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Potential mechanisms of carbon monoxide and high oxygen packaging in maintaining color stability of different bovine muscles

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ABSTRACT

The objectives were to compare the effects of packaging methods on color stability, metmyoglobin-reducing-activity (MRA), total-reducing-activity and NADH concentration of different bovine muscles and to explore potential mechanisms in the enhanced color stability by carbon monoxide modified atmosphere packaging (CO-MAP, 0.4% CO/30% CO_2/69.6% N_2). Steaks from *longissimus lumborum* (LL), *psoas major* (PM) and *longissimus thoracis* (LT) packaged in CO-MAP, high-oxygen modified atmosphere packaging (HiOx-MAP, 80% O_2/20% CO_2) or vacuum packaging were stored for 0 day, 4 days, 9 days, and 14 days or stored for 9 days then displayed in air for 0 day, 1 day, or 3 days. The CO-MAP significantly increased red color stability of all muscles, and especially for PM. The PM and LT were more red than LL in CO-MAP, whereas PM had lowest redness in HiOx-MAP. The content of MetMb in CO-MAP was lower than in HiOx-MAP. Steaks in CO-MAP maintained a higher MRA compared with those in HiOx-MAP during storage. After opening packages, the red color of steaks in CO-MAP deteriorated more slowly compared with that of steaks in HiOx-MAP.

1. Introduction

Meat color is frequently used as an indicator of freshness and wholesomeness, so color plays a critical role in determining consumers' purchasing decisions (Mancini & Hunt, 2005).

Modified atmosphere packaging (MAP) is sometimes used to maintain the attractive red color of fresh meat (Sørheim, Aune, & Nesbakken, 1997). The most commonly used MAP gas mixture for fresh beef in retail markets is 80% oxygen and 20% carbon dioxide (John et al., 2005; Resconi et al., 2012). High concentration of oxygen can slow myoglobin oxidation because oxymyoglobin cannot directly oxidize to metmyoglobin (George & Stratmann, 1952). However, high oxygen modified atmosphere packaging (HiOx-MAP) can increase lipid oxidation and protein oxidation, with negative effects on meat flavor (Jakobsen & Bertelsen, 2000; Jayasingh, Cornforth, Brennand, Carpenter, & Whittier, 2002) and texture (Lindahl, Lagerstedt, Ertbjerg, Sampels, & Lundstrom, 2010). To extend red color stability and avoid the drawbacks of aerobic packaging, an anaerobic MAP technology with 0.4% CO (CO-MAP) was approved (FDA, 2004) for use with fresh meats in the USA (Cornforth & Hunt, 2008). Carbon monoxide can bind to the 6th position of deoxymyoglobin and generate the bright red carboxymyoglobin (Brewer, Wu, Field, & Ray, 1994; Mancini & Hunt, 2005). Previous studies have proved that CO can significantly increase color stability of beef compared with other packaging methods (Jeong & Claus, 2010, 2011; John et al., 2005; Mancini, Suman, Konda, & Ramanathan, 2009). The possible reason that CO-MAP could enhance red color stability was related to the higher stability of the carboxymyoglobin than oxymyoglobin (Brewer et al., 1994; Mancini & Hunt, 2005), but other potential mechanisms have not been explored yet.

Gee and Brown (1978) reported that the red color of 1% CO treated ground beef was lost within a few days when the samples were exposed to air. Jeong and Claus (2011) also found that 0.4% CO-packaged ground beef became less red after opening the package, and the color of ground beef packaged in CO packaging was lighter compared with the color of ground beef packaged in vacuum packaging. However, the factors affecting color loss of beef steaks in CO-MAP upon opening the packages are still not completely understood. Additionally, the variable color stability of CO-packaged beef steaks from different muscles after opening the packages is not clear.

Color stability is affected by the intrinsic factors of muscle type and metabolism and extrinsic factors of packaging type (Bekhit & Faustman, 2005; Sammel, Hunt, Kropf, Hachmeister, & Johnson, 2002). Each muscle has the unique fiber type and metabolic function. Different muscles have different color stability when exposed to atmospheric oxygen due to different metmyoglobin reducing activity (MRA) (McKenna et al., 2005; Sammel et al., 2002; Seyfert et al., 2006). King, Shackelford, Rodriguez, and Wheeler (2011) also reported that MRA has a high correlation with color stability. Mancini et al. (2009) reported that color stability of different muscles is dependent on packaging type. High oxygen packaging has a negative effect on MRA from 0 day to 4 days (Seyfert, Mancini, Hunt, Tang, & Faustman, 2007). To our knowledge, no information has been provided about the MRA under the CO-MAP condition.

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Metmyoglobin can be enzymatically reduced to deoxymyoglobin in the presence of nicotinamide adenine dinucleotide (NADH) (Bekhit & Faustman, 2005). NADH plays an important role in the metmyoglobin reducing system and acts as a coenzyme and electron carrier in the conversion of ferric myoglobin to its ferrous form (Renerre, 1990). Many researchers have investigated the relationship between NADH and color stability (Bekhit, Geesink, Ilian, Morton, & Bickerstaffe, 2003; Kim, Keeton, Smith, Berghman, & Savell, 2009). However, little information is available about the effects of different packaging types especially CO-MAP and HiOx-MAP on NADH concentration.

In China, MAP was introduced in retail meat markets very recently and the cost is relatively high. Consequently, only *longissimus lumborum*, *psoas major* and *longissimus thoracis* which are highly priced would be packaged by MAP. The objectives of this study were to compare the effects of CO-MAP and HiOx-MAP on color stability and on the rate of red color loss of the aforementioned three muscles and to explore the potential mechanisms for enhanced color stability and decreased color loss by CO-MAP, and especially, to investigate the relationships between the packaging methods and the MRA and NADH concentration.

2. Materials and methods

2.1. Raw materials

The experiment was undertaken following the guidelines of the Animal Ethics Committee in Shandong Agricultural University and all experimental procedures were approved by the State Scientific and Technological Commission which was passed in Nov. 1988 in China.

Two separate experiments were conducted to investigate the objectives of this study. For both experiments, eight beef carcasses (Luxi × Simmental, 18–24 months old, 291 to 320 kg, pH_{24} 5.4 to 5.8) were selected from a commercial abattoir. The anterior and posterior carcass quarters were separated between the 11th and 12th ribs. On day 2 postmortem, the *longissimus lumborum* (LL, 12th rib to the last lumbar vertebrae), *psoas major* (PM) and *longissimus thoracis* (LT, 6th–12th ribs) on both sides of each animal were removed, vacuum packaged and transferred to the lab within 2 h.

2.2. Packaging and storage

In experiments 1 and 2, each muscle was portioned into 2 cm thick steaks by cutting perpendicular to the muscle length at 3 days postmortem. The steaks from each muscle were individually packaged in either vacuum packaging, HiOx-MAP (80% O_2, 20% CO_2) or CO-MAP (0.4% CO, 30% CO_2, 69.6% N_2). Vacuum packaging was performed using a MultiVac C200 (C200; Multivac Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany). The vacuum bag (SP21; Sealed Air Corp., Danbury, USA) was 62 μm thick, with the oxygen permeability of 50 $cm^3/m^2/24$ h at 23 °C and the water vapor transmission rate of 10 $g/m^2/24$ h at 38 °C. Steaks packaged in MAP were placed in trays (oxygen transmission rate: 10 $ml/m^2/24$ h at 23 °C/0% relative humidity, water vapor transmission rate: 15 $g/m^2/24$ h at 38 °C/90% relative humidity; TQBC-0775; Sealed Air Corp., Danbury, USA) with a Dri-Loc soaker pad (DLS-25; Sealed Air Corp., Danbury, USA) and trays were filled with the desired gas mixture using a DT-6D packaging machine (DT-6D; Dajiang machinery equipment Co. Ltd., Wenzhou, China). Trays were sealed with oxygen-barrier film (oxygen transmission rate: 25 $ml/m^2/24$ h at 23 °C/0% relative humidity, water vapor transmission rate: 10 $g/m^2/24$ h at 4 °C/100% relative humidity; Lid 1050; Sealed Air Corp., Danbury, USA). HiOx-MAP gas mixtures were mixed using the DT-6D packaging machine and CO-MAP gas mixtures were prepared and certified by the supplier (Xieli Special Gas Co., Ltd., Jining, China). In experiment 1, three steaks of each muscle remained unpackaged and were prepared for day 0 (initial color) instrumental color (after 45 min bloom) and chemical analyses. The packaged steaks were stored at 2 °C for 14 days in the dark. On days 4, 9 and 14 of storage, the surface of steaks was scanned immediately after packages were opened. In experiment 2, steaks were stored at 2 °C for 9 day in the dark and then packages were opened on day 9. After opening the packages, steaks were allowed to bloom for 45 min at 2 °C and overwrapped with an oxygen permeable polyvinyl chloride (PVC) film (oxygen transmission rate = 25,500 $cm^3/m^2/24$ h). PVC-overwrapped steaks were stored at 2 °C in darkness for 0 day (after 45 min bloom), 1 day, and 3 days before color and chemical analyses were taken.

2.3. pH

pH values of steaks were measured at 0 day, 4 days, 9 days and 14 days postmortem using a portable pH meter (SenvenGo, Mettler-Toledo, Switzerland) which was calibrated in buffers with pH 4.00 and 7.00 and inserted into the center of meat samples (Hou et al., 2013). Three measurements were taken from each steak and averaged.

2.4. Color measurement

All instrumental reflectance color measurements were performed using a X-Rite spectrophotometer (Model SP62, 8 mm diameter aperture, Illuminant A, 10° observer; X-Rite, Incorporated, Grand Rapids, USA). The average value of eight measurements on the surface was used. The instrument recorded reflectance values in the range of 400 nm to 700 nm at 10-nm intervals. Reflectance values that were not directly measured by spectrophotometer at specific wavelengths (474, 525 and 572 nm) were calculated by linear interpolation. The Kubelka–Munk K/S values were then calculated. The relative content of deoxymyoglobin (DeoxyMb) was estimated by the ratio (K/S474)/(K/S525), the relative content of oxymyoglobin (OxyMb) by the ratio (K/S610)/(K/S525) and the relative content of metmyoglobin (MetMb) by the ratio (K/S572)/(K/S525) (Hunt et al., 1991). In experiment 2, because the K/S ratio decreases when the relative content of corresponding myoglobin increases, the K/S ratio of DeoxyMb was transformed to [1.5 − (K/S474)/(K/S525)], the K/S ratio of OxyMb was transformed to [1 − (K/S610)/(K/S525)] and the K/S ratio of MetMb was transformed to [2 − (K/S572)/(K/S525)] (Li, Lindahl, Zamaratskaia, & Lundstrom, 2012).

2.5. Metmyoglobin-reducing activity

Metmyoglobin-reducing activity (MRA) was determined according to the procedure of Sammel et al. (2002) with minor modifications. A cube (2.54 × 2.54 × 2 cm^3) with no visible fat or connective tissue was removed from each steak on days 0, 4, 9 and 14. The samples were oxidized in 50 ml of a 0.3% sodium nitrite solution for 20 min at room temperature. Samples were removed, blotted dry, vacuum packaged and immediately scanned three times by using a SP62 spectrophotometer, as described above. Samples were placed in an incubator (SPX-158; Jiangnan Instrument CO., Ningbo, China) at 30 °C for 2 h and then rescanned. The MRA was calculated as: [(Initial %MetMb − Final %MetMb) / initial %MetMb] × 100.

2.6. Total reducing activity

Samples for analysis of total reducing activity (TRA) and NADH concentration were cut out from the surface of steaks and trimmed free of fat and any visible connective tissue, frozen in liquid nitrogen immediately and then stored at −80 °C until analysis.

The procedure of Lee, Cassens, and Fennema (1981) was used to measure total reducing activity (TRA). Duplicate 2 g of powdered frozen muscle tissue from steak surface was homogenized (T18, IKA, Werke GmbH & Co. KG, Staufen, Germany) in 10 ml of 25 mM PIPES (piperazine-1,4-bis (2-ethanesulfonic acid)) buffer. Then 5 ml of homogenate was mixed