Quality Changes of Beef Steaks Stored in Controlled Gas Atmospheres Containing High or Low Levels of Oxygen

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(Received for publication February 9, 1981)

ABSTRACT

Steaks from bovine Longissimus and Semimembranosus muscles were used to determine the influence of gas atmospheres on beef color, microbial growth and shrinkage during 9 days of retail display in two separate experimental trials. Steaks were displayed in one of four gas mixtures and were compared to steaks packaged under conventional vacuum and in a film wrap. Gas mixtures containing O₂ levels of 10% (one-half ambient) did not maintain a bright red color, but those with 40-75% O₂ (more than twice ambient) maintained acceptable color for 9 days of storage. Atmosphere stored steaks lost more moisture (P<0.05) than vacuum-packaged steaks. Psychrotrophic and mesophilic microbial counts from steaks stored 9 days in atmospheres containing 15% CO₂ were lower (P<0.05) than the counts for the control steaks.

Cuts of beef originating from usual channels of processing and packaging can be expected to have a retail shelflife of about 3 days limited by an undesirable color change or microbial growth leading to spoilage.

Several systems have been proposed to prolong beef shelf-life including: vacuum packaging, packing in gases such as CO₂, N₂, O₂ and air, as well as a mixture of several gases. In fact, vacuum packaging and CO₂ (in various forms) are extensively used commercially in beef distribution systems. Storage of meats in gas atmospheres may alter meat color and microbiology due to effects on the myoglobin pigment and on growth rate of gram-negative, psychrotrophic bacteria which are most often responsible for fresh meat spoilage. Myoglobin levels may affect meat color. Heme pigments may be oxidized by CO₂ resulting in deleterious effects on meat color. However, CO₂ does slow growth of organisms which cause surface deterioration of meat, especially Pseudomonas sp. Pure O₂ was used in England to transport beef from a central cutting plant to a retail outlet, extending shelf-life by at least 20 h. A modified atmosphere (60% CO₂, 25% O₂, 15% N₂) was used to ship beef cuts for a 5-6 day transport period but did not improve acceptability. Huffman also used a mixed gas atmosphere (25% CO₂, 5% O₂, 70% N₂) to store beef but found that after 13 days color was less desirable than beef stored in air, although bacterial counts were lower in the mixed atmosphere.

The objective of this study was to determine the effect of certain gas mixtures (containing low and high levels of O₂ and CO₂), as well as vacuum packaging on the color, microbiology and shrinkage of two beef muscles in an attempt to select the best controlled atmosphere for preservation of color and extension of shelf-life.

MATERIALS AND METHODS

Collection and handling of samples

Experimental steaks were obtained from the Swift Distribution Center in Tucson, Arizona. A top round and two short loins were obtained for each of two trials. Swift employees separated the bone from the otherwise intact short loin (major muscle, Longissimus dorsi, LD) and, subsequently, cut the loins and top round (Semimembranosus, SM) into 2.5-cm thick steaks. After transportation to a University lab, steaks were further reduced in size to 70-80 cm² of atmosphere-exposed surface area. All steaks were weighed, placed on styrofoam trays and packaged according to their randomly assigned treatment. Steaks treated in Atmosphere 1 were packed in 40% O₂, 15% CO₂, 45% N₂; Atmosphere 2-treated steaks in 10% O₂, 15% CO₂, 75% N₂; Atmosphere 3-treated steaks in 10% O₂, 10% CO₂, 80% N₂; and Atmosphere 4-treated steaks in 75% O₂, 15% CO₂, 10% N₂. Control steaks were wrapped in Borden's Resinite film (O₂ transmission of 310-387.5 cc/cm²/24 h and CO₂ transmission of 2480 - 2790 cc/cm²/24 h) and heat sealed. Vacuum-packaged steaks were placed in Nanophan bags (O₂ and CO₂ transmission both less than 50 cc/cm²/24 h) and evacuated. Steaks to be packaged with the various atmospheres were placed in the bags which were then evacuated, flushed twice and filled with approximately 900 cc of the gas atmosphere (certified ± 2% accuracy). Closure was attained with a metal clip.

After packaging, steaks were placed in one of three open top display cases and held at 4 ± 0.5°C throughout the storage period. Lighting was provided with cool white fluorescent bulbs alternating 12 h on and 12 h
Light intensity at the level of the steaks was 55 foot candles (ft-c) in the first experiment and 70 ft-c in the second experiment.

Four sampling days were used in these experiments; initial sampling (Day 0) followed by examination every 3 days for three more periods (Days 3, 6 and 9). Initial color measurements and microbial counts for Day 0 were taken from three non-treated fresh steaks of each muscle type. On sampling Days 3, 6 and 9, steaks were unwrapped, weighed and allowed to adjust to ambient atmosphere for 30 min before color determination.

Microbial evaluation
Sterile, 15.2-cm cotton swabs were used to sample the meat surface. A swab was rolled across a circular area (3.8 cm²) defined by a sterile aluminum template and rinsed in 99 ml of sterile Butterfield's buffered phosphate solution (22). This process was repeated once after which the cotton tip was broken off into the dilution blank. Two areas were swabbed so that total area sampled was 7.6 cm² per steak. Appropriate sample dilutions were then made and agar was poured plated. Psychrotrophic and mesophilic organisms were enumerated with Plate Count Agar (Difco, Detroit, MI). Determination of lactic acid producing bacteria was done with LBS agar (BBL, Cockeysville, MD). Mesophile count was determined on plates incubated at 23°C for 3 days, psychrotrophs at 5°C for 10 days and lactics at 23°C for 7 days. Total counts were reported as log₁₀ number of organisms per cm².

Color
Muscle color was determined with a Macbeth-Munsell disk colorimeter. Muscle color attributes of hue, value and chroma were designated using Munsell-CIE diagrams. Index of fading was calculated from the above attributes as was suggested by Nickerson (17) to describe changes in color.

Analysis of data
Experimental designs were completely randomized factorials with unequal replication of treatments. Significance testing of the main effects and interactions was done by analysis of variance (18). Duncan's new multiple range test (6) was used to isolate treatment differences. A method for extending Duncan's test to unequal replications was used when applicable (13). For comparisons for control versus several treatments, Dunnett's "t" test was used (7).

RESULTS

Experiment I

Data from Experiment I are presented in Table 1. As the color attribute hue increased, the shade of color changed from red to brown. Therefore, LD control steaks on Day 9 were the brownest with vacuum-and atmosphere 1-treated steaks having the lowest hue, thus the most desirable red color. These findings are reinforced by the results of index of fading calculations which indicate that Atmosphere 1 and vacuum-packaged steaks had the least overall change in color by the ninth day of storage.

In contrast Seideman (23) reported that prepackaged retail cuts (vacuum-packaged or packaged in CO₂-N₂ atmosphere) had more surface discoloration and lower overall appearance ratings than retail cuts derived from vacuum-packaged subprimal cuts. Changes in index of fading for each treatment throughout storage are shown in Fig. 1. The LD control steaks exhibited the greatest change in color beginning with Day 3, whereas the Atmosphere 1 and vacuum-treated LD steaks changed very little. The level of O₂ present (40%) in Atmosphere 1 apparently was sufficient to maintain the oxygenated form of myoglobin pigment. However, Atmosphere 2-and
Control LD steaks had more (P<0.05) mesophilic and psychrotrophic bacteria than any other treatment on Day 9 (Table 1). This demonstrated the effects of CO₂ or vacuum inhibiting bacterial growth. Clark and Lentz (5) found that CO₂ inhibited slime-producing bacteria on the surface of fresh beef, and Baran (2) noted that fresh meat packaged under vacuum had slower aerobic bacterial growth than cuts packaged in air.

After 9 days of storage, the lactic acid bacterial counts were significantly higher (P<0.05) on the Atmosphere 2 and vacuum-treated steaks than the control or other atmosphere-treated steaks. Previous reports (3,4,11,19) have shown that lactic acid bacteria become a significant and often dominant part of the microflora of vacuum-packaged meats and meats stored in gaseous environment. Based upon data collected from the present study, it appears that a gas mixture with less than 15% CO₂ or higher levels (>10%) of O₂ does not permit increased development of lactic acid bacteria.

Steaks from the Semimembranosus muscle (SM) darkened more during storage than steaks from the LD muscle regardless of treatment. As determined by the hue value for color, steaks from the SM muscle treated with Atmosphere 1 or vacuum-packaged had the most desirable color during the display period. Shrinkage values for the SM steaks were greater than for the LD steaks regardless of treatment. Microbial growth on the steaks from both muscles was similar within each treatment.

**Experiment II**

Data from Experiment II are presented in Table 2. As in Experiment I, LD control steaks had the least desirable hue and greatest change in color (P<0.05) by Day 9 of storage. Atmosphere 4 proved to be the best atmosphere for maintenance of color during storage. MacDougall (15) detected the formation of a thickened oxymyoglobin layer when oxygen was present in concentrations greater than normally found. A thickened oxymyoglobin layer may account for the red color obtained after 9 days storage in Atmosphere 4. Vacuum packaging also resulted in a desirable color because oxymyoglobin formed when the package was opened (19).

**TABLE 2. Means of color attributes, shrink and microbial numbers for steaks from the Longissimus muscle during storage in different atmospheres.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Atm. 4</td>
<td>Vacuum</td>
<td>Control</td>
<td>Atm. 4</td>
</tr>
<tr>
<td>Hue</td>
<td>7.5</td>
<td>9.6</td>
<td>6.7</td>
<td>10.0</td>
</tr>
<tr>
<td>Index of Fading</td>
<td>2.5</td>
<td>7.9</td>
<td>6.4</td>
<td>9.3</td>
</tr>
<tr>
<td>Shrink</td>
<td>3.4</td>
<td>1.8</td>
<td>1.6d</td>
<td>4.2</td>
</tr>
<tr>
<td>Mesophilesb</td>
<td>0.3</td>
<td>0.5</td>
<td>1.6</td>
<td>1.2c</td>
</tr>
<tr>
<td>Psychrotrophsb</td>
<td>0.3</td>
<td>0.4</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Lacticsb</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

aAtm. 4 = 75% O₂, 15% CO₂, 10% N₂; Vacuum = Nalophan wrapped; Control = film overwrap.
bReported as the number (log10) of microorganisms/cm² surface area.
c,d,e Means within the same day and variable bearing unlike superscripts differ significantly (P<0.05).
Shrinkage was greatest (P<0.05) from Atmosphere 4 steaks by Day 9 (Table 2). This followed results of Experiment I but disagreed with Marriott (16) who reported that a modified atmosphere (60% CO₂, 25% O₂, 15% N₂) did not significantly affect weight loss for 7-9 days of display. It is possible that the larger cuts shipped by Marriott (16) were more protected from moisture loss due to fat cover than were sample steaks from this trial.

Beginning with Day 6 and continuing through Day 9, mesophilic and psychrotrophic counts were lower (P<0.05) for the Atmosphere 4-treated LD steaks. In Atmosphere 4, the lag phase of growth was prolonged due to the CO₂ present (Figure 2) while bacteria on control steaks continued in logarithmic growth throughout storage. By Day 9, the psychrotrophic and mesophilic counts on the Atmosphere 4-treated steaks were lower than counts from the other two treatments. Lactic acid bacterial counts were highest (P<0.05) on vacuum-packaged steaks on Day 9 (Table 2) as compared to all the treatments. Possibly, these microbes did not increase in treatments containing sufficient oxygen because of the competition from the other more aerobic bacteria.

As in Experiment I, steaks from the Semimembranosus muscle produced similar results although muscle darkening and shrinkage was greater.

![Graph](image)

**Figure 2.** Change in number of psychrotrophs on Longissimus muscle during storage under different atmospheres.

**DISCUSSION**

High levels of oxygen (more than twice ambient concentration) can produce and maintain a desirable bright red color of beef cuts for a 9-day storage period. However, atmospheres with low oxygen levels (one-half ambient concentration) did not promote or maintain desirable color for an extended storage period. Seideman (23) found, using gas mixtures which did not contain oxygen, that as display time increased, surface discoloration of beef cuts also increased.

Carbon dioxide at concentrations of 15% in an atmosphere inhibited microbial growth but did not promote darkening of the meat. This suggests that CO₂ may be used in higher concentrations when combined with 40% or more oxygen without deleterious effects on meat color. A level of 15% CO₂ appears to be critical in reducing microbial growth and development during display periods of 3 to 9 days. Combining 15% CO₂ with higher levels of oxygen appears to provide a gas atmosphere mixture which is capable of increasing shelf-life of beef cuts at least to 9 days of retail display. This type of gas atmosphere provides a means whereby retail cuts of beef could be prepared at the meat packing plant level and distributed to retail markets with little loss of consumer appeal. Such a marketing scheme would be economically feasible especially with today's transportation costs. Furthermore, a marketing system of this type would allow for more efficient usage of waste fat and bone through accumulation of larger quantities in specific locations.

Retail display in controlled gas atmosphere promoted greater shrinkage of the steak cuts presumably by creating a drier atmosphere immediately above the steaks surface within the display container. Further research will be required to consider the use of controlled humidity and/or the use of film wraps to protect the exposed areas of the meat cut.

In comparing steak cuts treated with the various gas atmospheres from muscles of locomotion (SM muscle) versus muscles of attachment (LD muscle), it appears that the type of muscle has little or no influence upon shelf-life. Compared to the LD muscle, steaks from the SM muscle darken more during storage regardless of gas atmosphere treatments, presumably due to a higher concentration of myoglobin in muscles of locomotion as compared to attachment muscles.

**REFERENCES**

Seideman et al., con't. from p. 40


