Microbial Growth in Carcasses and Boxed Beef During Storage

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ABSTRACT

Fresh western-Canadian beef delivered to an eastern-Canadian terminal (Québec City, Qc) was evaluated for microbial contamination of carcasses (front and rear portions) and of boxed beef (heat and clip-sealed). Total microbial counts during a 12-month sampling varied from log₁₀ values of 6.28 in front portions of carcasses to 7.10 in clip-sealed boxed beef. Lactobacillus counts were higher in clip-sealed boxed beef (6.93) than in the front portion of carcasses (4.39). Total and fecal coliform counts were much higher in vacuum-packed beef than in carcass beef (4.42 and 0.97, respectively). Microbial species isolated from carcasses and vacuum-boxed beef varied markedly, with Pseudomonas spp. as predominant in carcasses andposal flora of Pseudomonas spp., Lactobacilli spp. and Aeromonas spp. in vacuum-packed beef.

Several modes exist for beef distribution from the packer to the retailer. Beef may be chilled and shipped in whole or halves of carcasses or cut and packaged in gas-impermeable films and shipped in boxes. It may subsequently be snowpacked, vacuum-packaged or gas-flushed.

Recently, vacuum packaging has been increasingly employed in the meat industry to realize economic advantages as well as to prolong shelf-life(8). Air evacuation from the gas-impermeable package maintains an anaerobic environment, thus reducing the growth of some microbial species and the evaporative losses in chilled fresh meat (7). The absence of oxygen and the release of CO₂ by meat tissues and microorganisms are responsible for the inhibition of most bacterial growth, especially that of aerobic psychrotrophs (3,4). It was observed, however, that growth of lactic acid bacteria is increased under anaerobic conditions while the initially predominant aerobic Pseudomonas spp. are decreased (5,13).

Little information is available comparing microbial growth in vacuum-packaged fresh meat to that in hanging carcasses. Johnston et al. (8) reported that vacuum-packaged beef generally has levels of indicator bacteria 10 to 100 times higher than those of hanging beef. Vacuum-packaging increases the shelf-life of meat to 7 weeks at 0°C, compared to 2 weeks in oxygen-permeable films (2). The current trend is away from carcass meat and toward primal cuts. The impetus for this trend is the vacuum pack, which accounts for more than 90% of the beef processed in Canada. Most of the beef consumed in eastern-Canadian cities is shipped from Western Canada, and there is a lack of information concerning the microbial load of these carcasses and of boxed beef arriving at eastern terminals.

The purposes of this study are (a) to compare the microbial load in front and rear portions of carcasses (hanging beef) throughout a 12-month period (b) to compare the microbial load of heat and clip-sealed vacuum-packaged beef, and (c) to compare representative microbial species of bacteria in carcasses and in boxed beef.

MATERIALS AND METHODS

Beef samples

Carcasses and boxed beef were received in refrigerated (4°C) freight-cars from Edmonton, Alta. Most of the meat was shipped less than 48 h after slaughtering and arrived in Québec City some 6 d later. Samples were obtained from a meat wholesaler (Gainers Inc., Québec City, Qc) on the following dates: June 1, 16, 30; August 4; September 19; December 15, 1981; January 26 and May 19, 1982, which correspond to sampling periods 1, 2, 3, 4, 5, 6, 7 and 8. At each interval, three of the following products were sampled: (a) heat-sealed vacuum-packaged beef, (b) clipped seal vacuum-packaged beef, (c) front, and (d) rear portions of hanging carcasses. Samples of approximately 150 g were cut aseptically from the boxed or hanging beef, delivered immediately in plastic bags to the laboratories, refrigerated at 4°C and analyzed the same day in duplicate. Samples of boxed beef were taken from both front and rear portions without distinction.

Microbiological methods

For microbiological determinations a 30-mm slice was aseptically excised from the meat surface sample which was in direct contact with air. Then a portion of this sample was mixed with 225 ml of sterile 0.1% peptone solution and homogenized in a Stomacher-400 (A. J. Seward Lab., London, UK) for 5 min to produce a slurry for plating. Psychrotrophic bacterial counts were determined by incubating on Plate Count Agar (Difco) at 7°C for 10 d. Lactobacilli were enumerated by incubation at 30°C for 4 d in Lactobacillus MRS broth (Difco) plus 1.5% agar in an anaerobic jar. (A Gas-Pak envelope introduced into the anaerobic jar ensured a gas atmosphere of 90% hydrogen and 10% carbon dioxide). For yeast and mold counts, a portion of the homogenate was incubated in acidified potato dextrose agar at 22°C for 4 d.
(1). All microbial counts are expressed as \( \log^{10} \) CFU (Colony forming unit) per gram (g) of beef sample

Total anaerobic and aerobic bacteria were enumerated on Plate Count Agar (Difco) incubated at 30°C for 3 d. Fecal and total coliforms were enumerated in violet red bile agar (Difco) pour plates incubated for 24 h at 44.5°C (6) and 37°C, respectively.

Seventy-two colonies were randomly isolated from psychrotrophic plates after plate counting to obtain representative isolates from beef. They were incubated on trypticase soy agar (BBL) slants at 25°C for 3 d before taxonomic characterization. These cultures were identified by gram stain, morphology, oxidase and catalase activities, \( \text{NH}_3 \) from arginine, motility and carbohydrate utilization according to Vanderzant and Nichelson (14).

Statistical analysis

Duncan type analysis (12) was used to determine differences among microbial growth as well as between hanging and boxed beef.

RESULTS AND DISCUSSION

As shown in Table 1, among the bacteria isolated as psychrotrophs \emph{Pseudomonas} spp. were predominant in all four types of fresh meat. They were especially important in both front and rear portions of carcasses. In the heat-sealed package, meat was found to also contain \emph{Lactobacillus} and \emph{Aeromonas}. If the packages were clip-sealed, at least five genera were present: \emph{Pseudomonas}, \emph{Lactobacillus}, \emph{Aeromonas}, \emph{Acinetobacter} and \emph{Brochothrix} spp., as shown in Table 1. Since less manipulation is required for distribution of carcasses, fewer bacterial species are likely to be present, when compared to boxed beef. Also, a higher atmospheric transfer with clipped packages than with heat-sealing may explain the higher number of bacterial species found in the former. There was, however, no apparent relationship between the number of species and bacterial counts.

The initial microflora in the front and rear portions of hanging beef and in heat- or clip-sealed beef packages are shown in Table 2. No significant difference in microbial counts was observed between front and rear portions of hanging carcasses. Psychrotrophs and total aerobic counts did not show any significant difference in the number of bacterial colonies isolated from meat samples packaged by these two methods. Total anaerobic and lactobacillus counts were higher in vacuum-packaged beef than in carcasses.

With the exception of psychrotrophs, microbial counts in vacuum-packaged beef (heat- or clip-sealed) were generally higher than microbial counts on carcasses. This is noticeable for total anaerobic bacteria, lactobacillus and coliform counts, which were significantly higher for vacuum-packaged fresh beef. Increased handling involved in preparing the boxed beef (cutting, packaging, evacuation, etc.), may result in a higher initial contamination. This is further illustrated by higher total and fecal coliform counts in the boxed beef (4 and 3 logs, respectively). Furthermore, depletion of oxygen favors growth of anaerobic bacteria in fresh meat.

The distribution of microbial species did not vary significantly between front and rear portions of carcasses. Since oxygen transmission may affect growth of the microflora present in the meat, the heat-sealed packaged meat was expected to contain more anaerobes and fewer aerobes than that sealed by clips. Results, however, showed that the clipping and heat-sealing had no effect on evolution of the microflora, even though the number of psychrotrophs, as well as yeast and molds, seemed to be slightly lower in clipped than in heat-sealed packages. It is possible that the clips are as efficient as the heat-seals over a reasonably short product distribution time.

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**TABLE 1. Microbial species isolated from carcasses and vacuum boxed beef**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Front</th>
<th>Rear</th>
</tr>
</thead>
<tbody>
<tr>
<td>\emph{Pseudomonas}</td>
<td>82</td>
<td>100</td>
</tr>
<tr>
<td>\emph{Brochothrix}</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

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**TABLE 2. The microbial flora of carcasses and vacuum boxed beef**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Carcasses</th>
<th>Vacuum-packaged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Front</td>
<td>Rear</td>
</tr>
<tr>
<td>Psychrotrophs</td>
<td>( 7.15 \pm 0.36 ) (^a)</td>
<td>( 6.17 \pm 0.42 ) (^a)</td>
</tr>
<tr>
<td>Total aerobic counts</td>
<td>( 6.73 \pm 0.33 ) (^a)</td>
<td>( 6.28 \pm 0.40 ) (^a)</td>
</tr>
<tr>
<td>Total anaerobic counts</td>
<td>( 5.17 \pm 0.30 ) (^a)</td>
<td>( 5.03 \pm 0.34 ) (^a)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>( 4.39 \pm 0.27 ) (^a)</td>
<td>( 4.66 \pm 0.36 ) (^a)</td>
</tr>
<tr>
<td>Yeast and molds</td>
<td>( 2.77 \pm 0.55 ) (^a)</td>
<td>( 2.83 \pm 0.44 ) (^a)</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>( 2.71 \pm 0.55 ) (^a)</td>
<td>( 2.14 \pm 0.73 ) (^a)</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>( 1.33 \pm 0.48 ) (^a)</td>
<td>( 0.97 \pm 0.38 ) (^a)</td>
</tr>
</tbody>
</table>

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\(^a\)Expressed as % of the total colony forming units.

\(^b\)Values on the same line superscripted with the same letter are not significantly different (\( P<0.05 \)).

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Psychrotrophs

Figure 1 shows the variation of psychrotrophs in carcass and boxed beef sampled at different periods of the year. Psychrotroph counts were lowest in June and February for the four types of beef (5.5-6.25 logs). In the spring, bacterial counts increased, except in the front portions of the carcasses, in which the counts remained unchanged. During the summer and autumn periods, an increase in microorganisms was observed. From July to December, microbial growth was highest for front carcasses and heat-sealed boxed beef, but decreased to 5.7 logs in clipped boxed beef. The microbial counts were lowest in winter. These variations may be explained by the fact that higher environmental temperatures during meat distribution in summer months favored bacterial growth in meat regardless of the type of packaging. Variations in psychrotrophic counts in boxed beef may have resulted from inhibition by CO₂ produced by animal and bacterial cell respiration in vacuum packaging (10). Psychrotrophs such as Pseudomonas spp. may be suppressed by antimicrobial agents produced by lactic acid bacteria (9).

Aerobic counts

Figure 2 shows that the rear portions of carcasses usually had lower aerobic counts. Maximum aerobic numbers occurred in the beginning of July and December. Important variations in cell counts were observed in the four different beef packages sampled in May (4.9 logs in the rear portions of carcasses and 7.6 logs in clip-sealed packaging).

In general, the aerobic counts were higher but not significantly different in the boxed beef. These results indicate that the presence of oxygen had some effect on the growth of the predominant microflora.

Anaerobic counts

Anaerobic bacterial counts varied from 4 to 8 logs and the highest numbers were observed in the beginning of July for the four different beef packages. Counts in vacuum-packaged meat sampled in May increased 100-fold with respect to those of January, while counts in carcasses increased by 10-fold.

Throughout the experiment, the pattern for anaerobic counts was similar to that of aerobic counts (Fig. 2 and 3); in the former, however, clipped and heat-sealed boxed beef contained significantly higher counts of anaerobes than the carcass portions.

Figure 3 also shows that anaerobic counts in heat-sealed and clipped packages did not differ significantly. This indicates that development of these microorganisms was not dependent on the degree of vacuum in these two processes, at the early stage of storage.
Lactobacillus counts

The pattern for lactobacillus counts (Fig. 4) was similar to that for anaerobes. Boxed beef had much higher counts than the carcass. As suggested earlier, increased manipulation and anaerobic conditions created by the vacuum probably favored multiplication of lactobacilli, while different degrees of vacuum (clips or heat-sealed) in the packages had no effect on these bacteria. On a few occasions, the number of lactobacilli was higher than that of anaerobes. Similar findings have been reported by Simard et al. (11). MRS is generally not a selective medium and the lactobacillus counts may include various groups of lactic acid bacteria and coliforms, resulting in overestimation.

Coliform count

High fluctuations were observed for fecal coliform counts in various samples of packaged beef (Fig. 5). Maximum counts of 3 to 4 logs were observed at the beginning of July for all four meats. In carcasses, the number of coliforms tended to decrease in beef sampled from July through May. Boxed beef samples, however, contained significantly more fecal coliforms in May. A peak count was also obtained in the beginning of August, similar to that of the aerobes, anaerobes and lactobacilli counts. Heat-sealed boxed beef contained the highest number of fecal coliforms throughout the experiment except in the beginning of August, where no cells were detectable by our plating methods. When compared to total coliform counts, the number of fecal coliforms was 100-fold less.

Total coliform counts were highest at the beginning of July for the four types of packaging (Fig. 6). Except in the rear portions of carcasses, the number of bacteria remained relatively constant throughout December. Until May, the level of contamination decreased drastically in carcasses (0 to 1 log) while counts in boxed beef remained high (3.1 to 5.8 logs). With a few exceptions, counts were usually lower in carcasses than in boxed beef. The increased development of total coliforms in vacuum packaging may be attributed to increased manipulations in the packing process.

Yeasts and molds

In this series of experiments, there was considerable variation in yeast and mold counts among samples taken at different periods of the year, although few differences in the number of yeasts and molds were found among the different packaging treatments. The microbial counts were lower in June and January, while peak counts were
oberved in July and October. Various factors such as pH as well as CO₂ produced by bacterial and animal cells may be involved in inhibiting yeast and mold growth in some vacuum-packaged boxed beef samples (10).

CONCLUSIONS

In our findings, the most common bacterial isolates were *Pseudomonas* spp. from carcasses, while *Pseudomonas*, *Aeromonas* and *Lactobacillus* constituted the greater portions of bacteria found in boxed beef. *Brochothrix* was also detected in clipped vacuum packages and constituted a significant portion of the front carcass microflora.

Vacuum-packaging, whether clip or heat sealed, essentially resulted in higher counts of total anaerobes, lactobacilli, and total and fecal coliforms in beef arriving at an eastern-Canadian terminal. No consistent differences were found among the four different packaging treatments concerning the numbers of total aerobes, yeast and molds.

The microbial shelf-life of vacuum-packaged freshly carved meat did not seem to be determined by the oxygen-tightness of the packages, i.e. whether clip or heat sealed.

REFERENCES