Changes in Microbial Parameters and Gas Composition During Modified Atmosphere Storage of Fresh Pork Loin Cuts

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ABSTRACT

The storage life of modified atmosphere packaged pork loin cuts in 40% CO₂ and 60% N₂ was determined at -1, 4.4, and 10°C in three packaging films with oxygen transmission rates (OTR) of 0.0, 12.6, and 26.5 ml/m²/24 h at 23°C, 0% relative humidity and 1 atm pressure. The pork loin cuts were “commercially” or “aseptically” prepared. Gas atmosphere of the packages, microbial load, and pH were monitored throughout storage. The principal factor influencing change in the headspace gases under the conditions of these studies was gas transmission through the packaging film. A 100-fold difference in initial microbial load dominated, regardless of packaging film. Under commercial packaging conditions in foil laminate packages with 0.0 or 12.6 OTR, storage life of pork cuts was 5 or 8 weeks at 4.4 or -1°C, respectively. This result was not definitive because of a difference among replicates. Samples in replicate 2 had a reduced storage life at each of the three storage temperatures.

Centralized prepackaging of meat in modified atmospheres using mixtures of carbon dioxide, oxygen, and nitrogen has been proposed as an effective method of packaging for retail sale. Modified atmosphere packaging (MAP) systems will not improve the quality of fresh meats; however, economic benefits, including the possibility of an extended storage life, could make MAP an alternative to current retail packaging (28). Extension of storage life with MAP depends on several factors, including the initial microbial load of the meat and the storage atmosphere and temperature. Studies have indicated that the lower the initial microbial load, the longer the storage life (5,25).

Packaging with elevated levels of CO₂ retards the growth of aerobic spoilage microorganisms, permitting lactic acid bacteria and other CO₂-resistant organisms to dominate the microbial population (3,9). It is generally accepted that a minimum of 20% CO₂ is needed to extend the storage life of MAP fresh meats (7,27). Christopher et al. (6) found that the growth of the spoilage microflora of pork loins was effectively inhibited during storage in an atmosphere containing 40% CO₂. The sensory characteristics of retail cuts prepared from the stored loins were not affected by storage in 40% CO₂ (17). However, retail cuts of pork, beef, or lamb stored at 2°C up to 21 d in 20 or 40% CO₂ became severely discolored (20). The presence of 5 to 10% O₂ in the retail packages may have promoted the development of metmyoglobin. An atmosphere of 100% CO₂ effectively extends the storage life of pork provided that O₂ is kept at very low concentrations throughout storage (15). However, Spahl et al. (22) found that a gas mixture of 30% CO₂/70% N₂ was as effective in extending storage life as 100% CO₂ when pork chops were stored at 2°C.

The minimum storage temperature for fresh meat without freezing is approximately -1.5°C. Modified atmosphere storage at temperatures close to -1°C markedly extends the storage life of fresh meat (1,4,15,21). Pork cuts stored in an atmosphere of 100% CO₂ at -1.5°C have a storage life of 12 weeks (15). Absorption of CO₂ by meat, respiration of meat and bacteria (with the evolution of CO₂), and gas exchange through the package contribute to changes in the gas atmosphere during storage (8,11). Reports on how the gas composition changes during storage in packs of MAP pork are limited and conflicting. Seideman et al. (20) reported that the CO₂ concentration decreases during storage of MAP retail pork cuts at 2°C in gas mixtures of either 20 or 40% CO₂ with 5 to 10% O₂. However, Spahl et al. (22) found that CO₂ levels increased during storage of MAP retail pork cuts.

This study examines the combined effects of storage temperature, gas permeability of packaging films, and microbial load on changes in the gas atmosphere and the storage life of MAP retail cuts of pork.
MATERIALS AND METHODS

Preparation of meat samples

Hogs from a selected producer were killed at a federally inspected packing plant on each of 3 successive days (replicates). Each replicate consisted of hogs from one producer. The carcasses were chilled to 0°C overnight before the *longissimus dorsi* muscles were excised and skinned of all fat. The pH of the excised lean muscles was determined by placing a glass electrode on the muscle surface. To avoid PSE (pale, soft, and exudative) meat, muscles with an appropriate physical appearance and a pH between 5.8 and 6.4 were selected. Each muscle was divided into two portions. At the packing plant, one portion was cut into six pieces approximately 1.5 cm thick (commercial product). The uncut portion was bagged separately, and all samples were held in a laboratory cooler at 4°C for packaging (not more than 6 h). In the laboratory the uncut half-loin was placed on a stainless steel tray, flamed with alcohol twice on each side, and aseptically cut into 6 x 1.5 cm portions with a sterile knife (aseptic product). The surface area of each cut was determined by aseptically tracing the outline on a sheet of aluminum foil and measuring the surface area with an Area Meter (Model LI-3100, LiCor Inc., Lincoln, NE). The mean surface area was 30.5 ± 5.4 cm². The meat cuts were placed on the bottom dish of a 150 x 15-cm sterile petri plate and packaged. Duplicate samples of commercial and aseptic product from each loin were randomly assigned to each of three package types.

Packaging and storage

The packaging materials used were (a) 12.2 μm polyester/9 μm foil/76 μm polyester laminate (Packall Packaging, Mississauga, Ontario, Canada; Fl); (b) a 17.8 μm nylon/71.1 μm polyvinylchloride coextrusion laminated to 48 gauge metalized polyester (Condor Laminations, Progressive Packaging Ltd., Aurora, Ontario, Canada; F2); and (c) a 50.8 μm nylon/76.2 μm polyvinylchloride coextrusion bag (Condor Laminations, Progressive Packaging Ltd.; P3) with the following manufacturer’s reported oxygen transmission rates (OTR): 0.0, 12.6, and 26.5 ml/m²/24 h at 23°C, 0% relative humidity under 1 atmosphere pressure. Packages (20 x 26 cm) were evacuated, flushed with a gas mixture of 40% CO₂ and 60% N₂, and heat-sealed using a Bizerba Packaging Machine (Model D66; Bizerba Inc., Mississauga, Ontario, Canada). Gases were mixed with a Smith Proportional Gas Mixer (Model 299-029; Smith Equipment, Watertown, SD). During preliminary work the volume of gas injected was ca. 500 ml which resulted in a gas/meat weight ratio of ca. 5 l/kg. Packaged samples were stored at each storage temperature and at 25°C. Sufficient samples were prepared for duplicate weekly microbial and gas analyses throughout the testing period. Geometric means were calculated for microbial (PCA, MRS, CFC, STAA) data. Microbial and pH data were statistically analyzed using ANOVA procedures. Where appropriate, means were ranked by Student Newman Keul’s multiple range test (23).

RESULTS

Preliminary work indicated the pH of the meat slurry to be within 0.1 pH unit of the pH on the meat surface. Means for the initial muscle pH of each replicate were 5.87 ± 0.19, 5.94 ± 0.17, and 6.03 ± 0.03 for replicates 1, 2, and 3, respectively. At all three storage temperatures, the mean pH of the samples decreased to ca. 5.5 during the first week of storage. No significant changes in pH occurred after this initial decrease, either with length or temperature of storage or with type of packaging.

Packages filled with 40% CO₂ and 60% N₂ without meat had similar amounts of gas exchange at -1, 4.4, and 10°C and slightly greater gas exchange at 25°C. At the lower storage temperatures, for example at 4.4°C, the decrease in CO₂ in Fl packs was marginal after 9 weeks storage; whereas in F2 packs, CO₂ decreased to 35%, and in P3 packs, to approximately 30%. Oxygen levels in the foil packs remained below 1% throughout storage, but in the plastic (P3) packs, O₂ was detected (2-3%) after 4 weeks and reached 4.5% after 9 weeks of storage.

Changes in the composition of headspace gases of meat samples stored at -1 and 4.4°C were similar. At -1 and 4.4°C there was little difference in the change in CO₂ during storage between the commercially packaged (CP)
and aseptically packaged (AP) samples. Fig. 1 shows the changes in CO₂ of headspace gases of meat samples stored at 4.4°C after a 3-5% drop in CO₂ during week 1 of storage. After a 3-5% drop in CO₂ during week 1 of storage, there was a greater loss of CO₂ in the P3 packs than in F1 or F2 over time. The percentage of O₂ detected in the F1 and F2 packs was less than 1% during 9 weeks of storage; in P3 packs O₂ levels increased from less than 1% during the first 4 weeks of storage to ca. 4% after 9 weeks of storage. Fig. 2 shows the change in CO₂ of headspace gases of meat samples stored at 10°C. At 10°C package and sample type (AP vs CP) affected the change in the gas composition over time. After 2 weeks of storage at 10°C, the CO₂ content of F1 and F2 packages with CP samples was much higher than AP samples.

The microbial quality of the meat samples was monitored at weekly intervals for 4 weeks and, for samples stored at -1 and 4.4°C, at biweekly intervals thereafter. Samples stored at 10°C became unacceptable after 2 to 3 weeks of storage and testing was discontinued after 4 weeks. Samples stored at 4.4 and -1°C remained acceptable for 5 and 8 weeks, respectively, and were tested up to 9 and 10 weeks of storage, respectively. Statistical analyses of microbial data for the pork samples indicated that storage time and temperature, package type, and initial microbial load (CP vs AP samples) were important factors affecting the microbial quality and hence the storage life of the meat samples. For a large proportion of the microbial data, there was a significant difference among replicates. The storage life of samples in replicate 2 was 1 to 2 weeks shorter than that of samples in replicates 1 and 3.

Table 1 lists initial mean counts for each microbial parameter for pork cuts prepared commercially and aseptically. There was a 1- to 2-log difference in total initial microbial load between meats prepared commercially and those prepared aseptically. This difference in microbial load was maintained throughout storage at -1 and 4.4°C because microbial populations were only approaching a maximum at the end of the storage period. Samples stored at 10°C had a maximum bacterial population within 2 to 3 weeks, and there was no difference between pork samples prepared commercially or aseptically.

Figure 3 shows the means for total aerobic and presumptive lactic acid bacteria, Enterobacteriaceae and pseudomonad counts, for CP samples stored at -1°C. Package type generally had little influence on these bacterial counts; therefore, data were pooled across package types.
The total aerobic microorganisms had a lag phase of about 4 weeks; thereafter the population increased with a generation time of approximately 2 d. After 6 and 8 weeks of storage, samples packaged in P3 had significantly higher total aerobic counts than samples packaged in F1 or F2, but the differences were less than 1-log unit. Presumptive lactic acid bacteria counts of samples stored at -1°C were 1 to 2 orders of magnitude lower than total counts and represented only 1% of the bacterial population. After a lag phase of approximately 4 weeks, Enterobacteriaceae numbers increased to a maximum population of $10^5$ CFU/cm² after 8 weeks of storage. There was only limited growth of pseudomonads during storage at -1°C. Growth of B. thermosphacta in MAP pork cuts stored at -1°C (Fig. 4) represented the principal change in microbial load. For both CP and AP samples stored for 6 or 8 weeks, those packaged in P3 had Brochothrix counts 1- to 2-log units higher (P<0.05) than those packaged in F1 and F2. CP samples packaged for 8 weeks had a slight sour meat odor and some discoloration. In contrast, AP samples only developed a slight sour odor at 10 weeks.

At 4.4°C, sample preparation method (CP vs AP) had a significant effect on all bacterial counts. Package type had no influence on total aerobic and presumptive lactic acid and Enterobacteriaceae counts for either CP or AP products. For the CP products the estimated generation time for the total aerobic population at 4.4°C was approximately 2 d, with little or no lag in bacterial growth. Total aerobic counts reached a maximum of $10^6$ CFU/cm² at 5 weeks. Generation time during the first 4 weeks of storage was 2 d for the lactic acid bacteria and 3 d for Enterobacteriaceae. By 4 weeks, lactic acid bacteria and Enterobacteriaceae reached maximum populations of $10^9$ and $10^4$ CFU/cm², respectively. Package type had a significant effect on the counts of pseudomonads and B. thermosphacta. Pseudomonads (Fig. 5) failed to grow on CP samples packed in F1 or F2; however, after a 3-week lag in growth, pseudomonad bacteria increased in numbers on products packaged in P3. This increase in pseudomonads coincided with an increase in $O_2$ to between 2 and 3%. Data obtained for B. thermosphacta followed the same pattern as that shown for pseudomonad growth, with increases in Brochothrix numbers in P3 after 4 weeks of storage. After 7 weeks of storage at 4.4°C, sulfur gas as well as sour meat odors were detected when the meat packages were opened. After 9 weeks of storage, samples stored at 4.4°C showed gross spoilage, characterized by strong off odors and severe discoloration.

Bacteria grew rapidly on both CP and AP pork samples stored at 10°C. Off odors were detectable after 2 weeks of storage. At this storage temperature, the principal group of bacteria was Enterobacteriaceae, which reached a maximum population of $10^8$ CFU/cm² by 2 weeks of storage. In contrast, lactic acid bacteria and pseudomonads reached maximum populations of $10^6$ CFU/cm² in the same period. Commercially prepared pork samples had a maximum B. thermosphacta count of $10^4$ to $10^5$ CFU/cm² after 1 week of storage. In contrast, Brochothrix counts for aseptically prepared samples increased gradually to $10^6$ to $10^7$ CFU/cm² over 4 weeks of storage.

**DISCUSSION**

A decrease in $CO_2$ in headspace gases of MAP meats is common and has been attributed to diffusion through the package (17,20) and absorption by meat (13). Changes in the gas composition of the packs without meat samples were proportional to the theoretical oxygen transmission rates of the packaging materials. In the meat packs, $CO_2$ absorption by meat and gas exchange across the package was responsible for the major proportion of the change in
the composition of the headspace gases. In the P3 packs (OTR 26.5) without meat, ca. 25% of the CO₂ was lost during storage at 4.4°C for 9 weeks. In the same package containing ca. 100 g of meat, after ca. 12.5% of the CO₂ was absorbed by the meat, a further ca. 25% CO₂ was lost over the 9-week storage period. The greatest difference in the composition of the headspace gases between packages with and without meat samples was noted at 10°C in that the CO₂ in the packs with meat either increased (as in the case of CP samples packed in F1 and F2) or decreased to a lesser extent (in P3 packs) than in packages without meat. Increases in CO₂ during storage can result from a lowering of CO₂ solubility in the meat tissue at higher temperatures, which results in an increase in CO₂ in the atmosphere (8,13). Carbon dioxide in meat packages can also increase due to microbial metabolism as Enterobacteriaceae and heterofermentative lactic acid bacteria produce CO₂ during growth. It has been suggested that for gas composition to change as a result of bacterial metabolism, a high bacterial count (10⁸ organisms/g) is required (11). In the present study, the Enterobacteriaceae count reached 10⁸ CFU/cm² at the time increases in CO₂ were noted.

The difference in storage life of samples “between” replicates is not readily explained. All samples from replicate 2 spoiled more rapidly than comparable samples from replicates 1 and 3. A possible reason for the difference among replicates could be variable muscle pH. High pH muscle spoils more rapidly than normal pH muscle because the carbohydrate available for microbial metabolism is limited (14). When growing on muscle with a high pH, compared with muscle at normal pH 5.3 to 5.7, bacteria use protein as an energy source and produce organoleptically obnoxious compounds much earlier during storage. However, the initial mean muscle pH of samples from the three experimental replicates differed by <0.2 pH units, and muscle samples from replicate 3 had the highest mean pH. During week 1 of storage, the pH in all samples dropped to between pH 5.3 and 5.7 and did not change significantly thereafter. Moreover, Gill and Harrison (15) reported that large differences in the initial pH of pork did not affect the development of spoilage during modified atmosphere storage. Thus, small differences in muscle pH in the present study should not account for the faster spoilage of the meat samples in replicate 2. Another possible explanation for the difference could be a higher initial microbial load or the types of microorganisms predominating on the meat. Higher microbial loads do reduce storage life of MAP meats (2,19); however, the initial microbial loads on samples from the three replicates (Table 1) were similar, and samples from replicate 3 had a higher load than samples from replicate 2. Thus, variation in the initial microbial load is not responsible for the difference in storage life of replicate 2.

Differences among replicates could represent differences in product from different producers, because samples for each replicate were taken from hogs obtained from a different producer each day. Perhaps, as a result of some factor in the feeding or raising of the hogs, the meat samples selected for replicates 1 and 3 contained an inhibitory substance not present in the samples for replicate 2. It was not possible to test this hypothesis. The failure of samples from replicate 2 to achieve the storage life of samples from replicates 1 and 3 raises questions about the reliability of obtaining an extended storage life for MAP pork. If such differences in the storage life of MAP pork are a result of production practices, then MAP of pork cuts may be of limited applicability.

The initial microbial load of the “commercial” samples was at the high end of the range normally acceptable in meat (15). Total aerobic counts of 10⁵ to 10⁶ CFU/cm², with presumptive Enterobacteriaceae counts of 10⁴ CFU/cm², indicate that improvements in plant sanitation could decrease these numbers. Aseptic laboratory preparation of samples reduced total bacterial load by 1- to 2-log counts and extended the storage life of the pork cuts by 1 week at 10°C and 2 weeks at 4.4 and -1°C. The success of MAP in extending the storage life of fresh meat relies heavily on low initial bacterial loads (12).

Storage in CO₂-enriched atmospheres inhibits or retards the growth of the aerobic spoilage microflora of fresh meats, such as pseudomonads and Enterobacteriaceae, and permits lactic acid bacteria and other CO₂ insensitive organisms to dominate the microbial population. The determining factor as to which organisms will predominate the bacterial population is dependent on storage temperature. At higher storage temperatures, Enterobacteriaceae grow in anaerobic environments (16). In this study, Enterobacteriaceae dominated the microbial population of pork samples stored at 10°C and by week 2 spoilage was noticeable. This confirms the work of Gardner et al. (11), who found that Enterobacteriaceae dominated the microflora of pork stored at 16°C in modified atmospheres. In meat samples

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<tr>
<th>Replicate Organisms</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
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<tr>
<td></td>
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<td>Total aerobic count</td>
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<tr>
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<tr>
<td>B. thermosphacta</td>
<td>&lt;1.8</td>
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TABLE 1. Initial mean log bacterial counts (CFU/cm²) obtained from each replicate for commercially (CP) and aseptically prepared (AP) pork cuts.
stored at 4.4°C, lactic acid bacteria dominated the microbial population regardless of package type, although growth of Pseudomonads and B. thermosphacta occurred in P3 packs. In contrast, in samples stored at -1°C, B. thermosphacta dominated the microbial population of samples packaged in P3 once a small amount of O2 (2 to 3%) was present. The presence of CO2, combined with low temperature storage, should inhibit the growth of B. thermosphacta (3,15). In samples stored at -1°C in foil packs, which contained very low amounts (<1%) of O2, growth of B. thermosphacta was limited even after 8 weeks and lactic acid bacteria tended to dominate. It would appear that even at -1°C, the presence of small amounts of O2 in the packs allowed B. thermosphacta to dominate the microbial population. This emphasizes the need to ensure that an anaerobic environment is maintained throughout storage to maximize the storage life of pork, as B. thermosphacta is a major spoilage organism of MAP and vacuum-packaged meats (15).

An increase in temperature from -1 to 4.4°C shortened the storage life of the pork cuts by 3 weeks. The potential for temperature abuse of MAP fresh pork cuts sold at the retail level raises concerns regarding the safety of such products. The temperature of household refrigerators is often above that recommended for safety (26). In the current study, after 2 weeks of storage at 10°C, the pork cuts had obvious signs of spoilage that would make them unacceptable for consumption. During storage at good refrigerator temperatures (5°C) potential pathogens, such as Yersinia enterocolitica, Listeria monocytogenes, and Aeromonas hydrophila, could grow in an anaerobic environment and possibly develop a health hazard prior to the development of spoilage. This problem has yet to be studied for fresh pork products.

This study indicates that a storage life of 10 weeks for individually packaged retail cuts of pork can be achieved by packaging in modified atmospheres with elevated CO2. Low temperature storage (-1°C) is the overriding control for long-term extension of storage life. The use of O2 impermeable films provides control of the dominating microflora which ultimately influences the storage life. Investigation of the reasons for differences in storage life between replicates is required to improve the reliability of obtaining an extended storage life of MAP pork cuts.

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