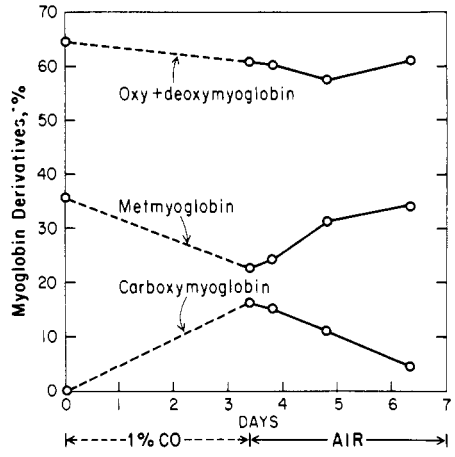

Figure 1. Relative amounts of myoglobin derivatives in ground beef samples exposed to carbon monoxide for 3.4 days, then stored in air: temperature, 2 °C; storage in air was under 15-W fluorescent illumination at 50 cm.

monoxide ingested as MbCO by the consumer can only be calculated if the rate at which CO dissociates from myoglobin occurs during the air storage period is known.

Since the rate of MbCO loss is approximately equal to the rate in increase of MetMb, the loss of the CO ligand may lead to rapid oxidation of the resulting Mb. Sampling beyond 7 days was not useful due to extensive microbial growth as evidenced by slime formation.

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Extension of Shelf Life in Refrigerated Ground Beef Stored under an Atmosphere Containing Carbon Dioxide and Carbon Monoxide

The microbiological and color shelf lives of ground beef patties exposed to a 1% carbon monoxide, 50% carbon dioxide, plus 49% air atmosphere were significantly increased compared to similar samples exposed to air at 2 °C. Total microbial plate counts indicated nearly a 100-fold difference in bacteria per gram of meat after 6 days between samples exposed to the modified atmospheres and those exposed to air and would appear to extend the microbiological shelf life approximately 4.5 days.. The fresh red color, measured with a Hunterlab Color/Difference Meter, was maintained for at least 6 days in samples exposed to modified atmospheres, while air-stored samples showed discoloration after 3 days.

In the modern day supermarket where virtually all fresh meats are prepackaged, the primary criterion used by today's consumer in meat selection is color. Although color is not a direct indicator of spoilage in meats, the formation of brown metmyoglobin (MetMb) may accompany extensive microbial growth. Spoilage in refrigerated meats is usually caused by the aerobic psychrophilic bacteria, primarily by species of *Pseudomanas*, *Flavobacterium*, and *Achromobacter* (Ayres, 1960; Jay, 1967; Haines, 1933). Spoilage has been shown to be retarded by use of atmospheres containing high concentrations of carbon dioxide (Haines, 1933; King and Nagel, 1967; Clark and Lentz, 1972; Huffman, 1974; Silliker et al., 1977). However, treatment of red meats, particularly beef, with high concentrations of carbon dioxide greatly accelerates the darkening of the meat's surface (Clark et al., 1969; Ledward et al., 1971; Silliker et al., 1977).

The use of carbon monoxide to extend the color life in fresh beef has been studied by El-Badawi et al. (1964). They showed that a mixture of 2% carbon monoxide and 98% air significantly preserved the color of fresh beef for 15 days. Under these conditions the pigment carboxymyoglobin (MbCO) is formed. MbCO, whose visual spectrum greatly resembles that of oxymyoglobin (MbO_2), is more resistant to oxidation than MbO_2. Clark et al. (1976) studying atmospheres containing carbon monoxide and nitrogen determined that continuous exposure of beef to this atmosphere substantially extended both the color and odor shelf life. They determined that a 1% CO atmosphere was the minimum concentration required for optimum color. Besser and Kramer (1972) also reported on the beneficial effect of CO treatment on the color of beef patties.

This paper reports the effects of an atmosphere containing both carbon dioxide and carbon monoxide on color changes and microbiological counts in fresh ground beef.

METHODS AND MATERIALS

Meat and Storage. Freshly ground beef of the leanest grade was purchased from a local retail market. Patties of uniform weight (50 ± 0.1 g), diameter (8.0 cm), and thickness (1 cm) were formed. Seven control patties were stored in an air tight 10-L desiccator jar on stainless steel mesh shelves containing an air atmosphere. Seven other patties were stored in a desiccator containing 50% carbon dioxide and 1% carbon monoxide, the balance being air. Storage was at 2 °C.

Microbiological Counts. At each sampling time, a 50-g patty was placed in a sterile blender jar with 150 mL of sterile 50 mM Tris–HCl buffer, pH 6.8, and blended for

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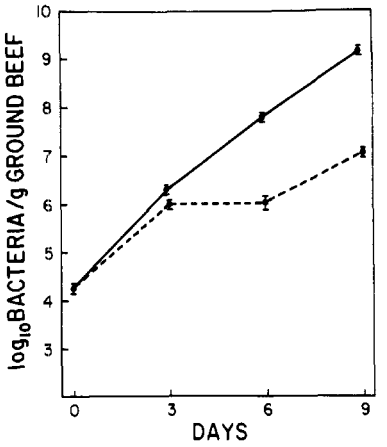

Figure 1. A comparison of bacterial growth between ground beef samples held in air (—) and samples held in a 50% CO_2 + 1% CO atmosphere (----). The values represent the average ● the standard deviation of four counts.

60 s. A series of dilutions into sterile buffer was made and 0.2-mL aliquots of three dilutions were placed into each of four plates containing Tryptone Glucose Extract Agar. The plates were incubated overnight at 20 °C after which the plates with between 30 and 300 colonies were counted.

Color Measurements. Three patties from each of the two desiccator jars were used repeatedly for color measurements. Color was measured using a Hunterlab Color/Difference Meter, Model D25D2. A pink standard (No. C21061) was used. The values for *L*, *a*, and *b* were recorded and averaged for each treatment.

RESULTS AND DISCUSSION

The results of the microbial counts are summarized in Figure 1. They show that there was a significant inhibition of microbial growth when the 50% CO_2 + 1% CO atmosphere was used. After 9 days, the microbial counts in patties stored in the 50% CO_2 + 1% CO atmosphere were over two log cycles lower than the counts found in the air controls. The counts at 9 days in samples held in the 50% CO_2 + 1% CO atmosphere correspond to counts that may be found, by interpolation, 4.5 days earlier in air controls. The differences in microbial counts found in this study correspond fairly well with differences in microbial counts reported by Silliker et al. (1977) when using a 60% CO_2 atmosphere (no CO) on pork and beef. Thus it appears that the presence of low levels of carbon monoxide has little effect on the microbial inhibitory properties of CO_2. This is further substantiated by work in progress in this laboratory on the lack of effect by CO-containing atmospheres on the growth of pure cultures of psychrophilic bacteria in liquid media. It should be noted, however, that Clark et al. (1976) demonstrated inhibition of bacterial growth by atmospheres containing CO but no oxygen.

Results of the color study using a Hunterlab Color/Difference Meter showed a marked difference between treatments in the Hunter "*L*" values and "a" values. Meat treated in the CO_2–CO atmosphere had higher "*L*" values than the meat stored in a normal air atmosphere, indicating increasing lightness (Figure 2). The Hunter "*a*" values, which measures, in the positive direction, the degree of redness, showed an increasing value in the samples stored in CO_2–CO atmosphere, for 6 days, followed by a sharp drop (Figure 3). The samples stored in air lost their bright red color within a few days, and this is reflected in their recorded Hunter "*a*" values. There was little difference between treatments regarding the Hunter "*b*" values which, in the positive direction, related to the degree of yellowness (Figure 4). Colorimeters have been shown

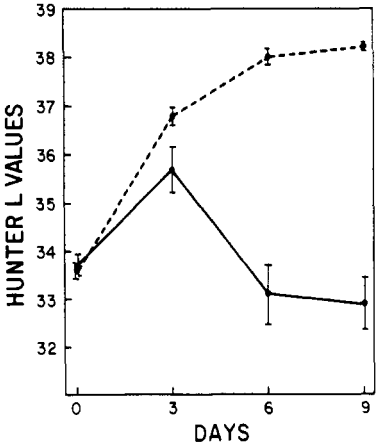

Figure 2. A comparison of Hunter *L* values between ground beef samples held in air (—) and samples held in a 50% CO_2 + 1% CO atmosphere (----). The values represent the average ± the standard deviation of three readings.

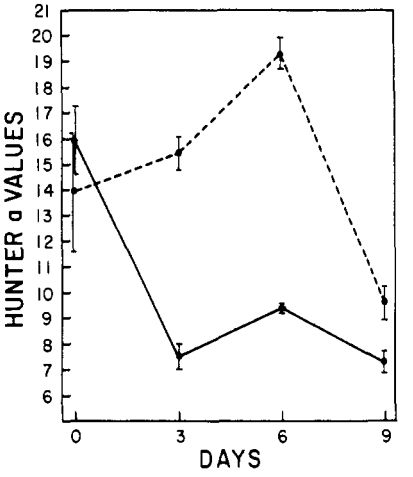

Figure 3. A comparison of Hunter "*a*" values between ground beef samples held in air (—) and samples held in a 50% CO_2 + 1% CO atmosphere (----). The values represent the average ± the standard deviation of three readings.

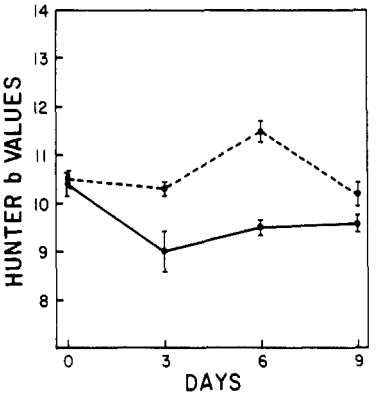

Figure 4. A comparison of Hunter "*b*" values between ground beef samples held in air (—) and samples held in a 50% CO_2 + 1% CO atmosphere (----). The values represent the average ± the standard deviation of three readings.

to provide a quick and accurate replacement for panels of trained observers (Strange et al., 1974).

The use of a 50% CO_2 + 1% CO atmosphere on refrigerated ground beef appears to extend the microbiological shelf life approximately 4.5 days while maintaining a desirable bright red color on the surface of the patties. Since ground beef is one of the worst cases in terms of

microbiological contamination, the use of modified atmospheres on primal or subprimal cuts of beef may demonstrate even better results.

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An Ion-Specific Electrode Analysis of Fluoride in Potato Tops Using an Ion-Exchange Pretreatment

A method for fluoride determination is developed which utilizes a hot-water extraction of inorganic F^- from dried potato tops. The interferences of F^- complexing species are effectively removed with an ion-exchange pretreatment, followed by a relatively simple specific-ion electrode determination. Recovery of F^- varied from 99–103%, compared to the AOAC procedure.

The use of a fluoride-specific electrode has become a convenient and economical tool for many types of samples, ranging from impurities in aluminum (Palmer, 1972) to contaminates in feeds (Torma, 1975) and plant matter (Baker, 1972; Vickery and Vickery, 1976). These procedures all have the common problem of complexation of fluoride by metals and hydrogen ions. The approach to solving this problem has been to find a complexing agent which will tie up the metals, buffer the solution to the correct range, and free fluoride ions, the only species to which the electrode responds (Durst, 1969). In this paper, a different approach is presented, that being to remove these metals by ion exchange, buffer the solution, and then determine the fluoride content.

EXPERIMENTAL SECTION

Apparatus. The fluoride-specific electrode was an Orion Research Model 94-09, and the reference electrode was an Orion Research Model 90-02 double-junction electrode. The pH meter used was a Beckman SS-1, with expanded millivolt scale.

Reagents. A 1000 μg/mL standard fluoride stock solution was made from reagent grade anhydrous potassium fluoride. Total ionic strength adjuster (TISAB) solution was made from reagent grade glacial acetic acid, sodium chloride, CDTA (cyclohexylenedinitrilotetraacetic acid), and sodium hydroxide according to the electrode manufacturer's instructions. Solutions of Al and Fe, 1000 μg/mL, were purchased from Fisher Scientific.

The ion-exchange resin used was Amerlite CG-120, 100–200 mesh. The resin was first washed in 7 N NH_4OH and then water washed until neutral. Next, it was washed in 6 N HCl and again washed with distilled water until neutral. The resin was stored in distilled water until used.

Procedure. The samples used for this study were potato tops which had been dried in open air sunlight for 1 week. The vegetation was then crushed to fit in gallon jars and dried at 105 °C for 48 h. The samples were then ground in an Osterizer finely enough to pass through an 80 mesh (180 μm) screen.

One-gram samples of these tops were extracted with 25 mL of distilled water on a steam bath for 30 min. The extracts were then filtered through glass fiber filters into a 50-mL volumetric flask and then diluted to volume with distilled water.

Ten milliliters of this solution were then placed onto 5 g of Amberlite which was packed in a 50-mL buret. The sample was washed off the column with an additional 15 mL of water. The column was eluted into another 50 mL volumetric flask which contained 25 mL of the TISAB solution. The contents were mixed, and the fluoride content was determined with the fluoride electrode according to the manufacturer's instructions.

The ion-exchange resin was then stripped by washing 5 mL of 7 N NH_4OH onto the column and washing with distilled water until the eluent was neutral to litmus paper. The resin was then recharged by washing 5 mL of 6 N HCl onto the column, and the distilled water wash was repeated until the eluent was again neutral. The same resin was used in this manner for three samples before discarding. The electrode calibration was done without ion-exchange pretreatment and covered the range from 0.1 to 20 μg/mL.

RESULTS AND DISCUSSION

A calibration curve was constructed to cover a wide range of concentrations since the approximate fluoride concentration of the potato tops were not known. The range covered was 0.1 to 20 μg/mL. The calibration gave a straight line with a slope of −57.8 (which indicates Nernstian behavior) and a correlation coefficient (r^2) of 99.98%.

Recovery studies of F^- through the ion-exchange column were conducted using 10-mL aliquots of 5, 10, and 20 μg/ml of F^- standard solutions. After ion exchange, the

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