

USE OF CARBON DIOXIDE AND OXYGEN FOR
EXTENDING SHELF-LIFE OF PREPACKAGED FRESH BEEF

by

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Summary

Storage of prepackaged fresh beef in an atmosphere containing 15% carbon dioxide, 50-100% oxygen or mixtures of the two gases at 5°C markedly increased color and odor shelf-life. Quarter-pound steaks of lean beef were uniformly inoculated with about 10^4 cells/sq cm of a mixture of psychrotolerant strains of *Pseudomonas* and *Achromobacter*, packaged in cardboard trays, overwrapped with a gas-permeable polyvinylchloride film and incubated in the desired gaseous environment for 20 days. A mixture of 70-85% oxygen and 15% CO₂ gave the best results, increasing the color and odor shelf-life by 9 and 10 days, respectively, compared to storage in air. None of the gases or gas mixtures studied affected the pH of the meat or weight loss due to weep. The results should have application in a central prepackaging operation including transportation.

Introduction

In commercial practice consumer cuts of fresh beef such as steaks and roasts individually packaged in gas-permeable plastic film frequently have a shelf-life of only 2 to 4 days (13,15) owing to development of undesirable color (brown) and/or odor (putrid). One of the main causes of these undesirable changes is the growth of common psychrotolerant bacteria which can cause meat myoglobin (red) to be changed to methmyoglobin (brown) and produce off-odor through breakdown of meat proteins (13,15,16). Retarding or preventing this growth would not only increase shelf-life in retail stores, but could provide sufficient lead time to allow central prepackaging of retail cuts, a practice which potentially affords greater economy, better quality control and less waste in terms of trimming losses (1).

Recent studies showed that storage in CO₂-enriched air or in a gas composed of CO₂ and O₂ increased the odor and color shelf-life of fresh beef substantially (8,10,12,14). Carbon dioxide retarded development of off-odor by inhibiting microbial growth while oxygen slowed down undesirable color changes by inhibiting the deoxygenation of oxymyoglobin. How-

ever, most of the tests were made with naturally-contaminated unpackaged meat or with meat packaged in special containers such as double-layered plastic pouches or rigid metal or plastic dishes in which the gaseous composition was not controlled throughout the storage period. Further studies are required to determine the effect of these gases more accurately under conditions where the inoculum and the atmospheric composition are controlled and to determine a way of applying gas treatments without involving special packaging materials or changes in current packaging procedures.

This paper describes tests on the effect of various concentrations of CO₂, O₂ and combinations of the two gases on the growth of pseudomonads and achromobacteria (the main spoilage organisms of refrigerated beef) and on development of off-odor and off-color at 5°C on the surface of fresh beef steaks packaged in molded paperboard trays and overwrapped with gas-permeable polyvinylchloride film (common commercial method of packaging retail cuts). Oxygen was tested separately as well as in mixtures, since earlier studies in this laboratory (9) indicated that this gas alone might be effective in preventing off-odor and off-color in fresh beef.

Methods

All tests were made with samples (approximately 7 cm sq and 1.5 cm thick) of lean meat sliced from rump muscle ("round") of red-brand fresh beef (aged 4-5 days) obtained from a local packing plant. To minimize contamination, the meat was trimmed with a sterile knife before slicing and the knife was swabbed with ethyl alcohol and wiped dry with a sterile cloth after each slice. All slices were weighed and those to be used as test samples were placed in Petri dishes and the top surfaces inoculated evenly in a spray-type inoculating chamber (3) with 10⁴ cells/sq cm of uniform mixture of 5 strains each of psychrotolerant *Pseudomonas* and *Achromobacter*. The inoculum was prepared as described previously (6) using cells of 24-hr-old cultures grown at 22°C on Standard Methods Agar (Difco). The bacterial strains used previously isolated from fresh beef (8). Weighed uninoculated samples were used as controls to determine the effect of CO₂ on weep and pH changes without interference from bacterial growth.

Both the inoculated and control samples were placed in paperboard trays, overwrapped with a polyvinylchloride (PVC) film, and incubated for up to 20 days at 5°C in a saturated atmosphere containing various concentrations of CO₂ (0, 10, 15 and 20%), O₂ (20, 50, 70, 85 and 100%) and mixtures of the two gases. The entire top surface of each sample was in contact with the film. The PVC film was a type

sold specifically for use with meat* and the trays were the molded pulp absorptive type commonly used for retail cuts in retail stores. The incubators consisted of 8-liter desiccators held in temperature controlled ($\pm 1^\circ\text{C}$) cabinets. Filtered, water-saturated gas containing the desired CO_2 and/or O_2 concentrations was metered continuously (150 cc/min) through the desiccators (8).

In all tests, up to 32 inoculated samples and controls were incubated for each condition studied. Three inoculated samples and 1 control were removed for analyses every 2 to 4 days over a total incubation period of 20 days. All tests were repeated once.

For analysis, the samples were tested for off-odor (by smelling) immediately after the film was removed and then, in succession, for weight loss due to weep and changes in pH, surface color and bacterial count. Odor that was noticeably different from that of fresh beef as obtained from the packing plant was considered "off". Previous studies (8) and preliminary tests in the present work showed that the first detectable off-odor was invariably a faintly putrid one and corresponded to a total bacterial count of 10^8 cells/sq cm of meat surface. The length of time a sample was stored before off-odor developed was recorded as its odor shelf-life.

To determine the amount of weep the samples were removed from the trays with sterile tweezers, allowed to drain for about 30 seconds and then weighed. The difference between the weight of a sample and its original weight was recorded as the weight loss due to weep. pH was determined with a semi-micro combination pH electrode inserted about 5 mm into the sample.

Color was assessed by visually matching the surface color of the exposed meat samples with Munsel color chips (glossy surface) in bright daylight. The results were noted in accordance with the Munsel system of color notation** which identifies color in terms of three attributes: hue, value, and chroma, specified in that order. The notation 7.5 R 6/8 for example, specifies the hue 7.5 R which is one of the intermediate steps between Red and Yellow-Red, the value 6 on the scale of lightness and darkness where 10 is White and 0 is Black, and 8 as level of chroma, where the chroma (or intensity) of the color increases from 0 (or neutral) to

* Vynar MW1; 0.68 thousand inch thick; oxygen permeability, 1300-1700 cc/meter²/24 h/atoms; courtesy of TCF of Canada Ltd., Cornwall, Ontario

** Munsel Color Company, Inc., 2441 North Calvert Street, Baltimore, Maryland 21218, U.S.A.

16 or 18, depending on the hue. Color analysis of the meat samples at the start of the tests showed that the normal variation for hue, value and chroma among muscles and between the same muscles of different carcasses was 7.5 R - 10 R, 3-5 and 6-10, respectively. Samples that subsequently gave readings outside the normal range of any one of the three attributes were considered "off" in color. The length of time a sample was stored before off-color occurred was recorded as its color shelf-life.

To determine the total bacterial count, three 2 sq cm areas of sample surface were each washed with 75 ml of 0.1% peptone solution using the spray-gun technique (4,5). The three washings were pooled, diluted with 0.1% peptone solution as required and plated on Standard Methods Agar (Difco) using the surface plate method (7). The plates were incubated at 22°C for 48 h.

Results

Shelf-life

Carbon dioxide continuously present in the atmosphere around the packaged samples extended the odor shelf-life by 5-10 days and the color shelf-life by 2-3 days depending on the CO₂ concentration (Table 1). Fifteen percent CO₂ was the most practical level of treatment since it was nearly as effective as 20% and about twice as effective as 10%. Color change occurred before odor change in all cases. For example, the difference between color shelf-life and odor shelf-life was only about 1 day in the absence of CO₂ but was 8 days in the presence of 15% CO₂. The effect of CO₂ on the growth rate of the inoculum used is shown in Figure 1.

Tests with O₂-enriched air and pure O₂ showed that both the odor and color shelf-life increased with increased oxygen concentration above 50% (Table 2). Unlike the results obtained with carbon dioxide, change in color and odor occurred at about the same time. Bacterial count determinations showed that the extension in odor was the result of an inhibitory effect of high oxygen concentration on growth (Figure 1). It is assumed that oxygen increased color shelf-life by increasing the time the muscle myoglobin was held in the oxygenated form (oxymyoglobin, cherry red) (2).

Storage in gas mixtures composed of 15% CO₂ and 50-85% O₂ gave longer odor and color shelf-lives than did storage in either gas alone (Table 3). In tests giving the best results (15% CO₂ + 70-85% O₂), the increase in odor and color shelf-life over the corresponding shelf-lives in air was 13 (200%) and 9 (180%) days, respectively. Off-color limited the overall shelf-life in all cases. The results indicate that the separate beneficial effects of CO₂ and O₂ on odor and

color shelf-life are approximately additive when the gases are used together.

Weep and pH

Neither carbon dioxide, oxygen nor bacterial growth affected weight loss due to weep. Statistical analysis (t-test) of results showed that weight loss differences between samples stored in air and those stored in 15% CO₂, 50-85% O₂ or in mixtures of the two gases and between inoculated and uninoculated samples were not significant at the 5% level at any of the storage times tested.

Bacterial growth caused a sharp increase in the pH of samples containing a large population of cells (count above 10⁸/sq cm) while carbon dioxide did not change pH significantly. t-test analysis revealed that differences between pH of uninoculated samples stored in air and similar samples stored in 15% CO₂, 50-100% O₂ or mixtures of the two gases were not significant at the 5% level. Carbon dioxide had an indirect effect on pH change in inoculated samples, however, inasmuch as it inhibited bacterial development (Figure 1).

Discussion

It appears that storage in CO₂- or O₂-enriched air or in mixtures of CO₂ and O₂ could be used in a central pre-packaging operation, including transportation, to increase the shelf-life of fresh beef prepackaged in a gas permeable film of the type used in this study (PVC, in common use commercially). The greatest increase in shelf-life would be obtained with CO₂-O₂ mixtures since the beneficial effects of each gas are additive when used together. Storage in a 15% CO₂-85% O₂ mixture, for example, could mean a shelf-life increase of 9 days at 5°C compared to storage in air. As applied in this study, such a method would not require the use of unconventional packaging materials or a change in current packaging procedures. The prepackaged meat would need only be kept refrigerated in an atmosphere containing the desired gas composition during the necessary storage and transportation periods prior to retail display.

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Table 1

Effect of CO₂ on odor- and color shelf-life

CO ₂ concentration (%)	Odor shelf-life (days)	Color shelf-life (days)
0 (air)	6	5
10	11	7
15	16	8
20	17	8

Table 2

Effect of O₂ on odor and color shelf-life

O ₂ concentration (%)	Odor shelf-life (days)	Color shelf-life (days)
21 (air)	6	5
50	7	7
85	8	8
100	10	9

Table 3

Effect of CO₂-O₂ mixtures on odor and color shelf-life

Atmosphere	Odor shelf-life (days)	Color shelf-life (days)
Air	6	5
15% CO ₂ -21% O ₂ (balance, nitrogen)	16	8
15% CO ₂ -50% O ₂ (balance, nitrogen)	17	12
15% CO ₂ -70% O ₂ (balance, nitrogen)	19	14
15% CO ₂ -85% O ₂	19	14

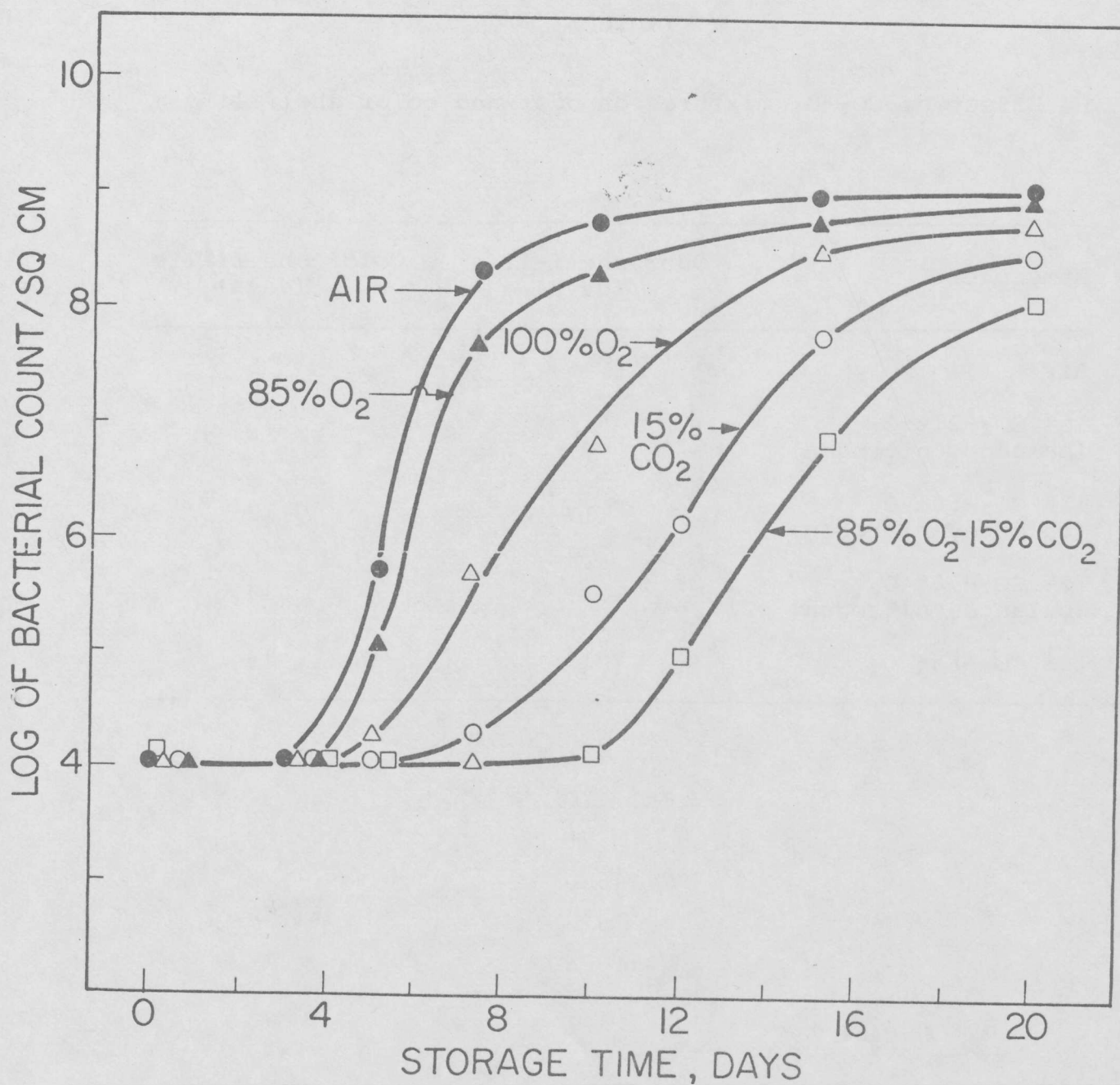


Fig. 1. Effect of CO₂ and O₂ on the growth of pseudomonads and achromobacteria on fresh beef at 5°C.