, MN) were used and incubated at 35°C for 24±2 h. Results were reported as colony forming units per cm^2 (CFU/cm^2) of brisket surface. Qualitative analysis for *Salmonella* spp. was performed by pre-enriching samples in 25 mL PW-T at 37°C for 24 h. Aliquots (0.5 mL) of PW-T preenriched samples were transferred to 10 mL of Hajna and Damon tetrathionate (TT) broth (Difco, Detroit, MI). Aliquots (0.1 mL) of the preenriched cultures also were transferred to 10 mL Rappaport-Vassiliadis (RV) broth. TT and RV broths were incubated at 42°C for 24 h. One loopful of sample from each selective enrichment was streaked onto Double Modified Lysine Iron agar (DMLIA, Oxoid) and Brilliant Green Sulfapyridine agar (BGS; Difco, Detroit, MI) plates and incubated at 37°C for 24 h. BGS plates with no presumptive colonies were incubated for an additional 24 h. Suspect colonies on BGS and DMLIA plates were streaked on Triple Soy Iron agar (TSI; Difco, Detroit, MI) and Lysine Iron Agar (LIA; Difco, Detroit, MI) slants. Isolates giving typical *Salmonella* spp. reactions were confirmed by serological tests using *Salmonella* O antiserum poly A-I, Vi (Difco, Detroit, MI) and *Salmonella* H (A-Z) antisera. All colonies showing positive agglutination were further confirmed as *Salmonella* spp. using API 20E test kits (bioMerieux, Hazelwood, MO).

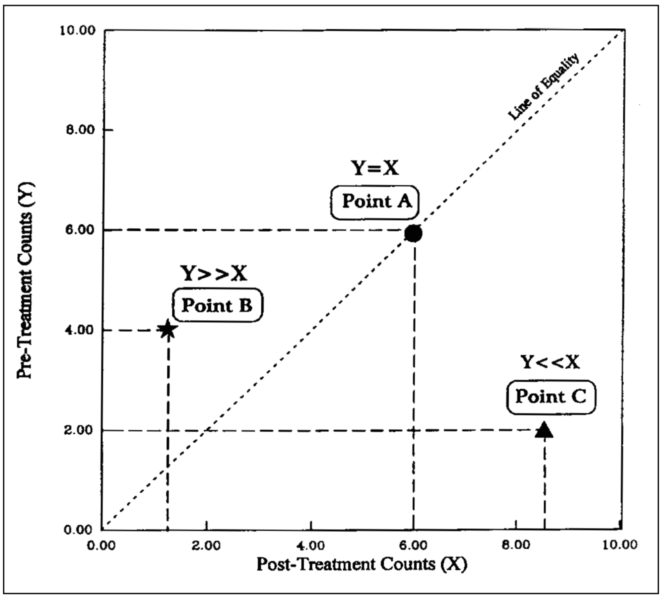

Fig. 2—Example agreement plot showing counts before (Y) and after (X) a given treatment.

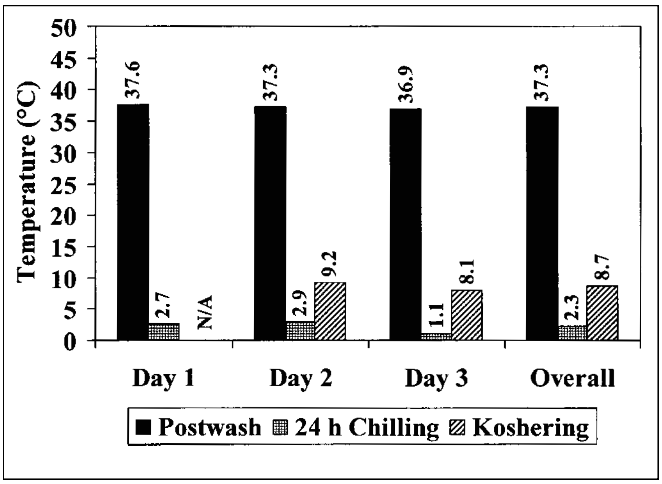

Fig. 3—Temperature profile for beef briskets at four sampling stages in carcass processing (postwashing, after 24 h chilling, and after koshering) at the koshering facility.

Temperature profile

The temperature of each brisket was recorded after sample collection at PS-W, after 24 h chilling, and after koshering to determine if the slaughtering facility had careful control over temperature. The temperature was recorded manually by inserting a digital thermometer (Koch, Kansas City, MO) about 3.8 cm into the brisket immediately after, and at the location where, a 25 cm^2 area was excised. All carcasses were stored in the same cooler.

Statistical analysis

A randomized complete block experimental design was used to select carcasses for treatments in which a block was a day. Repeated measures were taken on briskets. Differences among least square means (LSM) were determined at $p \leq 0.05$. Experimental data were analyzed using PROC MIXED and GLM of the Statistical Analysis System (SAS Institute, Inc., 1996).

Pre-post plots for data analysis

In addition to statistical analysis for microbial data, we developed pre-post plots for comparison of data. As carcasses moved from one

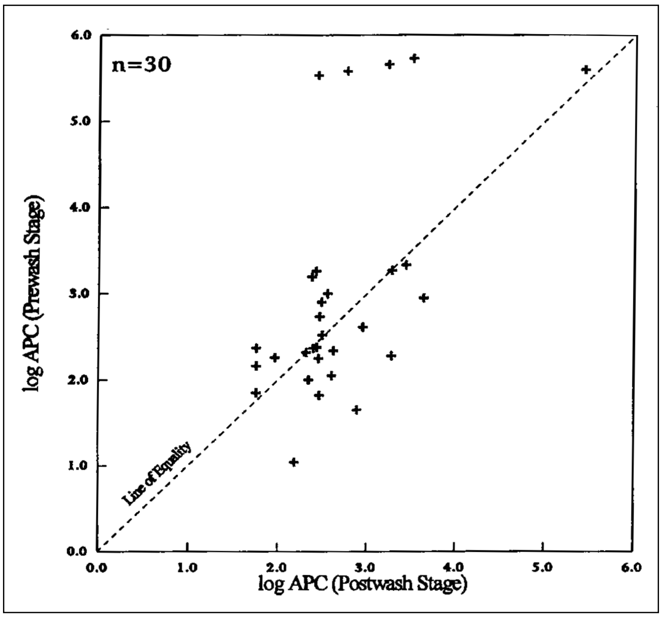

Fig. 4—Agreement plot for beef brisket APC data from excised samples collected at prewashing and postwashing stages. Points above line=15, points on line=2, points below line=13. When plotting data, some points coincided.

MICROBIOLOGY

stage in the slaughtering process to another, preventative measures such as washing and chilling were used to inhibit or control contamination. Therefore, we expect that samples collected after treatment (e.g., postwashing stage) would have lower (or equal) microbial counts than samples collected prior to that treatment. Thus, samples after carcass chilling should have microbial counts lower than or equal to postwashed samples, and koshered samples should have lower or equal counts to those of chilled samples. The relationship between pre-treatment and post-treatment counts could be one or more types:

(1) Type I: post-treatment counts are higher than corresponding pre-treatment counts. This indicates possible contamination of sampled carcasses at some time after treatment. It is inconsequential whether any additional contamination was introduced by the specific treatment applied or sometime before applying the next treatment (such as handling/hauling of meat). However, such situations would indicate inadequate control at the facility.

(2) Type II: post-treatment counts are equal to pre-treatment counts. Contamination may have occurred after applying the treatment only if treatment process was known to reduce the microbial count. The lack of change in counts could imply an ineffective treatment method.

(3) Type III: post-treatment counts are lower than pre-treatment counts. Depending on the magnitude of the pre-treatment count and the reaction time, the treatment is *exceptionally* effective if post-treatment counts are reduced to zero or below detection limit. Deviation of post-treatment counts from zero may pinpoint some contamination source and/or partially effective treatment.

For comparison between stages in carcass handling, data were plotted on agreement plots showing counts before and after treatment (pre-post plots). Pre-treatment microbial counts (y-axis) were plotted against their post-treatment analogs (x-axis) on agreement plots (see Fig. 2). A line connecting equal pre- and post-treatment counts is referred to as line of equality (or the 1:1 line). Points that plotted above the 1:1 line (e.g., point B) indicate effective treatment, which agrees with our hypothesis that the applied treatment tends to reduce microbial load (Type III). Conversely, points below the 1:1 line (e.g., point C) indicate cases where microbial counts increased after treatment (Type I). Alternatively, points on the 1:1 line (e.g., point A) refer to cases where no change in counts occurred (Type II). The pre-post plots for the meat samples were developed to evaluate quantitatively the effectiveness of treatments and the quality control practiced.

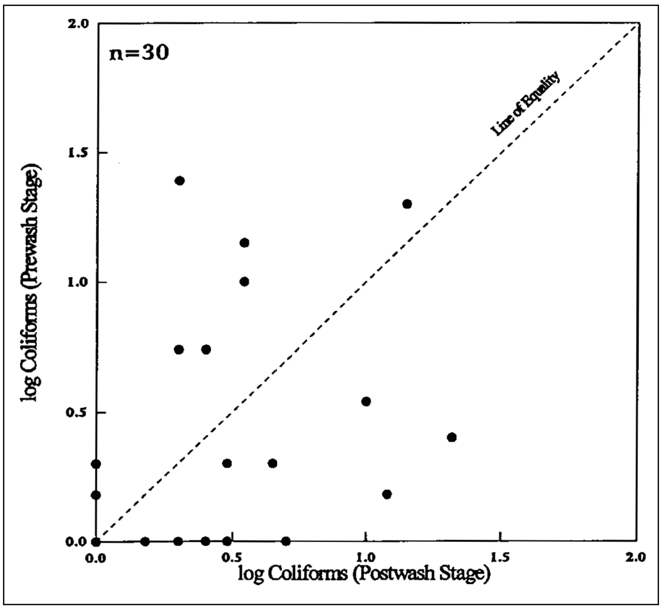

Fig. 5—Agreement plot for beef brisket coliform data from excised samples collected at prewashing and postwashing stages. Points above line = 9, points on line = 9, points below line = 12. When plotting data, some points coincided.

RESULTS & DISCUSSION

Temperature profile

Data indicated that the temperature of carcasses (n=30) dropped from an average 37.3°C directly after slaughter to 2.3°C after 24 h chilling (Fig. 3). The average temperature of kosher briskets (n=10) increased by 6.4°C after salting which followed chilling. The increase in koshered briskets temperatures was probably due to their soaking for 30 min in tap water (average temperature 12.2°C), or to the generation of heat by salt which had not been chilled to the temperature of meat.

Microbial load analysis for prewashing vs postwashing

Samples collected from the commercial packing facility at prewashing (PR-W) were compared with postwashing (PS-W) samples. Hose rinsing of carcasses with plain water (11 MPa and 11.1°C) after

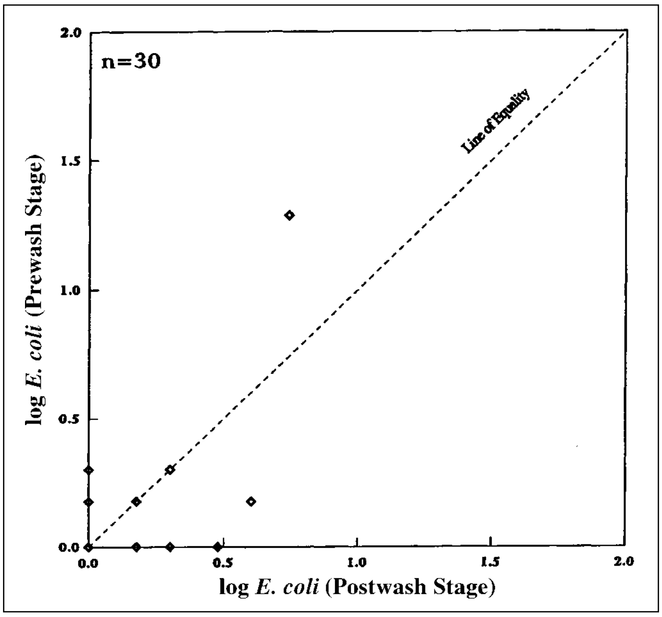

Fig. 6—Agreement plot for beef brisket *E. coli* data from excised samples collected at prewashing and postwashing stages. Points above line=4, points on line=21, points below line =5. When plotting data, some points coincided.

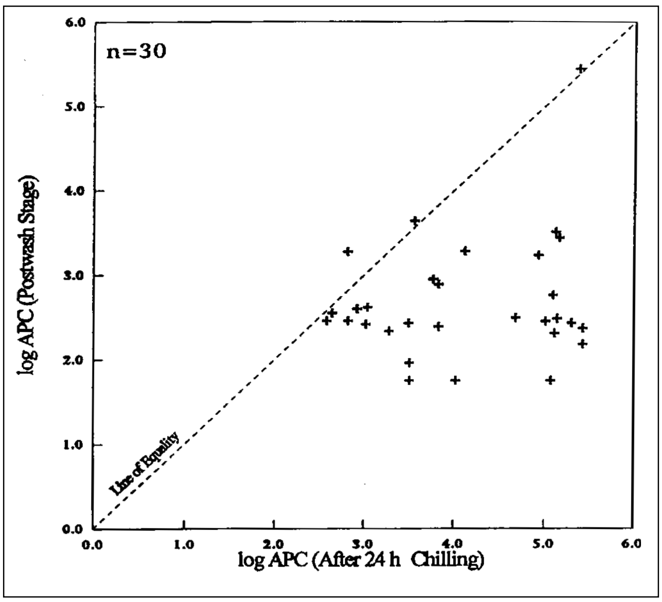

Fig. 7—Agreement plot for beef brisket APC data from excised samples collected at postwashing and after 24 h of chilling stages. Points above line = 3, points on line = 0, points below line = 27. When plotting data, some points coincided.

carcass splitting and inspection did not always reduce microbial counts. Average aerobic plate counts at PS-W were higher by 0.5 $\log_{10}$ ($p<0.04$) than APCs of PR-W. Comparison between PR-W and PS-W samples on pre-post plots (Fig. 4) showed that 13 of 30 briskets sampled for APCs (~40%) fell below the 1:1 line (i.e., increased counts), indicating that washing was not always effective. After washing, coliforms increased by 0.1 $\log_{10}$ ($p>0.69$), and *E. coli* counts dropped by 0.02 $\log_{10}$ ($p>0.70$). The pre-post plots (Figs. 5 and 6) showed that washing was effective in 30% of the cases for coliforms and in 6.6% for *E. coli*.

Contrary to our findings, Siragusa (1995) had reported that rinsing carcasses with water was effective in removing at least 1 $\log_{10}$ APC units from the carcass surface, and the reduction could exceed 3 $\log_{10}$ for APCs or other organisms after hot water washing. Other studies have shown that washing was effective in reducing bacterial populations on meat, including pathogens (Kotula et al., 1974; Anderson et al., 1981; Reagan et al., 1996). For our study, the increase in APCs might have been due to ineffective carcass washing, contamination above initial counts by possible mishandling after washing, or both. At several occasions during sample collection, we observed that carcasses were not washed thoroughly, especially at times approaching employee recess. Inadequate washing of carcasses, especially at the brisket area, would likely transfer contaminants from another contaminated area (e.g., rump) to a potentially less contaminated area such as the brisket.

Microbial load analysis for postwashing vs chilling

After 24 h carcass chilling, APCs increased (relative to post-wash) by 1.24 $\log_{10}$ CFU/cm^2 ($p<0.0001$), coliforms by 0.43 $\log_{10}$

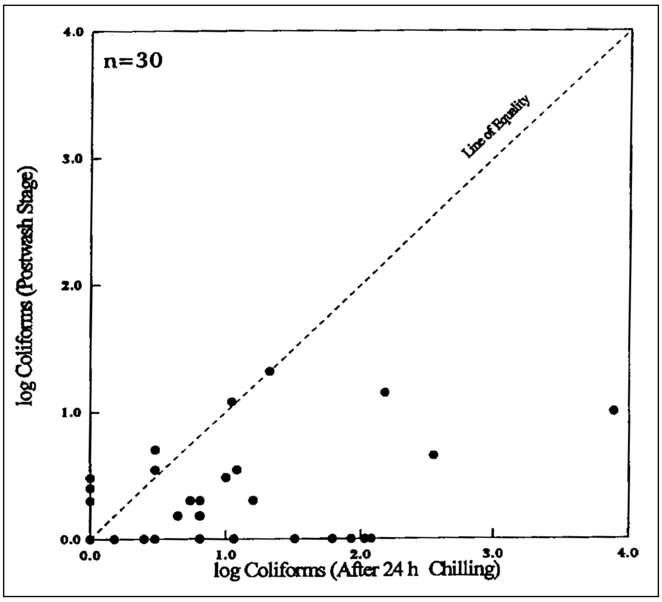

Fig. 8—Agreement plot for beef brisket coliform data from excised samples collected at postwashing and after 24 h of chilling stages. Points above line = 7, points on line = 3, points below line = 20. When plotting data, some points coincided.

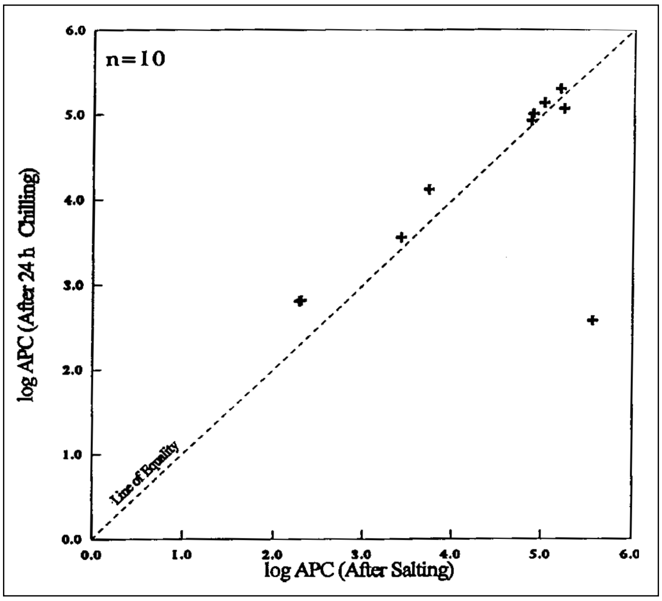

Fig. 10—Agreement plot for beef brisket APC data from excised samples collected after 24 h of chilling and after koshering steps (water soaking, salting and water rinsing of briskets). Points above line = 8, points on line = 0, points below line = 2. When plotting data, some points coincided.

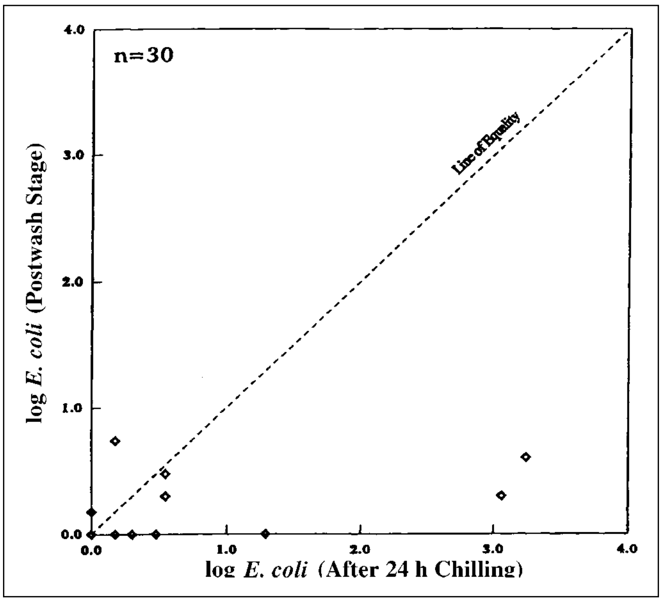

Fig. 9—Agreement plot for beef brisket *E. coli* data from excised samples collected at postwashing and after 24 h of chilling stages. Points above line = 4, points on line = 18, points below line = 8. When plotting data, some points coincided.

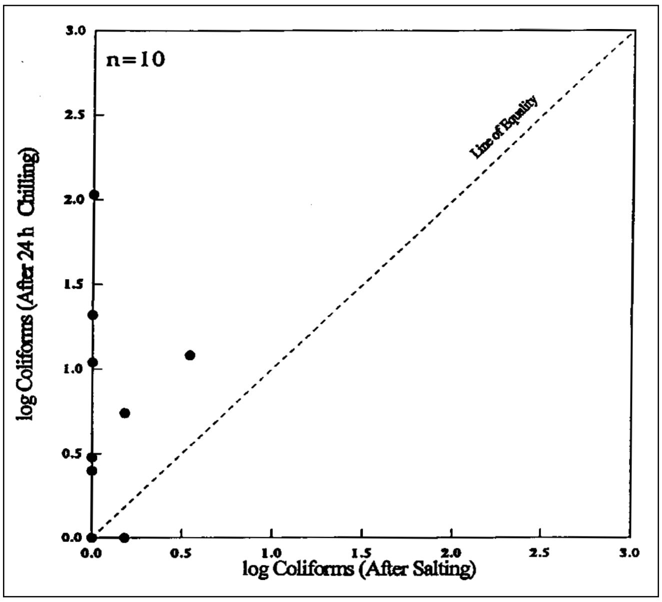

Fig. 11—Agreement plot for beef brisket coliform data from excised samples collected after 24 h of chilling and after koshering steps (water soaking, salting and water rinsing of briskets). Points above line = 7, points on line = 2, points below line = 1. When plotting data, some points coincided.

MICROBIOLOGY

($p<0.01$), and *E. coli* by 0.01 $\log_{10}$ ($p>0.91$). The pre-post plots (Fig. 7) indicated that chilling was ineffective in controlling APCs in almost 90% of cases. Similarly, it was ineffective in 66.6% of the cases for coliforms, and 80% of the cases for *E. coli* (Figs. 8 and 9). Since temperature profile (Fig. 3) for data collected at the commercial facility indicated that carcasses were chilled to <2.3°C , microbial growth should have been minimized over the 3 day period. However, the rate of temperature decline was not monitored, and there might have been time for some psychrotrophs to proliferate during the transition from 37°C after washing to 2.3°C after 24 h chilling. Nothingham (1982) reported that further contamination of carcasses could occur during chilling through contact with walls or surfaces, by splashing during cleaning, or from the air. Also, bacterial level at the end of chilling depends on initial contamination, particularly by psychrotrophs, and chilling conditions such as air velocity. The surfaces of carcasses were in contact with moist shrouds, which might have allowed proliferation of psychrotrophs. The sampling facility did not have a mechanized rail system to move carcasses. Plant personnel had to rail the carcasses manually, thus increasing the potential for recontamination, especially at the brisket area: which is the most probable site where employees would place their hands when railing carcasses.

Microbial load analysis for chilling vs koshering

Although the pre-post plots (Fig. 10) indicated that in 80% of the cases salt treatment reduced APCs, the magnitude of reduction was small 0.11 $\log_{10}$ (points were close to the 1:1 line; $p=1.000$). Coliforms dropped by 0.7 $\log_{10}$ ($p<0.0003$) after koshering with 70% effectiveness (Fig. 11). On average, *E. coli* also decreased by only 0.09 $\log_{10}$ ($p>0.19$) after salt application. This small decrease might be explained by the fact that in 7 of 10 briskets sampled, microbial analysis for *E. coli* recovered zero counts before and after treatment (Fig. 12). The pre-post plot of *E. coli* indicated no source of *E. coli* to highly contaminate carcasses after treatment. However, few carcasses were affected by some contamination source, which resulted in no change in counts across treatments (the on-line points).

Analysis for *Salmonella*

Qualitative analysis for *Salmonella* spp. indicated that 10 carcasses of 30 sampled over the 3-day period were *Salmonella*-positive. Three *Salmonella*-positive samples came from PR-W stage, 1 from PS-W, and 6 from 24 h after chilling stage. Four of the 10 that were *Salmonella*-positive came from rabbi-certified carcasses. After salting, the same four (*Salmonella* positive) certified briskets tested negative for *Salmonella*, indicating that koshering had an inhibitory effect. Jay (1992) reported that *Salmonella* were unable to tolerate high salt. Because only four briskets (certified) were salted, the data were not sufficient for firm conclusions regarding the antimicrobial effects of salt on *Salmonella*.

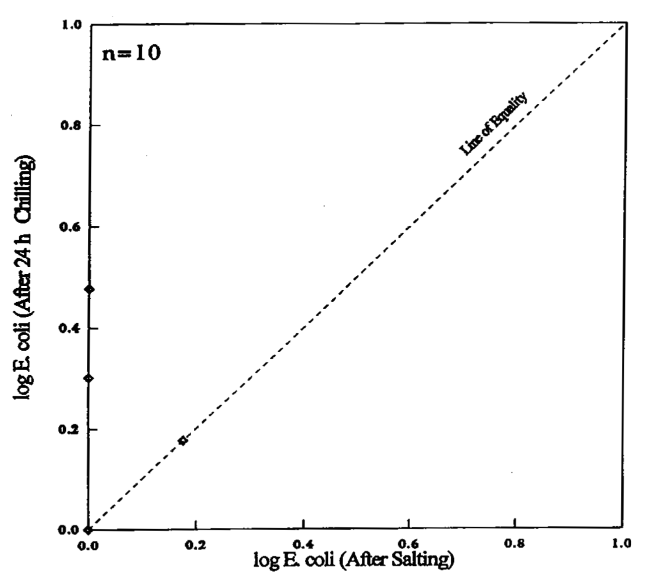

Fig. 12—Agreement plot for beef brisket *E. coli* data from excised samples collected after 24 h of chilling and after koshering steps (water soaking, salting and water rinsing of briskets). Points above line=2, points on line=8, points below line=0. When plotting data, some points coincided.

CONCLUSIONS

THIS ON-LINE STUDY INDICATED THAT SALT APPLIED DURING koshering had a potential for microbial reduction, however, a plant based study cannot provide a valid scientific evaluation for line effectiveness of koshering. This is because at several stages, the process may have not been controlled. Carcasses might have been recontaminated by handling while decontamination procedures were applied. This could explain the anomalies in results. Laboratory scale studies are essential to determine effectiveness of such processes, and translated to field conditions such findings should be applied with excessive measures for safety. Validation of salt effectiveness in microbial reduction on fresh beef might prove beneficial for controlling *E. coli* O157:H7 and *Salmonella*.

In the future, the water soaking, salting, and water rinsing (koshering) may be used in conjunction with other techniques such as trimming, hot water rinsing, or steam pasteurization to achieve possible synergistic decontaminant effects, especially if salt is applied at the end of the fabrication process. Also, the koshering process may be used by small plants since it is fairly low cost as compared to other methods.

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