

Effect of Carbon Dioxide and Oxygen Enriched Atmospheres on the Shelf-Life of Refrigerated Pork Packed in Plastic Bags

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(Received for publication August 12, 1987)

ABSTRACT

Changes in bacterial numbers, metmyoglobin percentage and 2-thiobarbituric acid number during the chill storage of pork longissimus dorsi packed with air, carbon dioxide, carbon dioxide and oxygen or vacuum-packed in plastic bags of high (polyethylene) and low (Cryovac BB-1) permeability to gases were studied. The fast increase of viable counts in polyethylene bags showed that plastic films of high permeability are not suitable to extend the shelf-life of meat using modified atmospheres. In Cryovac bags both carbon dioxide-enriched atmospheres and vacuum-packaging inhibited aerobic organisms, keeping the bacterial flora (mainly lactobacilli and *Brochothrix thermosphacta*) below the level of $10^8/\text{cm}^2$ for about 3 weeks. Metmyoglobin formation was not affected by 20% carbon dioxide, whilst 80% oxygen significantly decreased its rate. Metmyoglobin accumulation in vacuum-packed samples was even slower than in the oxygen-enriched atmosphere, but meat color was less attractive in the former than in the latter. Lipid oxidation was not the limiting factor of shelf-life in either of these two atmospheres. Off-odors appeared in both at approximately 20-22 d of storage.

The slime-producing psychrotrophic gram-negative bacteria that are usually responsible for spoilage of refrigerated meat (9,15) are inhibited by 20% CO_2 (6). This flora is replaced, to a large extent, by CO_2 -resistant organisms, e.g. lactic acid bacteria and *Brochothrix thermosphacta* (1,20).

The major chemical problem when holding fresh muscle foods in CO_2 -enriched atmospheres has been related to surface discoloration (10). To overcome this, use of O_2 -enriched atmospheres has been proposed (19), because levels of oxygen (e.g. 80%) delay the oxidation of Mb and OxiMb (19,22).

Use of high levels of these gases requires systems in which the original composition of the atmosphere can be kept within a certain range as long as the storage period lasts. To accomplish this, two types of systems can be used (31). In one, called controlled-atmosphere systems, the selected gas concentrations can be kept constant throughout storage by flushing the appropriate gas mixture into a storage chamber. In the other method, called modified-atmosphere systems, the meat is packed in appropriate bags which are filled with the selected gas mixture before sealing.

In both instances, some dynamic changes of the composition of the atmosphere due to gas permeation, leakage, absorption and/or biochemical conversion via respiratory activity must be considered (31). Obviously, plastic films with low diffusion coefficients to gases will be more appropriate to maintain a given atmosphere composition throughout the storage period. Nevertheless, plastic films of high diffusion coefficient to oxygen have been used as wrapping material to keep high levels of oxiMb (23).

Oxidative rancidity is not considered the limiting factor of refrigerated meat shelf-life, but it may become a problem if higher-than-normal levels of oxygen are used (10,22).

The purpose of this work was to study the change in factors limiting the shelf-life of refrigerated pork packed in plastic bags of low and high permeability to gases, filled with CO_2 - and CO_2 - O_2 -enriched atmospheres.

MATERIALS AND METHODS

Pork longissimus dorsi 24-h post-mortem (pH 5.3-5.6) was aseptically cut into ca. 1 cm thick slices and individually packed in bags of 20 x 22 cm of two different plastic films: Polyethylene (PE) (diffusion coefficient of 7,700 ml/m²/24 h/atm to O_2 and 21,000 ml/m²/24 h/atm to CO_2) and a laminated film, Cryovac BB-1 (BB-1) (diffusion coefficient of 25 ml/m²/24 h/atm to O_2 and 100 ml/m²/24 h/atm to CO_2).

Gas mixtures consisted of 20% CO_2 + 80% air (v/v) and 20% CO_2 + 80% O_2 (v/v) supplied by the Sociedad Española del Oxígeno (Spain).

Plastic bags were flushed for 5 s at a flow rate of ca. 6,000 ml/min and after being filled with ca. 1,000 ml of gas mixture, heat-sealed with a Moulinex 629 therm-sealer. As controls, for both types of plastic bags, a batch was packed with air, and another one vacuum-packed using an EUVAC 70/40. All samples were stored at $1 \pm 1^\circ\text{C}$.

Microbial analyses were done on duplicate samples by swabbing a 2 x 2-cm area of the meat surface with sterile cotton swabs.

Total viable counts (TVC) were determined with Plate Count Agar (PCA) incubated at 24°C for 36 h. To characterize the dominant organisms, 20% of the colonies from PCA plates were randomly chosen (21) and subcultured in Brain Heart Infusion (BHI). Characterization of gram-negative and gram-positive microorganisms was carried out following the methodology described by Dainty et al. (7).

MetMb percentage was estimated spectrophotometrically by measuring the reflectance at 525 and 572 nm (27). The maximum value of $\frac{K/S_{572}}{K/S_{525}}$ in the first day of the experiment was taken as 0% MetMb, while 100% MetMb was obtained after oxidizing a sample in a 1% (w/v) solution of potassium ferricyanide (17). Each MetMb value is given as the mean of at least 10 determinations, taken from a minimum of 5 meat slices. All results are compared using the Student's t-test. Lipid autooxidation was monitored by the 2-thiobarbituric acid (TBA) number following the method described by Tarladgis et al. (28).

At each sampling time, the odor of the headspace gas of the bags used for microbial analysis was assessed, after 10 min of equilibration at room temperature, by an untrained panel of 6 members. The panel was asked to tell if any rancid or off-odor was present.

RESULTS AND DISCUSSION

Microbial analysis

As expected, the change in TVC of samples packed with air (Fig. 1) in both types of bags (PE and BB-1) was quite similar to that obtained in aerobically stored meat by other authors (1,2), reaching a level higher than 10^8 CFU/cm² in 10-12 d. The characterization of organisms when off-odors were present showed that *Pseudomonas* spp. were the dominant bacteria (90%). These results suggest that, even though oxygen is partially depleted by microbial and post-mortem metabolism (11,16), the amount of this gas in both types of bags is enough to allow growth of aerobic organisms at a rate similar to that observed in a non-restricted atmosphere of air. Therefore, the type of plastic film has no relevant influence on the shelf-life of refrigerated pork when the bags are filled with air.

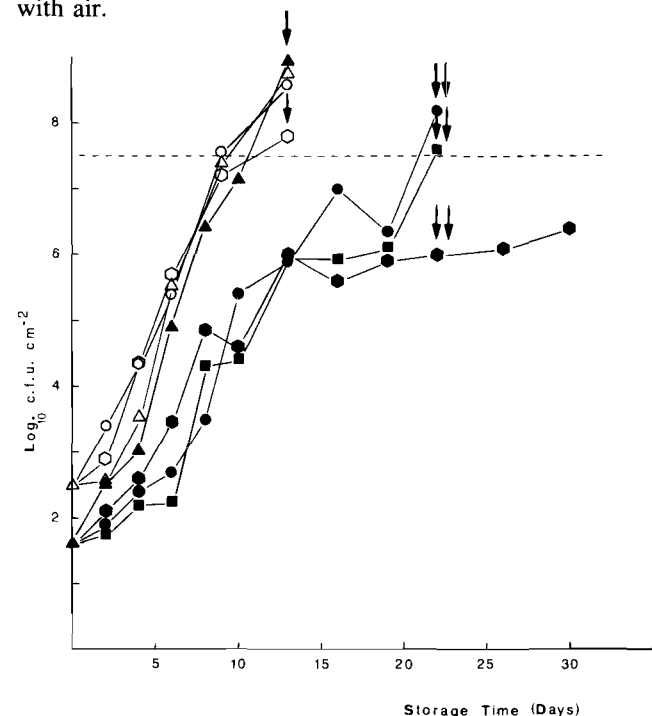


Figure 1. Change in TVC on refrigerated pork (1°C) packed with air (triangles), 20% CO₂ + 80% air (squares), 20% CO₂ + 80% O₂ (circles) or vacuum-packed (hexagons) in PE (open symbols) and BB-1 (closed symbols) bags. The arrows indicate when off-odors were detected (▲ -Putrid odors, ▼ -Dairy/cheesy/sour/acid odors).

Microbial growth on samples packed in PE with CO₂-O₂ showed a change similar to that observed for samples packed with air (Fig. 1). Tests made to identify the main groups of organisms when bacterial numbers reached ca. 10^8 CFU/cm² (9-13 d of storage) showed that approximately 90% of the strains isolated from this meat belonged to the genus *Pseudomonas*, the remainder being *B. thermosphacta* (gram-positive, catalase-positive, non-motile, non-sporeforming rods that weakly fermented glucose). This fact may be explained by the high diffusion of CO₂ and O₂ through PE. Consequently, any difference in the concentration of these gases between the atmosphere inside the bag and the surrounding air will virtually disappear in a short time.

Figure 1 also shows the evolution of microbial counts on pork packed in BB-1 with CO₂-air or CO₂-O₂. The increases of TVC were much slower than those in PE with CO₂-O₂ or in BB-1 with air, and the time required to reach numbers of about 5×10^7 CFU/cm² was twice that required in air filled bags. The dominant organisms (>90%) on samples packed in BB-1 bags with CO₂-enriched atmospheres after 21-22 d of storage were gram-positive bacteria, most of which (>95%) were *B. thermosphacta*. Similar results have been described for meat stored in CO₂-enriched atmospheres (20-100% of CO₂), irrespective of the remaining gases in the gas mixture (1,5,18). These changes are due to the effective inhibition of CO₂ on gram-negative organisms (14).

Similar microbial change observed in BB-1 with each CO₂-enriched gas mixture suggests that oxygen concentration, at least between 16% (in CO₂-air) and 80% (in CO₂-O₂), has no influence on the predominant organisms.

The TVC of pork vacuum-packed in PE (Fig. 1) showed a similar pattern to that observed with air or CO₂-O₂ in the same type film. Likewise, pseudomonads and *B. thermosphacta* were the organisms responsible for spoilage (78 and 22%, respectively). Thus, from a microbiological point of view, this kind of plastic is not suitable to pack meat under modified atmospheres with the aim of delaying microbial growth.

When pork was vacuum-packed in BB-1, the evolution of TVC during the first 15-20 d (Fig. 1) was quite similar to those observed in CO₂-air or CO₂-O₂. However, after this first period, TVC of vacuum-packed samples remained at levels around 10^7 CFU/cm² for at least 10 more days. This observation agrees with the results of other authors (5,25,29), who have established that TVC of vacuum-packed meat are typically below 10^8 CFU/cm².

Characterization of the dominant flora in vacuum-packed samples showed a high percentage (70%) of *Lactobacillus* spp. and *B. thermosphacta* in a proportion close to 2:1. The remaining 30% were gram-negative oxidase-negative motile rods that weakly fermented glucose, tentatively characterized as *Enterobacteriaceae*. This selection of slower-growing, gram-positive bacteria has been attributed to the combination of restricted oxygen levels (<1%) and high carbon dioxide concentrations of about 20% developed in vacuum-packs (8). The predominance of lactic acid bacteria (5,7,25) and the presence of *B. thermosphacta* and *Enterobacteriaceae* after storage of vacuum-packed

meat have been reported (25,29). However, in other instances, the latter organisms have not been detected (5). The presence of these organisms on vacuum-packed meat has been associated with elevated temperatures (3,9) and high pH and high oxygen transmission rates of the packaging film (4,12).

Chemical analysis

When PE bags were used, the MetMb formation in vacuum-packed samples or kept in air-filled bags (Fig. 2) did not show significant differences ($p > 0.05$) throughout the storage (9 d). However, the level of MetMb in $\text{CO}_2\text{-O}_2$ was significantly lower ($p < 0.01$) at day 9. Consequently, at the 9th day, when TVC were higher than 10^8 CFU/cm², (Fig. 1), the percentage of MetMb in the former was 60%, but only 40% in the latter. The delay of MetMb oxidation by 80% of oxygen, while 20% of CO_2 did not affect the microbial growth might be explained by the higher concentration of the latter and its lower diffusion through this type of film.

MetMb change in pork packed in BB-1 bags with CO_2 -air showed no significant difference ($p > 0.05$) with that observed in air (Fig. 3). Therefore, 20% CO_2 in the original gas mixture does not affect MetMb formation rate, neither in controlled atmosphere (1,22) nor in bags of low permeability to gases (Fig. 3). In BB-1 bags, MetMb accumulation after the 5th day of storage was significantly lower (usually $p < 0.025$) under $\text{CO}_2\text{-O}_2$ than in air or CO_2 -air (Fig. 3). Consequently, a MetMb level of 60% was not reached in the first 20 d of storage.

According to Greene et al. (13) consumers will reject beef with a MetMb percentage higher than 40%, while Solberg (26) offered a value between 50 and 75% of MetMb. If a 60% of MetMb is considered as the threshold for discoloration of pork, and 5×10^7 CFU/cm² the limit for microbial spoilage, the shelf-life of pork packed in BB-1 bags with $\text{CO}_2\text{-O}_2$ (20-80) atmosphere is extended to at least 15-20 d.

Pork vacuum-packed in BB-1 (Fig. 3) showed the lowest Mb oxidation rate throughout the storage period (30 d) without reaching percentages of 60%. However, MetMb evolution for the first 5 d did not differ significantly ($p > 0.05$) from those in BB-1 bags filled with any of the other gas mixtures. This may be related to the residual oxygen inside the bag after vacuum packaging. After this first period, when the oxygen concentration around the meat equilibrates to a value lower than a 1% (8), MetMb reduction activity increases (30) and Mb oxidation occurs at a very low rate (17).

The delay in Mb oxidation reported here has also been observed in pork in different modified atmospheres by Seideman et al. (24). These authors obtained the lowest levels of MetMb, after 35 d of storage, in oxygen-free atmospheres (vacuum-packed and 20% CO_2 + 80% N_2), followed by those packed in O_2 -enriched atmospheres (50% CO_2 + 50% O_2 ; 20% CO_2 + 80% O_2 ; 100% O_2). However, the Mb of vacuum-packed meat, is in its reduced state (unattractive purple red color), while the Mb in O_2 -enriched gas mixtures is mainly in the oxygenated form, (attractive bright red color).

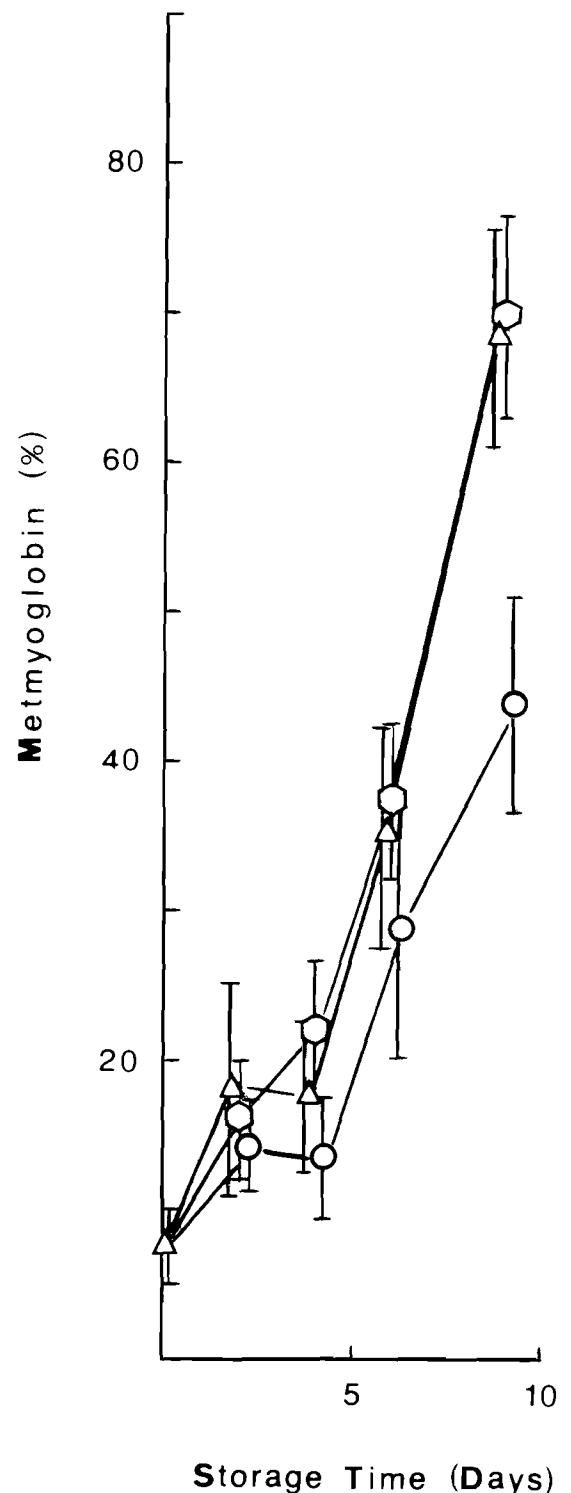


Figure 2. Metmyoglobin accumulation on refrigerated pork (1°C) packed in PE bags with different atmospheres. Vertical bars represent S.D. of each batch of samples. Symbols as in Fig. 1.

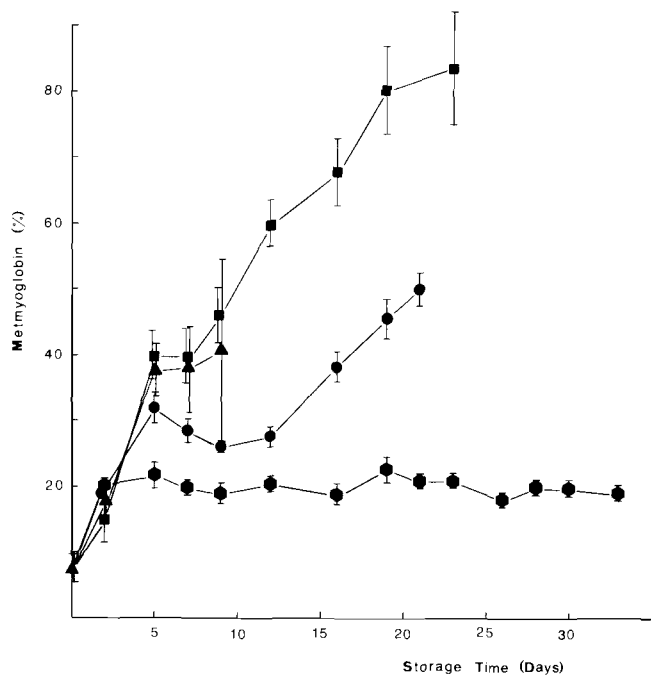


Figure 3. Metmyoglobin accumulation on refrigerated pork (1°C) packed in BB-1 bags with different atmospheres. Vertical bars represent S.D. of each batch of samples. Symbols as in Fig. 1.

In PE bags, there was no extension of the shelf-life in any of the different conditions tested. Therefore, lipid autooxidation was monitored only in samples packed in BB-1 bags. In this type of plastic film, the change in TBA values in pork packed with air or CO₂-air were quite similar (Fig. 4). It may be seen from the figure that lipid autooxidation was not affected by 20% of CO₂ in the original gas mixture, at least for the first 10 d of storage. The TBA value at which the rancid odors became detectable is not known. Furthermore, given the variability of this technique, it is not easy to set a threshold value. However, it has been considered (22) that rancid odors became apparent in pork at a TBA number of 5. In spite of the different change in TBA values found in the two experiments, TBA values never reached 5 as long as TVC were lower than 10⁷ CFU/cm² (Fig. 1).

As expected, TBA numbers of samples stored in CO₂-O₂ were always higher than those obtained in air or in CO₂-air. However, TBA values of 5 were reached only after 20-22 d (Fig. 4), when TVC were higher than 5 × 10⁷ CFU/cm². As autooxidation rate depends on the muscle used (22), lipid autooxidation could limit the shelf-life of muscles other than longissimus dorsi stored in CO₂-O₂ gas mixtures.

In samples vacuum-packed in BB-1 bags, TBA numbers were very low throughout storage (Fig. 4). Therefore, lipid autooxidation will never be the limiting factor of the shelf-life of refrigerated pork vacuum-packed in plastic bags of low permeability to oxygen. However, this meat

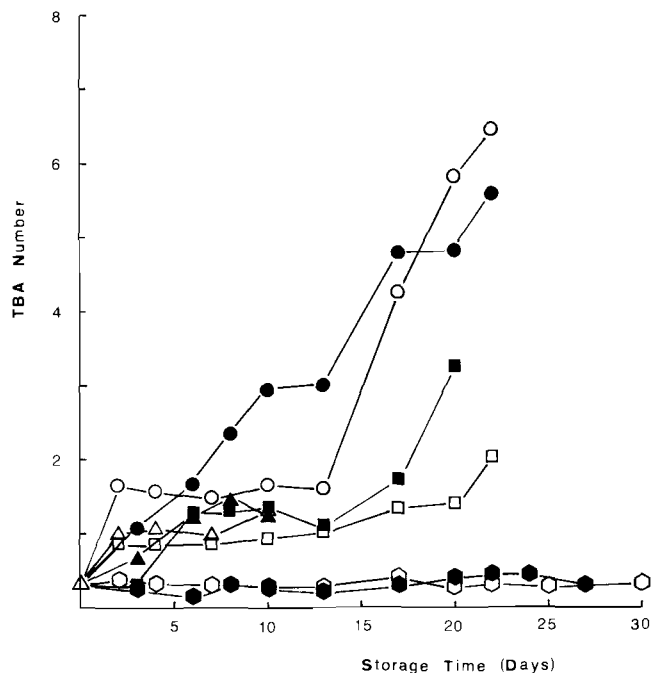


Figure 4. TBA value change in refrigerated pork (1°C) packed in BB-1 bags with air (triangles), 20% CO₂ + 80% air (squares), 20% CO₂ + 80% O₂ (circles) or vacuum-packed (hexagons) in two separate experiments (open and closed symbols).

developed off-odors, which were characterized as dairy/cheesy/sour/acid, in about 20 d (Fig. 1). These types of off-odors appeared, by the same time, in meat stored under CO₂-O₂ enriched atmosphere (Fig. 1). Therefore, the CO₂-O₂ atmosphere has the advantage that meat presents the more attractive bright red color due to the oxygenated form of Mb.

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