Use of Carbon Monoxide Combined with Carbon Dioxide for Modified Atmosphere Packaging of Pre- and Postrigor Fresh Pork Sausage To Improve Shelf Life

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ABSTRACT

Fresh pre- and postrigor pork sausage patties were manufactured in the Iowa State University Meat Laboratory and packaged either in modified atmosphere (MAP) with 0.4% carbon monoxide (CO) and 99.6% carbon dioxide (CO2) or on foam trays overwrapped with oxygen-permeable film (OW). Packages were stored at 2 to 4°C under fluorescent lights for up to 31 days. Aerobic, anaerobic, and psychrotrophic plate counts, raw and cooked color, purge, and lipid oxidation were measured during storage. Results indicated that both pork sausage products in MAP had lower aerobic and psychrotrophic counts and less lipid oxidation throughout storage (P < 0.05). Raw color of both products in MAP was redder than the OW patties (P < 0.05), but the prerigor pork sausage in MAP benefited more from the CO atmosphere in terms of raw color than the postrigor pork sausage in MAP. Cooked color of the prerigor pork sausage in MAP was significantly redder than cooked color of the postrigor pork sausage. Both pork sausage products in MAP were also lighter (L* value) than the OW patties for raw and cooked color. Therefore, the combination of CO and CO2 in MAP was beneficial in extending the shelf life of pre- and postrigor fresh pork sausage by reducing aerobic and psychrotrophic microbial growth and improving oxidative stability and color, compared to conventional OW packaging. However, increased purge, increased anaerobic growth, and changes in cooking behavior were also observed for the products in MAP during storage (P < 0.05).

The United States consumes over 1 billion lb (450 million kg) of fresh pork sausage each year, representing a multimillion dollar segment of the meat industry (14). Pork sausage has a relatively short shelf life in the fresh form because of color loss, rapid onset of lipid oxidation, and rapid microbial growth (6, 12). Traditionally, pork sausage manufacturers have used natural and synthetic antioxidants, prerigor meat, reduced fat and salt content, and additional nonmeat ingredients to extend the shelf life. Prerigor meat, in particular, is preferred over postrigor meat for pork sausage because prerigor meat has the significant advantages of improved color and lower aerobic microbial counts (21). Prerigor meat has also been reported to have greater water-holding capacity because of its high pH and lower weight loss during chilling (13, 21, 25).

While these approaches all contribute to the shelf-life extension of fresh pork sausage, the expected shelf life is still relatively short in comparison to other processed meat products. New, innovative methods are needed to further extend pork sausage shelf life.

Modified atmosphere packaging (MAP) has been used for many years to extend the shelf life of fish, vegetables, and fruits. This packaging concept is defined as “the packaging of a perishable product in an atmosphere which has been modified so that its composition is other than that of air” (5). For the meat industry, MAP can be dated back to the 1930s, when Australia and New Zealand utilized MAP to ship fresh beef cuts to the United Kingdom (9). By the late 1930s, 26% of the beef in Australia and 60% of the beef in New Zealand were commercially shipped in large containers with carbon dioxide (CO2) atmospheres (32).

High concentrations of CO2 in MAP have been shown to reduce microbial growth and lipid oxidation in meat products (19, 20, 22, 27). Low concentrations of carbon monoxide (CO) in MAP result in a bright cherry-red pigment in fresh meat products that extends product color life (12, 19, 31). Consequently, these two gases, in combination in MAP, offer significant potential to increase the shelf life of fresh meats by improving both bacterial control and color stability.

Viana et al. (31) evaluated pork loins packaged in 99% CO2–1% CO, 100% CO2, 100% CO, and 100% O2 and reported that the highest consumer acceptance score for raw color after 24 h was observed for the 99% CO2–1% CO treatment. In these packages (99% CO2–1% CO), Pseudomonas sp. growth was limited, and psychrotrophic organisms did not reach 107 CFU/g until after day 20. Krause et al. (19) reported a reduction in lipid oxidation and improved color stability for pork loins packaged with 0.5% CO in combination with CO2 and nitrogen in MAP.

These studies as well as several others have demonstrated that MAP with high CO2 concentrations and low CO concentrations can effectively extend the shelf life of

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fresh meat products. While the application of MAP with CO has been shown to have distinct advantages for fresh meats, there has been little information comparing the effects of MAP with CO for pre- and postrigor meat. Further, pork sausage, with the addition of salt, represents a particularly fragile meat color environment in which the use of CO-MAP may be particularly advantageous. Addition of salt typically accelerates the rate of discoloration of fresh meat, and CO may provide a very effective countermeasure for this form of color loss. Consequently, the objectives of this study were to evaluate the effects of MAP with 99.6% CO2 and 0.4% CO on microbial properties, color stability, and quality attributes of pre- and postrigor fresh pork sausage. While most commercial MAP systems with CO and CO2 for fresh meat utilize about 70% CO2, we chose to use 99.6% CO2 in order to assess the maximum antimicrobial impact that CO2 could contribute to a MAP system.

**MATERIALS AND METHODS**

**Sample preparation.** Prerigor pork sausage patties were prepared from coarse-ground, salted, prerigor 70/30 pork trim supplied by Pine Ridge Farms of Des Moines, Iowa. The prerigor meat was boned immediately after harvest, blended with 1.5% salt, and then blended with CO2 snow for chilling. Chilling was complete within 45 min of harvest. The meat was delivered to the Iowa State Meat Laboratory in insulated containers with cold packs within 24 h of harvest. Fat content was 30% in the prerigor meat with a pH of 6.2. Postrigor pork sausage patties were prepared by coarse grinding 50/50 pork trim and 85/15 pork trim separately through a 0.38-cm plate and then blending to a final fat content of 30%. All pork trimmings for the postrigor pork sausage were supplied by the Iowa State University Meat Laboratory from chilled fresh pork 48 h after harvest. The pH of the postrigor meat was 5.6. Both products were mixed in a Hopper mixer (Hopper KLM 110, Koch Equipment, Kansas City, Mo.) with spices (Pork Sausage Blend TG-04-417-00, AC Legg, Calera, Ala.) and reground through a 0.32-cm plate before forming patties. The prerigor meat was delivered to coincide with the availability from chilled fresh pork 48 h after harvest. The pH of the postrigor meat was 5.6. Both products were mixed in a Hopper mixer (Hopper KLM 110, Koch Equipment, Kansas City, Mo.) with spices (Pork Sausage Blend TG-04-417-00, AC Legg, Calera, Ala.) and reground through a 0.32-cm plate before forming patties. The prerigor meat was delivered to coincide with the availability

Each batch of pork sausage was formed into patties with an extruder (Colossimo Press Model 400, Colossimo, Magna, Utah). Each patty weighed between 85 and 95 g and measured approximately 10 by 10 by 0.69 cm. One half of the patties were placed on individual foam trays (TF1 02S0, White 20.83 by 14.48 by 1.52 cm; Pactiv, Lake Forest, Ill.) and overwrapped with oxygen-permeable film (OW), while the other half of the patties were placed in bags (vacuum pouch B620, 20.3 by 30.48 cm; Cryovac Sealed Air Corporation, Bolingbrook, Ill.). A Multivac model C500 (Koch) packaging machine was used to flush and fill the bags with a food-grade gas blend of 0.4% CO and 99.6% CO2 at 400 MPa before sealing. Gas cylinders with the 0.4% CO–99.6% CO2 food-grade gas blend were supplied by the Iowa State University Chemistry Stores and obtained from the Linweld Company in Lincoln, Nebr. All packages were stored under fluorescent lights at refrigerated temperature (2 to 4°C) for periodic evaluation during storage. The MAP packages were stored for 31 days post-packaging, while the overwrapped packages were stored for 21 days postpackaging. The overwrapped packages were removed from the study after day 21 because of obvious spoilage, as evidenced by discoloration and odor.

**Microbiological assessments.** Aerobic, anaerobic, and psychrotrophic plate counts were monitored by standard microbiology methods (30). Total aerobic plate counts were determined with tryptic soy agar with incubation at 35°C for 48 h. Psychrotrophic plate counts also utilized tryptic soy agar but were incubated at 10°C for 5 days to determine the number of aerobic microorganisms present at refrigerated temperatures. Anaerobic plate counts were plated on deMan Rogosa Sharpe agar in candle-oats jars with anaerobic packets (B71040, Fisher Scientific, Pittsburgh, Pa.) at 32°C for 6 days.

**Quality assessments.** The TBARS (2-thiobarbituric acid reactive substances) procedure by Tarladgis et al. (28) was used to measure lipid oxidation in the products. The TBARS value of milligrams of malonaldehyde per kilogram of sample was determined by multiplying the absorbance of the malonaldehyde–thiobarbituric acid complex at 532 nm by 7.8, in accordance with Beer's Law. The 7.8 conversion factor was derived with the extinction coefficient of 1.56 × 105 for the TBARS complex, the molecular weight of malonaldehyde of 72.04 g/mol, and a dilution factor of 1:10 for 10 g of sample. Duplicate TBARS measurements were recorded for each treatment.

Purge was measured by weighing separated water in the packages (18). For the OW products, purge was calculated as follows: [total package weight — foam tray (prior to packaging) — OW (day of sampling) — unpackaged patty weight] divided by the prepackaged patty weight. For MAP products, purge was calculated as follows: [total package weight — MAP bag (prior to packaging) — postpackaged patty weight] divided by the prepackaged patty weight.

Color stability was measured with a HunterLab LabScan instrument (model LS<1500, Hunter Associated Laboratories Inc., Reston, Va.) (2). An illuminant D75, 10-degree observer light source (representing daylight at 7,500 K) and a 2.54-cm port insert were used as the parameters for the HunterLab LabScan. Raw and cooked patties were held at room temperature for about 20 min prior to color measurement. Three measurements of L*, a*, and b* values were taken on random locations of the surface of each patty measured for each treatment.

Patties were cooked to assess cooking properties and cooked color. The cooking procedure followed that established for pan boiling and pan frying of fresh beef patties (3). An industrial-sized electric griddle (model 351, Star Manufacturing Company, St. Louis, Mo.) was preheated to 163°C and monitored for surface temperature with a stove-top thermometer (model A48210, Red Hill, Hillsville, Va.). The patties were turned each minute for the first 2 min and then every 15 s afterward until an internal temperature of 77°C was reached. A hand thermometer (model 11025, DeltaTrak, Pleasanton, Calif.) was used to determine the internal temperature of the patties.

**Statistical analysis.** The experiment was replicated three times, and a mixed linear model was fit with PROC GLM (version 9.1, SAS Institute, Inc., Cary, N.C.) (26). Comparison of the least-squares means was used to determine the effect of meat type and packaging on the product attributes. When a fixed effect was significant (P < 0.05), post hoc tests of differences were calculated and then adjusted with the Tukey procedure. In addition, the least significant difference test with a level of 5% was performed to determine significant differences between the meat type and the packaging treatment for the product attributes.

**RESULTS AND DISCUSSION**

**Microbial assessment.** CO2 is generally recognized as a bacteriostatic agent and is effective in reducing microbial
growth (8, 10). This is an important potential effect of MAP for pork sausage, because pork sausage spoilage is closely linked to the growth of aerobic and psychrotrophic microorganisms, e.g., Lactobacillus sp. and Pseudomonas sp. (29). Figure 1 displays the impact of the MAP treatment on reducing aerobic microbial counts during storage. The MAP treatment resulted in lower aerobic microbial counts after 6 days \((P < 0.05)\) in both the pre- and postrigor pork sausage products. Figure 2 shows the growth of aerobic psychrotrophic microbes in MAP and OW samples. The pre- and postrigor pork sausages in MAP had lower psychrotrophic counts than their overwrapped counterparts after the first 6 days \((P < 0.05)\). Additionally, the prerigor pork sausage in MAP resulted in lower psychrotrophic microbial counts than the postrigor pork sausage in MAP throughout storage \((P > 0.05)\). These results are similar to other studies of prerigor meat that reported reduced aerobic and psychrotrophic microflora during storage relative to postrigor meat (21). These findings also are supported by Jayasingh et al. (17), who observed reduced microbial growth with a packaging treatment of 5% CO for 24 h, followed by vacuum packaging. The microbial loads remained below \(10^6\) CFU/g for 7 weeks after packaging. In our study, we observed that the prerigor pork sausage in OW had greater psychrotrophic counts than the postrigor pork sausage in OW, which was not expected, because postrigor meat is generally characterized as having lower levels of aerobic growth than postrigor meat (21).

![FIGURE 1](image1.png)

**FIGURE 1.** Total aerobic plate counts for post- and prerigor fresh pork sausage stored at 4°C under fluorescent light. ▲, Postrigor pork sausage in oxygen-permeable overwrap packaging; X, postrigor pork sausage in MAP (99.6% CO₂–0.4% CO); —, prerigor pork sausage in oxygen-permeable overwrap packaging; ●, prerigor pork sausage in MAP (99.6% CO₂–0.4% CO).

The elimination of oxygen from fresh meat packages is expected to cause a shift in the microbial population (16). Several studies have shown that the reduction of oxygen in the packaging environment will shift microflora from aerobic to facultative anaerobic growth during storage, resulting in higher anaerobic plate counts (5, 8, 10). Figure 3 shows the microbial shift from aerobic to anaerobic growth in MAP that was observed in this study. The OW packages resulted in lower anaerobic counts than the MAP treatments for both meat types \((P < 0.05)\). Additionally, the postrigor pork sausage in MAP had lower anaerobic microbial counts than the prerigor pork sausage in MAP, particularly after 20 days of storage \((P < 0.05)\). This was also true for the postrigor pork sausage in OW packaging compared with the prerigor sausage in OW. The greater anaerobic growth in postrigor pork sausage was not expected, because the predominant microorganisms are lactic acid bacteria, which prefer a more acidic environment. Therefore, it was expected that the postrigor pork sausage, which had a lower pH (5.6), would result in a greater anaerobic microbial count than the postrigor pork sausage (pH 6.2). However, in this case, the initial counts were lower for postrigor samples, and because the pre- and postrigor meats came from two different sources and were produced in two different facilities, differences in the initial counts or in the bacterial profile of the two meat sources may have contributed to the differences observed during storage.

**Quality assessment.** Lipid oxidation was accelerated to a greater extent in the OW pork sausage than in the same product in MAP in this study (Fig. 4). With an arbitrary TBARS value of 1.0 as a rancidity threshold, the prerigor OW patties exceeded this value by day 15, while the MAP

![FIGURE 2](image2.png)

**FIGURE 2.** Aerobic psychrotrophic plate counts for post- and prerigor fresh pork sausage stored at 4°C under fluorescent light. ▲, Postrigor pork sausage in oxygen-permeable overwrap packaging; X, postrigor pork sausage in MAP (99.6% CO₂–0.4% CO); —, prerigor pork sausage in oxygen-permeable overwrap packaging; ●, prerigor pork sausage in MAP (99.6% CO₂–0.4% CO).

![FIGURE 3](image3.png)

**FIGURE 3.** Total anaerobic plate counts for post- and prerigor fresh pork sausage stored at 4°C under fluorescent light. ▲, Postrigor pork sausage in oxygen-permeable overwrap packaging; X, postrigor pork sausage in MAP (99.6% CO₂–0.4% CO); —, prerigor pork sausage in oxygen-permeable overwrap packaging; ●, prerigor pork sausage in MAP (99.6% CO₂–0.4% CO).
FIGURE 4. TBARS measured for post- and prerigor fresh pork sausage stored at 4°C under fluorescent light. ▲, Postrigor pork sausage in oxygen-permeable overwrap packaging; ×, postrigor pork sausage in MAP (99.6% CO2–0.4% CO); ■, prerigor pork sausage in oxygen-permeable overwrap packaging; ○, prerigor pork sausage in MAP (99.6% CO2–0.4% CO).

FIGURE 5. Uncooked a* color values expressed for post- and prerigor fresh pork sausage stored at 4°C under fluorescent light. ▲, Postrigor pork sausage in oxygen-permeable overwrap packaging; ×, postrigor pork sausage in MAP (99.6% CO2–0.4% CO); ■, prerigor pork sausage in oxygen-permeable overwrap packaging; ○, prerigor pork sausage in MAP (99.6% CO2–0.4% CO).

FIGURE 6. Cooked a* color values expressed for post- and prerigor fresh pork sausage stored at 4°C under fluorescent light. ▲, Postrigor pork sausage in oxygen-permeable overwrap packaging; ×, postrigor pork sausage in MAP (99.6% CO2–0.4% CO); ■, prerigor pork sausage in oxygen-permeable overwrap packaging; ○, prerigor pork sausage in MAP (99.6% CO2–0.4% CO).

products did not yet reach this level at day 30. Similarly, the postrigor OW pork sausage reached a TBARS level of 1.0 by day 4, while the MAP samples did not reach this value until day 19 of storage. Krause et al. (19) reported similar results for the reduction of lipid oxidation in pork loins with MAP with 0.5% CO in combination with CO2 and nitrogen. Luno et al. (22) also reported less lipid oxidation in beef products when CO, in combination with CO2 and oxygen, was used for MAP. The prerigor pork sausage in both packaging treatments had a lower TBARS value (P < 0.05) than the postrigor pork sausage, as expected.

Kusmider et al. (20) reported that, in addition to reduced TBARS values, MAP-CO packaging increased the lightness and redness (L* and a*, respectively) of uncooked beef products. When CO binds with myoglobin to produce carboxymyoglobin for raw meat color, instrumental color measurements are typically shifted to lighter and redder color values. Both the pre- and postrigor pork sausage products in MAP were lighter (higher L* value) than the OW patties for both the raw and cooked color measurements throughout storage. Figure 5 shows that the postrigor pork sausage in MAP was very similar to the OW patties for the redness (a*) of raw and cooked color (Fig. 6). On the other hand, the prerigor pork sausage in MAP was very significantly redder in both raw and cooked color than the postrigor pork sausage in either MAP or OW packaging. These findings suggest that the prerigor pork sausage benefited much more from CO than the postrigor sausage in terms of raw color. This may be because of the greater proportion of meat pigment present in prerigor meat as reduced deoxymyoglobin. Our experience with CO packaging has suggested that CO binds readily to reduced deoxymyoglobin but not with oxidized metmyoglobin. Further, it is not clear whether CO will displace oxygen on oxygenated oxyhemoglobin.

The prerigor pork sausage in MAP also retained greater redness after cooking, which may imply that the carboxymyoglobin in the prerigor meat did not denature in the same fashion as the oxyhemoglobin in the overwrapped prerigor pork sausage. John et al. (18) suggested that the persistent redness after the cooking of meat held in a CO atmosphere was due to a heat-denatured globin–CO hemochrome pigment. Studies have also suggested that because prerigor meat has higher water-holding capacity, it is able to hold more carboxymyoglobin pigment, producing a redder and lighter appearance (4, 12). Honikel and Fischer (13) found that because hot-boned (prerigor) meat had a less severe pH decline, the color was more uniform and stable. These factors may also have contributed to the greater redness in the prerigor pork sausage.

A visual change observed in this study during the cooking of products from MAP was expansion and swelling to create a puffy appearance. These effects were probably due to the high amount of CO2 used in the packaging. CO2 is absorbed readily by the water phase in meat (24). During the cooking process, CO2 gas can be released to produce gas pockets in the meat, thus giving it a “puffy” appearance. This appeared to be primarily an internal development in the patties, while the surface was largely unaffected. Surface color did not appear to be altered by the swelling induced by the release of the gas. Other studies have reported...
pore formation in cooked meats when the CO2 concentration (80 to 90%) are elevated (15).

A potential disadvantage of the high CO2-MAP used in this study is increased purge, which was observed for both products in MAP compared to the overwrapped products throughout storage ($P < 0.05$). Figure 7 displays the differences between the MAP products and the overwrapped products, as well as differences between the pre- and postrigor pork sausage in MAP. Throughout storage, the MAP products had significantly more purge in the packages than their overwrap counterparts. In addition, the prerigor pork sausage in MAP had significantly more purge that the postrigor pork sausage in MAP. This observation was unexpected, because prerigor pork is known to have a higher water-holding capacity than postrigor meat; therefore, the opposite effect for purge from prerigor pork sausage in MAP was anticipated. The increased purge in MAP may well be the result of the very high CO2 concentration used in this study. Researchers have found that the absorption of large amounts of CO2 by meat tissue can cause a small decrease in pH because of the formation of carbonic acid. Aberle et al. (1) explained that when the pH of muscle decreases, such as before and during rigor mortis, the proteins in muscle have less space available for water because of steric effects, thus reducing the water-holding capacity. A higher pH, such as that observed (6.2 for prerigor versus 5.6 for postrigor) in this study, is one reason why the prerigor meat is preferred for pork sausage (21).

When CO was used in combination with a high concentration of CO2, significant advantages of attractive color and extended shelf life for both pre- and postrigor pork sausage were provided. Prerigor pork sausage remained superior to postrigor pork sausage, even in the MAP-CO system, and prerigor meat benefited more from the CO atmosphere in terms of raw color than did postrigor meat. These results suggest that MAP with CO and CO2 for fresh pre- and postrigor pork sausage can extend the shelf life by both the reduction of lipid oxidation and the reduction in aerobic and psychrotrophic microbial counts. The disadvantages of increased purge and product expansion during cooking could be resolved by reducing the concentration of CO2 used and by incorporating nitrogen as part of the MAP gas blend (7, 23).

REFERENCES


