



Effect of pretreatment with carbon monoxide and ozone on the quality of vacuum packaged beef meats



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ABSTRACT

Beef meats without pretreatment (CK) or pretreated with different volume ratios of carbon monoxide and ozone of 100%CO (T1), 2%O₃ + 98%CO (T2), 5%O₃ + 95%CO (T3) and 10%O₃ + 90%CO (T4) using modified atmosphere packages for 1.5 h, after that they were vacuum-packaged and stored in 0 °C refrigerator for 46 days. The surface color a* values and sensory scores of T1, T2, T3 and T4 were significant higher than CK ($p < 0.05$) during storage. In the mid and later storage, the drip loss, total viable counts (TVC), metmyoglobin (met-Mb), thiobarbituric acid reactive substances (TBARS), total volatile basic nitrogen (TVB-N) and pH of T1, T2, T3 and T4 were significantly lower than CK ($p < 0.05$), and these values of T2, T3 and T4 were significantly lower than T1 in the later storage. In conclusion, O₃ in the combination didn't affect the color-developing effect of CO, and could help CO maintain the meat quality. Therefore, the pretreatment of CO combined with O₃ at certain concentrations can be a promising technique to maintain the quality of beef meats.

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1. Introduction

Beef meats, one of the most popular consumption meats in the world, are low fat and high protein content, have the nutritional composition of key nutrients, such as minerals, fatty acids and vitamins (Hambidge & Krebs, 2007), and are a major source of high quality dietary proteins for human metabolic processes. However, due to its biological composition, beef represents a favorable environment for microbial growth and is highly susceptible to spoilage (Udenigwe & Howard, 2013). A few preservation technologies have been applied to maintain its safety and quality, and extend the shelf life, which will be useful for both regional farmers and consumers around the world (Aymerich, Picouet, & Monfort, 2008).

Packaging beef meat protects it against deterioration. Vacuum package (VP) lacking of O₂ in packages can minimize the oxidative deteriorative reactions. Modified atmosphere package (MAP) consists in the replacement of air in the packaging atmosphere by a mixture of different gases (mainly composed of O₂, CO₂ and N₂), can be used to extend the shelf life of meat (Chaix et al., 2015; Nair, Kiess, Nannapaneni, Schilling, & Sharma, 2015). Polyvinyl chloride film (PVC), which is used for retail storage of meat and is extremely permeable to oxygen, and so on. However, for at least two decades, low O₂ MAP has been less-used, on account of VP is the most cost-effective package (Brewer, Jensen, Prestat, Zhu, & McKeith, 2002).

A recent research indicated that color and appearance are the most frequently used factors in consumer purchase decisions and judging shelf life of fresh meat (Brewer, Wu, Field, & Ray, 1994). As we all know, enzyme-mediated reactions termed metmyoglobin-reducing activity always dissipates making the color of meat terrible during storage (Kropf, 2003), but carbon monoxide (CO) can displace O₂ from oxymyoglobin (MbO₂), once it has been bound and carboxymyoglobin (COMb) is more stable than deoxymyoglobin (Elbadawi, Anglemey, Cain, & Samuels, 1964). In order to impart a desired red color, meat may be exposed to CO before sealing or in low CO atmosphere (CO-MAP) be sold out. Nevertheless, Cornforth and Hunt (2008) reported that there were two major disadvantages of CO-MAP. As the potentially hazardous gas, CO might bring negative images to the consumers. In this way, the pretreatment of CO for meat may be more suitable than CO-MAP for consumers. Another is the color of meat also looks fresh, even though the bacterial levels of beef are high. Therefore, meat preservation techniques should be developed to control the risk of microbial contamination.

Sometimes, the main cause of fresh meat spoilage is the growth of bacteria, and increasing bacteria will modify the color and quality of the meat (Renner, 1990). We know that ozone (O₃) is highly reactive and a strong oxidizing agent, in the meantime, classified as "GRAS" (Generally Recognized as Safe) by the United States Environmental Protection Agency (USEPA). People all over the world have used O₃ to kill bacteria, sanitize drinking water and food, and decrease aflatoxin contamination (Inan, Pala, & Doymaz, 2007). Moreover, Kim and Yousef listed a number of bacteria species that are commonly found on food and are very susceptible to O₃ (Kim & Yousef, 2000). It means that in

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some cases, O_3 can have better sterilization ability than the other gas. Several studies revealed O_3 exposure prevents microbial growth and extends the shelf life of treated produce. [Dondo, Nachtman, Doglione, Rosso, and Genetti \(1992\)](#) found that the O_3 treatment can greatly inhibit the amount of microbes of fishes, and improve the effect of its sensory quality. Treatment with O_3 water resulted in about 1.25 (log CFU/g) reductions of total bacteria of Pacific oyster before storage respectively, and enhanced the shelf life of 2 days ([Rong, Qi, Yin, & Zhu, 2010](#)). Furthermore, other studies showed that half-life of gaseous O_3 is 12 h at atmospheric pressure, and gaseous O_3 is more stable than aqueous O_3 ([Weavers & Wickramanayake, 2001](#)).

The color-stabilizing effect of CO and the sterilization effect of O_3 have been reported previously. However, limited information is available on the effects of co-treatment of CO and O_3 on quality of beef stored, no published results from experiments combining both technologies are available. Thus the objectives for this study were to investigate the effect of the combination gases of CO and O_3 pretreatment on the microbiological, chemical, physical and sensory characteristics of beef meats during storage at 0 °C and to find the best way to extend the shelf life and maintain good qualities for vacuum packaging beef meats.

2. Materials and methods

2.1. Raw materials

A total of 8 Luxi × Simmental steer (18–24 months old, 286–323 kg) were selected randomly from a local farm, and slaughtered on a commercial abattoir. The longissimus lumborum and psoas major of the tenderloin were removed from both sides of the carcasses after 48 h post-mortem, and were trimmed of all visible fat and cut into beefsteaks about 50 g with 2 cm thickness.

O_3 was produced from O_3 generator (QD-OS-S-300L, Guangzhou, China). CO was supplied by Hangzhou Distribution Company (Hangzhou, China) from a gas cylinder, the gas purity was above 99.5%.

2.2. Gas pretreatment

Steaks were pretreated by different gas volume ratios of CO and O_3 . The pretreatments are showed as follows:

1. Steaks without gas pretreatment (CK);
2. Steaks were pretreated by 100% CO MAP for 1.5 h (T1);
3. Steaks were pretreated by 2% O_3 + 98%CO MAP for 1.5 h (T2);
4. Steaks were pretreated by 5% O_3 + 95%CO MAP for 1.5 h (T3);
5. Steaks were pretreated by 10% O_3 + 90%CO MAP for 1.5 h (T4).

After the pretreatments of CO and O_3 , the MAP was removed and the meats were vacuum packaged and stored in 0 °C refrigerator for use.

The gas concentrations of CO and O_3 were controlled on the basis of gas volume. At first, steaks of T1, T2, T3 and T4 were modified atmosphere packaged with 100% CO. The volume of the MAP tray was 635.25 cm³ (not including the volume of steaks and water absorbent pad). After CO MAP, steaks of T2, T3 and T4 were injected O_3 with the volume of 12, 34, and 71 mL as soon as possible to arrive the ratios of CO and O_3 , respectively. Modified packaging machine (MAP-H360, Senrui Preservation Equipment Co., Ltd., Suzhou, China) was used to fill the 100% CO, and gas injector was used to achieve the required approximate volumes of O_3 . [Fig. 1](#) showed the O_3 injecting process using the syringe with 20 mL volume. The MAP used trays were made of polystyrene with O_2 permeability of 0.1 g O_2 /1000 cm²/24 h at 0 °C and H_2O permeability of 0.0 g H_2O /1000 cm²/24 h at 0 °C, and films used were the nylon/polyethylene film with O_2 permeability of 0.8 g O_2 /1000 cm²/24 h at 0 °C and H_2O permeability of 0.1 g H_2O /1000 cm²/24 h at 0 °C. The volume of the MAP boxes was 635.25 cm³.

Before MAP pretreatments, rubber septa (one side is polytetrafluoroethylene and the other side is rubber) were stuck on the MAP film by EVA adhesive, with the side of rubber outside. We



Fig. 1. Operation for the combination gases of CO and O_3 .

stuck the rubber septa every 12 cm on the films, so that each tray automatically was packaged with the film having a rubber septa after MAP. Water absorbent pad (125 mm × 52 mm × 0.8 mm, PE/cellulose series) was put into the packing trays, and then the steaks were put in aseptic conditions and packaged with 100% CO using the modified packaging machine. Meanwhile, the O_3 generator was turned on and the gas outlet of O_3 generator was put in the water, running 3 min to eliminate internal oxygen. After that, the outlet of O_3 generator was put into the bottom of a gas collect bottle with a capacity of 2 L. The collect bottle of O_3 was a narrow-mouthed bottle with an opening on the top. The generating O_3 velocity was 0.04 m³/h. The time of the gas injecting into the bottle was 5 min enough to eliminate internal atmosphere and injecting O_3 gas. Then the bottle was sealed with a rubber plug immediately. The collection of O_3 was put in a dark place with a low temperature, and used in 5 min. There was a micro-channel in the rubber plug, it was easy to acquire the O_3 gas from this channel. When the O_3 gas was acquired using the syringe, the channel was sealed with a gas-insulated sticker quickly. After MAP, prepared O_3 was injected into the packing box immediately, and the rubber septum was the location of the needle of the syringe, and then put into the refrigerator of 0 °C for 1.5 h. After the pretreatment of different concentrations of CO and O_3 , the sirloin steaks were vacuum packaged, using a vacuum packaging machine (DZQ-400, Afapa Vacuum Equipment Co., Ltd., Shanghai, China). The packaging film was clear nylon-polyethylene with O_2 permeability of 0.8 g O_2 /1000 cm²/24 h at 0 °C and H_2O permeability of 0.1 g H_2O /1000 cm²/24 h at 0 °C. Beef were stored at 0 °C for 46 days up to the end of the shelf life of all samples.

Each two steaks were pretreated with CO and O_3 in one MAP pack, and then vacuum packaged together in one vacuum pack. 90 packs (namely 180 steaks) were randomly assigned to each experiment group. All vacuum packaged steaks were stored in 0 °C refrigerator. 3 vacuum packs, namely 6 steaks were removed from each treatment randomly at each of the relevant sampling times, days 0, 1, 7, 13, 22, 28, 34, 40 and 46 for the analysis of L*, a*, b*, total viable counts, TBARS, met-Mb, pH and TVB-N, and days 1, 7, 13, 22, 28, 34, 40 and 46 for the analysis of drip loss. Three steaks were used for microbial sampling alone, and the left 3 steaks were used for drip loss and color instrumental analysis, and thereafter for the determination of chemical analysis of pH, met-Mb, TBARS, and TVB-N. In addition, 2 vacuum packs, namely 4 steaks were removed from each treatment randomly at days 0, 1, 4, 7, 10, 13 and 16 for CO penetration depth analysis. Meanwhile, 3 vacuum packs, namely 6 steaks were removed from each treatment randomly at each of the 11 sampling times were used for sensory analysis (3 steaks used for fresh meat sensory analysis, the other used for cooked meat sensory analysis).

2.3. Physical analysis

2.3.1. Carbon monoxide penetration measurements

CO penetration depth (bright red band) was measured with a caliper in micron (Fisher, SLC, UT). Penetration depth values were the mean of

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