Effect of Aerobic Storage before Vacuum Packaging on the pH, Color and Bacterial Flora of Beef

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ABSTRACT

Beef was stored aerobically for 2 or 6 d at 1 ± 1°C before vacuum packaging. Total numbers of aerobic bacteria, Pseudomonas spp., Brochothrix thermosphacta and lactic acid bacteria were determined by selectively plating stored samples. Meat color was evaluated with a color difference meter and a color panel. Aerobic plate counts, numbers of pseudomonads and lactic acid bacteria and minimize the number of pseudomonads. The population for 21 d and in pH for 7 d, and there was no significant effect of packaging treatment in color. Results indicate that the initial treatment variations were lost as storage time progressed.

Early work concerning meat spoilage established that the off-odors and slime produced with spoilage were evidence of aerobic growth of Pseudomonas spp. (1). Restriction of oxygen from the meat appeared to extend shelf-life by limiting the growth of pseudomonads. Growth rates of Pseudomonas spp., discoloration and putrefactive odors of meat are directly related to the oxygen permeability of the packaging film (30). Vacuum packaging produces a semi-an aerobic environment which extends shelf-life by restricting the growth of Pseudomonas spp. and allowing lactic acid bacteria to predominate (4,8,9,21,34,36). An anaerobic environment which would restrict spoilage in vacuum-packaged, normally colored beef would not prevent spoilage. This study investigated the effect of an increased initial bacterial population caused by aerobic storage before vacuum packaging on the bacterial flora, pH and color of vacuum-packaged beef.

MATERIALS AND METHODS

Source and treatment of meat samples

Beef short loins used in the study were obtained from a local packer. Sixteen short loins from 8 carcasses were selected on the basis of similar size and color. Short loins were excised 24 h postmortem and transported to the University of Missouri Meat Laboratory where they were stored unpackaged in a cooler at 1 ± 1°C. Short loins were separated into two groups of 8, both groups having one short loin from each of the 8 carcasses.

At 2-d postmortem, one group (8 short loins) was transported to a local retail store and fabricated into 1 in. thick steaks. Processing in this man-
ner allowed for typical retail contamination of the meat. After fabrication, they were transported to the University Meat Laboratory where the bones and bone dust were removed. Smaller muscles were removed, leaving the *Longissimus* muscle with some subcutaneous fat. The steaks were placed in B-620 Cryovac barrier bags (oxygen transmission at 23°C/30-40 ml/m²/day) and vacuum-packaged (69 cm Hg/bag), using a Kenfield model C-14 vacuum sealer. The bags were not heat shrunk to allow bones and bone dust were removed. The bags were not heat shrunk to allow for easy observation of vacuum loss in case of seal or bag failure. Immediately after packaging, the steaks were stored at 1 ± 1°C.

At 6-d postmortem, the second group was processed, packaged and stored in an identical manner as was the first group. Samples from each group were analyzed at 0, 7, 14, 21, 28 and 35 d after packaging. At each sampling date, 8 steaks, one from each loin, taken at corresponding loin locations were analyzed. The relative loin position was randomized among the six sampling periods.

Bacteriological analysis

The bag was removed by cutting with a sterile scalpel through the film along the perimeter of the meat and using sterile forceps to aseptically separate the bag from the meat. Two cores, each 2.54 cm in diameter and approx. 1.5 cm thick, were removed by means of a sterile corer, forceps and scalpel. The cores were aseptically blended with 99 ml of 0.1% peptone solution for 60 s. Additional dilutions were made in 0.1% peptone.

Samples were plated in duplicate with plate count agar (Difco). Plates were incubated for 3 d at 21°C to determine aerobic plate counts. A modification of the nitrite, actidione and polyoxyxyn B (NAP) medium described by Davidson and Cronin (10) was used for selective enumeration of lactic acid bacteria. The modified medium consisted of (g/L): peptone, 5.0; yeast extract, 5.0; beef extract, 2.5; agar, 5.0; MgSO₄ 7 H₂O, 0.575; MgSO₄ 4 H₂O, 0.05; Bacto lactobacilli agar, 48.0; and distilled water, 1L. Medium preparation and antibiotic and nitrite addition were as reported by Davidson and Cronin (10). The MG medium of Masurovsky et al. (26), with yeast extract (0.25 g/L), was used for selective enumeration of *Pseudomonas* spp. STAA medium, described by Gardner (19), was used for selective enumeration of *B. thermosphacta*. For all three selective media, duplicate pour plates were used and numbers were determined after incubating 5 d at 21°C.

At each sampling period, one plate of each selective medium was retained for confirmation of identity of colonies. Two colonies were randomly selected from each plate and placed in culture tubes containing 10 ml of APT broth (18). After incubating 48 h at 21°C, the isolates were Gram-stained and observed for morphological properties. Each isolate was streaked from 48-h APT broth cultures onto duplicate APT agar plates (APT broth plus 15 g agar/L) and incubated for 48 h at 21°C. One plate was used for detection of catalase by flooding the plate with 5% hydrogen peroxide. Benzidine and oxidase tests were run on colonies from the second plate. A single colony was transferred to a piece of Whatman No. 1 filter paper and tested for oxidase by the method of Kovacs (25). The benzidine test of Deibel and Evans (14) was performed on other colonies of the plate. Oxidation/fermentation was detected using the medium of Baird-Parker (2) distributed in the plate. Oxidation/fermentation was detected using the medium of Baird-Parker (2) distributed in 10 ml volumes in culture tubes. Forty-eight-hour cultures were inoculated into duplicate tubes, stabbing through the medium to the bottom of the tube. One tube was overlaid with sterile mineral oil. The tubes were incubated for 48 h at 21°C and observed for oxidation or fermentation.

Measurement of pH

Meat (10 g) was blended with 100 ml of deionized water and filtered through cheesecloth. The pH of the filtrate was measured with a Fisher Accumet model 320 pH meter.

Measurement of color

A Hunterlab digital color difference meter (D2S02), standardized with a pink (C2-3395) plate, was used to determine "a" and "b" values. Steaks were removed from the vacuum package and allowed to bloom for 1-3 h at 1 ± 1°C before taking a color reading. An 8.3 x 10.2-cm Kodak projector slide cover glass was placed on the meat surface and the surface was adjusted flush with the sample opening.

An experienced 9-member panel evaluated the steaks at each sampling period for color desirability. The panel members were screened for red-green color blindness using pseudo isochromatic plates (Beck Engraving Company, Inc., Philadelphia, PA). No formal training was performed, to allow for a "consumer-like" judgment. The steaks were displayed on white polystyrene trays under white tungsten lighting and evaluated with a 5-point scale, ranging from undesirable to desirable.

Statistical analyses

Analysis of variance was used to statistically analyze the data (38) and the test of Duncan (15) was utilized in separating means that had significant (P<0.05) treatment variation.

RESULTS AND DISCUSSION

Numbers of aerobic bacteria (Fig. 1) illustrated that the additional 4 d of storage before packaging resulted in a significantly (P<0.05) greater population on the day of packaging. At 7 d, there were no significant differences among treatments, nor was there a difference during the remaining storage time.
Table 1. pH of vacuum-packaged beef stored at 1°C for 35 d.

<table>
<thead>
<tr>
<th>Period between slaughter and packaging</th>
<th>Storage period (d)</th>
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<tbody>
<tr>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>2 d</td>
<td>5.45&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 d</td>
<td>5.68&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abcd</sup>Means not bearing a common superscript in columns and rows do not differ significantly (<sup>P<0.05</sup>).

Figure 2 shows the growth of *Pseudomonas* spp., lactic acid bacteria and *B. thermosphacta* over a 35-d storage period. The 6-d postmortem treatment had a *Pseudomonas* spp. population that was 1 log<sub>10</sub> greater than the 2-d postmortem treatment at the time of packaging. The *Pseudomonas* population remained significantly (<sup>P<0.05</sup>) greater than the 2-d postmortem treatment from 0 to 21 d, then did not differ significantly from 28 to 35 d. Within each treatment, there was no significant change in numbers of *Pseudomonas* during the 5-wk storage. Populations of lactic acid bacteria increased in both treatments by more than 4 log<sub>10</sub> cycles during the first 7 d of storage. There was significant (<sup>P<0.05</sup>) growth from 0 to 21 d in the 2-d postmortem treatment and from 0 to 14 d in the 6-d postmortem treatment. The only significant difference between treatments was at 14 d. Initially, the 6-d postmortem treatment had a significantly (<sup>P<0.05</sup>) greater *B. thermosphacta* population than the 2-d postmortem treatment, but by day 7 there was no difference between treatments. Numbers of *B. thermosphacta* decreased significantly (<sup>P<0.05</sup>) in both treatments with increased storage time.

Table 1 shows the results of pH measurements. The pH of the meat packaged 2-d postmortem increased during 7 d of storage, did not differ between 7 and 21 d, and decreased at 35 d (<sup>P<0.05</sup>). The meat stored for 6 d before packaging had higher initial pH values than did the 2-d postmortem packaged meat (<sup>P<0.05</sup>). The pH for the 6-d treatment did not differ from 0 to 21 d but decreased at 28 d (<sup>P<0.05</sup>) as compared to 0 and 7 d.

Table 2 presents color difference meter “*a*” and “*a/b*” values and color desirability scores for steaks from both treatments. The “*a*” value is a quantitative measurement of the meat’s redness, with decreasing values representing decreasing amounts of redness. Significantly (<sup>P<0.05</sup>) lower “*a*” values were recorded at 0 and 28 d than for other times of storage of steaks packaged 2-d postmortem. No significant variation was found among the “*a*” values within the 6-d postmortem treatment. A decrease in “*a/b*” value is indicative of increasing amounts of metmyoglobin (<sup>39</sup>). The “*a/b*” values of both treatments were similar, showing a slight initial decrease from 0 to 21 d then remaining constant until day 35. The data show that color scores within packaging and between treatments did not differ significantly over the entire storage period.

The results of biochemical and morphological tests on isolates removed from selective media are presented in Table 3.

The numbers of aerobic organisms suggest that the extended storage period before packaging allowed for high initial numbers of bacteria but, once vacuum-packaged, differences in counts were minimized within a week of storage. Hodges et al. (23) illustrated a merging of total bacterial counts between prepackageing treatments of 1 and 15-d postmortem vacuum packaging, at 14 d of storage. Our data and those of Hodges et al. (23) infer that a delay before vacuum packaging affects growth of aerobic or facultative bacteria for the first 7 to 14 d after vacuum packaging.

Similar counts of the three types of bacteria were evident in both treatments. *Pseudomonas* spp. dominated at the time of packaging, but numbers decreased slightly with time of storage. Number of *B. thermosphacta* were initially within 1 log<sub>10</sub> of numbers of *Pseudomonas*, but the former tended to die off faster than the latter. By 7 d, lactic acid bacteria represented the majority of the flora, and, by 14 d, they completely dominated. *Pseudomonas* spp. and *B. thermosphacta* remained less than 1% of the flora of 14 to 35 d.

The lack of *Pseudomonas* spp. growth indicated that the higher population associated with extended aerobic storage was not sufficient to change the environment to allow for growth of *Pseudomonas* spp. in vacuum. Others (<sup>31,32</sup>) have reported that *Pseudomonas* spp. did not grow on anaerobically packaged beef, whereas Sutherland et al. (<sup>41</sup>) observed an overall increase in pseudomonads after 9 wk of storage at -2°C in vacuum packages made of a film with similar oxygen permeability to that used in this study. The variation among studies may be due to the initial microbial flora and the gas and meat environments.

The decreases in *B. thermosphacta* populations were similar to findings of a previous study (<sup>32</sup>) yet conflicted with others (<sup>9,41</sup>). In each study, numbers of *B. thermosphacta* followed similar trends to that of *Pseudomonas* spp. In this study, there was growth between 0 and 7 d in the 2-d postmortem treatment that corresponded to a slight increase in *Pseudomonas* spp. and a significant (<sup>P<0.05</sup>) increase in pH from 5.45 to 5.60. To the contrary, Campbell et al. (<sup>7</sup>) reported that *B. thermosphacta* does not grow anaerobically below pH 5.8. *Lactobacillus* spp. have been reported to produce an inhibitor to *B. thermosphacta* (<sup>28,33</sup>), and undissociated lactic acid has been reported to specifically inhibit *B. thermosphacta* (<sup>22</sup>). Thus, the inhibition of growth seen from 7 to 21 d without a significant change in pH was probably due to lactic acid from the lactic acid bacteria, but the quantity of acid was not sufficient to overcome the buffering capacity of the meat.

NAP medium permitted enumeration of *Lactobacillus* spp., *Streptococcus* spp., *Leuconostoc* spp. and *Pediococcus* spp. The maximum observed population (log<sub>10</sub> colony forming units) was 7.36 CFU/cm<sup>2</sup>. These results are in agreement with those of Dainty et al. (<sup>9</sup>), where log<sub>10</sub> 7.29 CFU/cm<sup>2</sup> were present after 35 d at 1°C, and Gill (<sup>20</sup>), who proposed that a maximum cell density of approx.
TABLE 3. Results of biochemical and morphological tests on isolates recovered from selective media.

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Selective media</th>
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<tbody>
<tr>
<td></td>
<td>MOV&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gram stain</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>28</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
</tr>
<tr>
<td>Rod</td>
<td>28</td>
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<tr>
<td>Cocci</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Benzidine</td>
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<td>Catalase</td>
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<tr>
<td>Positive</td>
<td>28</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Glucose utilization</td>
<td></td>
</tr>
<tr>
<td>Oxidative</td>
<td>27</td>
</tr>
<tr>
<td>Fermentative</td>
<td>1</td>
</tr>
<tr>
<td>Growth only</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Selective for *Pseudomonas* spp.

<sup>b</sup>Selective for lactic acid bacteria.

<sup>c</sup>Selective for *Brochothrix thermosphacta*.

$log_{10} 8.0 \text{ CFU/cm}^2$ is regulated by the diffusion rate of fermentable substrates to the meat surface in vacuum-packaged beef. Characteristics of NAP isolates were typical of members of the genus *Lactobacillus* and the family *Streptococcaceae* (6,40). The high proportion of gram-positive cocci isolated from NAP medium could explain the greater populations of lactic acid bacteria observed in this study compared to those studies which enumerated only *Lactobacillus* spp. (4,8,34,36,41). Lactic cocci were observed by Sutherland et al. (42) when characterizing organisms isolated from vacuum-packaged beef held 0 to 9 wk at 0 to 2°C. Five isolates from NAP showed growth only and were cocci in pairs and chains, suggesting that they were species of *Leuconostoc*.

Characteristics of agar isolates on MGV were typical of members of the genus *Pseudomonas* (6,12). One isolate was fermentative, thus atypical of *Pseudomonas*. Masurovsky et al. (26) indicated that members of the genera *Serratia* and *Proteus* survived on MGV medium, but both genera are oxidase-negative. The isolate appeared to have been closely associated with the genera *Aeromonas* and *Vibrio*.

Confirmatory tests indicated that all isolates from STAA medium were *B. thermosphacta* (6,11,13,19,37).

Unpackaged storage instigated the greatest effect on the pH at time of packaging and for the first 7 d of storage. This can be attributed to large numbers of pseudomonads present in the 6-d postmortem treatment. At 14 d, the meat packaged 6-d postmortem contained greater numbers of *Pseudomonas* spp. (P<0.05), but there was no difference between the pH values of the two packaging treatments.
This was probably related to buffering caused by the production of lactic acid, since the population of lactic acid bacteria had risen to greater than $\log_{10} 6.0 \text{ CFU/cm}^2$ in both treatments.

The packaging treatments did not have a significant effect on meat color based on color difference measurements and evaluation by the panel. However, Seideman et al. (35) reported an increase in surface discoloration of vacuum-packaged beef steaks after a 7-d storage period followed by a 1-d retail display. This difference may be due to use of a trained panel in the latter study. Snyder (39) reported that both metmyoglobin and myoglobin cause the "a" value to decrease while a decrease in the "a/b" value is indicative only of metmyoglobin. Thus the lower "a" values observed at 0 and 28 d in the 2-d postmortem treatment appear to be due to myoglobin.

There was an initial decline in the "a/b" value then it leveled off. This was similar to observations in a sterile meat system (3), indicating the observed color change may be due to the meat, package environment and storage room lighting rather than the microbial flora. Bala et al. (3) demonstrated that, when $P. \text{ fragi}$ was inoculated onto sterile meat and allowed to grow aerobically for 10 d, the population increased to approx. $\log_{10} 4.5 \text{ CFU/cm}^2$ with a corresponding decrease in "a/b" value to 0.9. We observed similar numbers of pseudomonads in the 6-d postmortem treatment, but "a/b" values did not decrease, indicating that anaerobic growth, mixed flora or the species of $Pseudomonas$ may play a role in metmyoglobin formation.

REFERENCES


