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Effects of packaging systems and fat concentrations on microbiology, sensory and physical properties of ground beef stored at 4 ± 1 °C for 25 days

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ABSTRACT

This study evaluated effects of modified atmosphere (MAP, 0.4% carbon monoxide [CO], 30% carbon dioxide, and 69.6% nitrogen), vacuum (VP) and polyvinyl chloride (PVC) packaging systems and fat levels (10, 20 and 30% fat) on ground beef stored at 4 ± 1 °C for 25 days for microbiology, sensory, pH, thiobarbituric acid reactive substances (TBARS), objective color, headspace and residual CO. As storage time increased, pH decreased ($P < 0.05$) for MAP and VP and increased ($P < 0.05$) for PVC. TBARS varied ($P < 0.05$) among MAP and VP treatments. Except for day 1, CO headspace concentrations were similar among fat concentrations, and residual CO absorption in meat increased ($P < 0.05$) for all MAP treatments. In all treatments, degree of lightness was similar, redness decreased and brown discoloration increased during storage. As psychrotrophic bacteria counts increased, panelists detected color and off-odor deterioration in all systems. The CO treatment had no effect on maintaining the carboxymyoglobin "cherry red" fresh meat color during meat spoilage.

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1. Introduction

Packaging systems play a major role in color enhancement and stability of muscle food products during storage. The three major packaging systems include vacuum packaging (VP), modified atmosphere packaging (MAP) and polyvinyl chloride film (PVC). Fresh meat is susceptible to discoloration by low levels of oxygen (Sebranek & Houser, 2006). Partial oxygen pressure in the range of 5–10 mm of mercury will rapidly convert the myoglobin pigment in meat to metmyoglobin. Therefore, the residual oxygen (O_2) requirement for vacuum packaged meat is very strict and must be below 0.05% after packaging, and essentially zero within 24 h following packaging (Venturini, Contreras, Sarant'Opoulos, & Villanueva, 2006). PVC packaging is used for retail storage of meat and is extremely permeable to oxygen. The high oxygen and moisture permeability of PVC function to maintain the desirable bright 'cherry red oxymyoglobin color' associated with fresh meat. The VP system involves exhaustion of oxygen from the package and sealing immediately or flushing the package with a single gas or combination of gasses. MAP is defined as packaging of a product in an atmosphere which has had a one-time modification of gaseous composition so that it is different from that of air, which normally contains 78.08% nitrogen, 20.96% oxygen, and 0.03% carbon dioxide (USDHHS USFDA, 2009). Modifications of the package atmosphere could involve changing the composition of the gasses or inclusion of other gasses such as carbon monoxide (CO).

A distinct advantage of meat color stability resulting from carbon monoxide in MAP is that discoloration from elevated levels of carbon dioxide is no longer a problem (Sorheim, Nissen, Aune, & Nesbakken, 2001). It is well documented that CO functions to stabilize the bright red color, carboxymyoglobin, in beef (Jayasingh, Cornforth, Carpenter, & Whittier, 2001; Krause, Sebranek, Rust, & Honeyman, 2003), pork (Viana, Gomide, & Vanetti, 2005) and tuna (Kowalski, 2006). Brooks et al. (2008) reported that packaging ground beef in a low-oxygen-carbon monoxide (0.4% CO, 30% CO_2 and 69.6% N) system resulted in formation of the stable carboxymyoglobin pigment and prevention of surface discoloration. The growth of psychrotrophic aerobic bacteria, total aerobic bacteria and *Lactobacillus* counts were significantly decreased when compared to the conventional PVC packaging system. The lower microbial counts could also be attributed to the presence of CO_2 in the package. Dixon and Kell (1989) determined that lowering of meat pH is a result of CO_2 absorption and production of carbonic acid that dissociates to bicarbonate and hydrogen ions. A decrease in meat pH has been shown to negatively affect the rate of oxidation processes such as pigment and lipid oxidation as well as water holding capacity (WHC) of meat (Juncher et al., 2001).

Carbon monoxide is approved for use in MAP for meat systems (USDA FSIS, 2013) at levels of 0.4% in combination with 20 to 30% CO_2. Carboxymyoglobin is much more stable toward oxidation than

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oxymyoglobin due to the stronger affinity of CO for the iron porphyrin site on the myoglobin molecule. It is believed that this stable red color may conceal spoilage and place consumers at risk (Pattron, 2007). Cornforth and Hunt (2008) reported that the two major disadvantages of CO-MAP included a negative image of CO by consumers because it is a potentially hazardous gas, and concern that products might look fresh even though bacterial levels are high and the product is spoiled. However, Watts, Wolfe, and Brown (1978) found that CO-treated ground beef did not remain red indefinitely. When ground beef was treated with 1% CO and then exposed to air, CO was slowly lost (T1/2 = 3 days), and there was an 85% loss of CO after cooking. CO is also considered a processing aid and therefore, is not required to appear on the product label to inform consumers of its use in the product.

The objectives of this study were to evaluate the effects of MAP (0.4% CO, 30% CO_2 and 69.6% N_2), conventional VP, and retail PVC overwrap packaging systems and three fat levels (10, 20 and 30%) on storage stability of fresh ground beef, and to ascertain the effects of the treatments on microbiology (aerobic plate count, total psychrotrophs, Gram negatives, *Escherichia coli* O157:H7, total coliforms, generic *E. coli*, and lactic acid bacteria), pH, onset of rancidity, objective color, headspace and residual carbon monoxide, and sensory characteristics.

2. Materials and methods

2.1. Formulation, packaging and storage of ground beef

Fresh beef was obtained from carcasses after 48 h chilling at 0 °C in a USDA inspected processing facility. Beef containing 50% fat (50/50) and 10% fat (90/10) were blended in the appropriate proportions using the Pearson's Square calculation method (Pearson & Tauber, 1984) to obtain 20 and 30% fat ground beef (uncooked basis). No blending was necessary for the 10% fat ground beef. After blending, the meat was ground (Butcher Boy, Berkel Model 100.42, Laser Co., Inc., Los Angeles, CA) through a 0.3175 cm grinding plate, divided into three equal aliquots and packaged in either Genpak 2 styrofoam trays and over wrapped with one layer of PVC film (companions, product # 12073, oxygen transmission rate — 1400 cc/m^2/24 h/22.8 °C; water transmission rate — 32 g/24 h @ 37.8 °C); vacuum packaged in 22.86 cm × 45.72 cm Cryovac B4770 barrier bags (oxygen transmission rate — 1 cc/m^2/24 h atm @ 40 °F; water transmission rate — 0.5–0.6 g/24 h @ 37.8 °C, Simpsonville, SC), or vacuum packaged in Cryovac B4770 barrier bags containing a manually, aseptically installed septum valve (Cole-Parmer, Vernon Hills, IL 60061-1844, catalog no. 00095XR) to allow for the injection of the CO gas blend. Each MAP bag was injected with 3 L of a custom gas blend composed of 0.4% CO, 30% CO_2 and 69.6% N_2 (Airgas Specialty Gases, Gainesville, FL) which was measured using a mass flow meter (model M-50SLPM-D, Alicat Scientific, Inc., Tucson, AZ 85745). All samples were stored in a walk-in cooler (4 ± 1 °C) and analyzed after 0, 1, 3, 5, 7, 14, 21 and 25 days for microbiology (aerobic plate count [APC], total psychrotrophs [TPSY], total Gram negative organisms [GN], *E. coli* O157:H7, total coliforms and generic *E. coli*, and lactic acid bacteria [LAB]), pH, thiobarbituric acid reactive substances [TBARS], headspace and residual carbon monoxide, color and sensory characteristics. The temperature of the storage cooler was monitored daily and continuously using a circular-chart thermometer installed in the cooler (model RTF, Partlow, Elizabethtown, NC 28337). The fluorescent lights inside the cooler were kept on at all times.

2.2. Microbial analyses

All media (Difco Laboratories, Detroit, MI 48232) and materials used for the cultivation and maintenance of the bacteria were purchased from Fisher Scientific (Pittsburgh, PA). Twenty-five grams of ground beef from each treatment was placed in sterile 18 × 30 cm Fisherbrand stomacher bags (400 mL, Fischer Scientific, Pittsburgh, PA 15238) along with 225 mL of sterile 0.1% peptone water (catalog no. DF1807-17-4) and massaged manually for 2 min to produce the sample homogenate which was used to prepare the appropriate dilutions using sterile 0.1% peptone water. One microliter of the dilutions was pipetted and spread onto the surface of prehardened Trypticase Soy Agar (TSA, catalog no. B11043) for aerobic plate and total psychrotrophic counts, Sorbitol MacConkey agar (SMAC, catalog no. OXCM0813B) supplemented with a Cefixime–Tellurite supplement (CT, catalog no. OXSR0172E) for *E. coli* O157:H7, Lactobacilli MRS agar (MRS, catalog no. DF0882170) for lactic acid bacteria and GN Broth (GN, catalog no. DF0486-17-4) with added granulated agar (catalog no. DF0145-17-0) for Gram negative bacteria counts. One milliliter of each dilution was added onto 3 M Petrifilm *E. coli*/Coliform Count Plates (3 M Company, St. Paul, MN 55144, catalog no. 6414) for generic *E. coli* and total coliforms.

The TSA plates for total plate counts, and GN and MRS plates were incubated at 35 ± 1 °C for 48 h, CT-SMAC plates were incubated at 37 ± 1 °C for 24 h, 3 M Petrifilm *E. coli*/Coliform Count Plates were incubated at 35 ± 1 °C for 24 h, and TSA plates for total psychrotroph counts were incubated at 7 ± 1 °C for 10 d. After incubation, colony forming units (CFU) were counted, recorded, averaged and reported as log colony forming units per gram (log CFU/g).

2.3. pH analysis

The pH analyses were conducted immediately after the microbiological analyses were completed using the same sample homogenates. Two pH measurements were recorded for each sample using and Accumet pH meter (model AB15, Fisher Scientific, Pittsburgh, PA). The probe was placed into the 10^{-1} sample homogenate and allowed to reach equilibrium for 1 min before the readings were recorded.

2.4. Carbon monoxide analyses

2.4.1. Carbon monoxide headspace

The headspace CO concentration (Miyazaki et al., 1997) was measured using the Agilent Technologies 6890 N Network Gas Chromatograph System. Two 100 μL samples from each package (two packages per treatment) were collected and injected into the GC using a 100 μL Hamilton syringe (Cat. No. 14-815-80). One peak was obtained for each 100 μL sample that was injected into the GC. The two peaks per bag for the ground beef were then averaged and used in the following formulas to determine CO concentration in the headspace of the package:

$$\% \text{ CO} = 0.000196\ X – 0.001458$$

(where X = average peak area); ppm CO = % CO * 1,000,000.

2.4.2. Residual carbon monoxide

Residual CO was measured as outlined in the method of Miyazaki et al. (1997). Two 6 g ground beef samples from each package (two packages per treatment) were each placed into a 60 mL tube (I-Chem Economy 100-series tubes, catalog no. 05-719-398) with three drops of octanol (antifoaming agent, catalog no. S80109), 12 mL of 10% sulfuric acid solution (catalog no. 815032), and shaken for 10 seconds manually, followed by incubating the mixture for 5 min at 40 °C, then shaking mechanically for 15 min. Duplicate 100 μL aliquots of the solution were injected into the GC using a 100 μL Hamilton syringe (catalog no. 14-815-80). One peak was obtained for each 100 μL sample that was injected into the GC. The data obtained for the two peaks per tube were averaged and used to determine ppm CO/g meat using the following equations: % CO = 0.000196 X − 0.001458 (where X = average peak area); g CO/g meat = (0.000001145 * % CO * 430)/6; adjusted g CO/g meat = (6 * g CO/g meat)/weight of Sample; ppm CO/g meat = adjusted g CO/g meat * 1,000,000.