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Shelf Life of Fresh Sausages Stored under Modified Atmospheres

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ABSTRACT

The aim of this study was to investigate differences in modified atmosphere packaging (MAP) for the improvement of the shelf life of fresh meat products. Three different conditions for preserving fresh sausages were tested: MAP1 (20% CO_2, 70% O_2, and 10% N_2), MAP2 (40% CO_2 and 60% O_2), and MAP3 (40% CO_2, 30% O_2, and 30% N_2). Samples from the MAP2 group had fewer spoilage bacteria, stable red color (no change of a* value), and good physical attributes (high water-holding capacity, little loss from cooking, and low shear force needed for cutting) compared with samples from other treatment groups. Thus, high concentrations of CO_2 (40%) and O_2 (60%) resulted in a longer shelf life for fresh sausages.

Changes in dietary habits and the subsequent need for distribution channels to satisfy consumer requests have led researchers to study new methods for preservation of fresh meat products. Currently, consumers pay close attention to the quality and safety of fresh sausages, particularly ingredients, additives, and storage conditions of the products.

Microbiological and biochemical characteristics of fresh sausages are very similar to those of fermented sausages during the first phase of ripening, when microbial activities can be responsible for modifications with subsequent shelf-life reduction of the products *(24).* Over the past few years, researchers have explored ways of using natural treatments to reduce bacterial counts and, consequently, extend the shelf life of fresh meat products. Djenane et al. *(3)* showed small but significant inhibition of the growth of psychrotrophic microorganisms with the application of rosemary extract on the surface of beef steaks. In contrast to those findings, Sánchez-Escalante et al. *(20)* demonstrated a lack of effect of antioxidants on microbial growth. Zhang et al. *(25)* reported the reduction of the initial number of spoilage bacteria in the presence of lactic acid during the storage of beef. Modified atmosphere packaging (MAP) is a well-known method for extending the shelf life of meat products *(3, 8, 9, 12, 16, 21, 23).*

In a recent study by Djenane et al. *(4),* beef steaks treated with lactic acid and a mixture of natural antioxidants and then packaged in 40% CO_2 had an extended shelf life and lactic acid bacteria and *Brochothrix thermosphacta* were significantly inhibited. However, the use of a modified atmosphere with high CO_2 and low O_2 concentrations can cause meat bleaching *(14, 15).* This aspect was the object of some debate. Some authors *(5, 7)* have assumed that high

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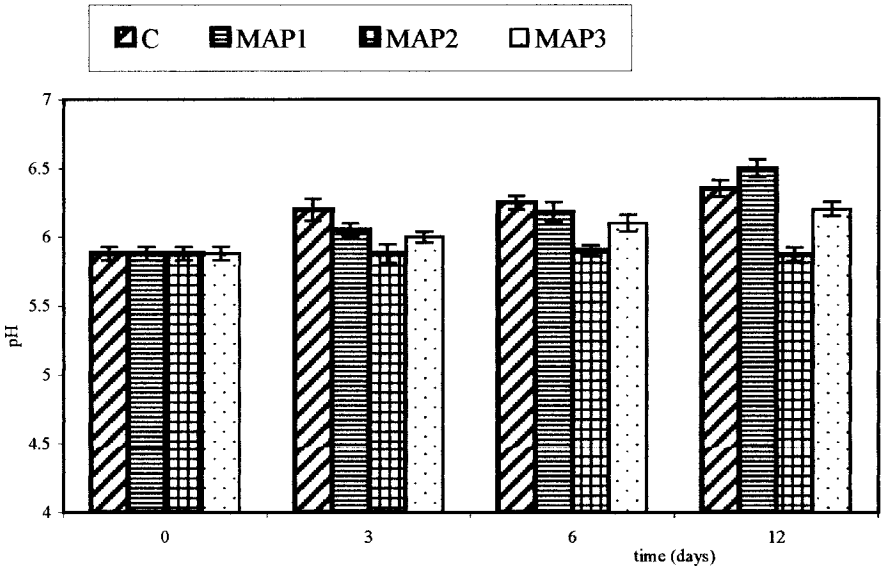

FIGURE 1. *pH values for fresh sausages during storage under different MAP conditions. The results are expressed as the mean of four determinations performed on different parts of three samples for each batch. MAP1, 20% CO_2, 70% O_2, and 10% N_2; MAP2, 40% CO_2 and 60% O_2; MAP3, 40% CO_2, 30% O_2, and 30% N_2; C, control.*

TABLE 1. *L*, a*, and b* values on the surface of fresh sausages stored under different MAP conditions*[a]

	Storage time (days)	C	MAP1	MAP2	MAP3
L*	0	38.37 (±0.12)	38.37 (±0.12)	38.37 (±0.12)	38.37 (±0.12)
	3	32.09 (±0.92)	35.25 (±0.54)	39.53 (±0.24)	32.8 (±0.38)
	6	28.95 (±0.38)	33.38 (±1.01)	37.78 (±0.57)	30.8 (±0.75)
	12	33.76 (±0.76)	36.99 (±0.93)	40.13 (±0.85)	32.79 (±0.63)
a*	0	13.3 (±0.07)	13.3 (±0.07)	13.3 (±0.07)	13.3 (±0.07)
	3	8.63 (±0.08)	9.91 (±0.11)	13.4 (±0.08)	7.6 (±0.09)
	6	7.19 (±0.06)	7.99 (±0.09)	13.2 (±0.08)	6.88 (±0.12)
	12	4.99 (±0.09)	4.92 (±0.08)	13.2 (±0.10)	4.65 (±0.05)
b*	0	5.52 (±0.08)	5.52 (±0.08)	5.52 (±0.08)	5.52 (±0.08)
	3	5.55 (±0.06)	5.60 (±0.06)	5.00 (±0.08)	5.42 (±0.09)
	6	5.65 (±0.04)	5.70 (±0.08)	4.24 (±0.10)	5.50 (±0.09)
	12	6.00 (±0.09)	5.80 (±0.11)	4.20 (±0.05)	6.20 (±0.11)

[a] Results are expressed as the mean (±standard deviation) of three determinations performed on different parts of six samples for each batch. MAP1; 20% CO_2, 70% O_2, 10% N_2; MAP2; 40% CO_2, 60% O_2; MAP3, 40% CO_2, 30% O_2, and 30% N_2; C, cotrol.

O_2 concentrations could be used in MAP to maintain the meat pigment myoglobin to oxygenated state, whereas others *(2)* have suggested that high concentrations of O_2 retard but do not prevent the oxidation of red oxymyoglobin to brown metmyoglobin. From a sensory point of view, color is the most important parameter that influences consumers in the choice of fresh meat products *(6, 7, 13)*.

Jayasingh et al. *(12)* reported that color stability of ground beef, packaged with 0.5% CO, was strongly improved. MAP systems with low CO concentrations do not represent a risk of toxicity for consumers *(22)*. However, marketing research has indicated that consumers require safe and natural products *(11)* without additives such as preservatives and antioxidants and without the use of potentially toxic gases.

For MAP systems to be most effective, the optimal

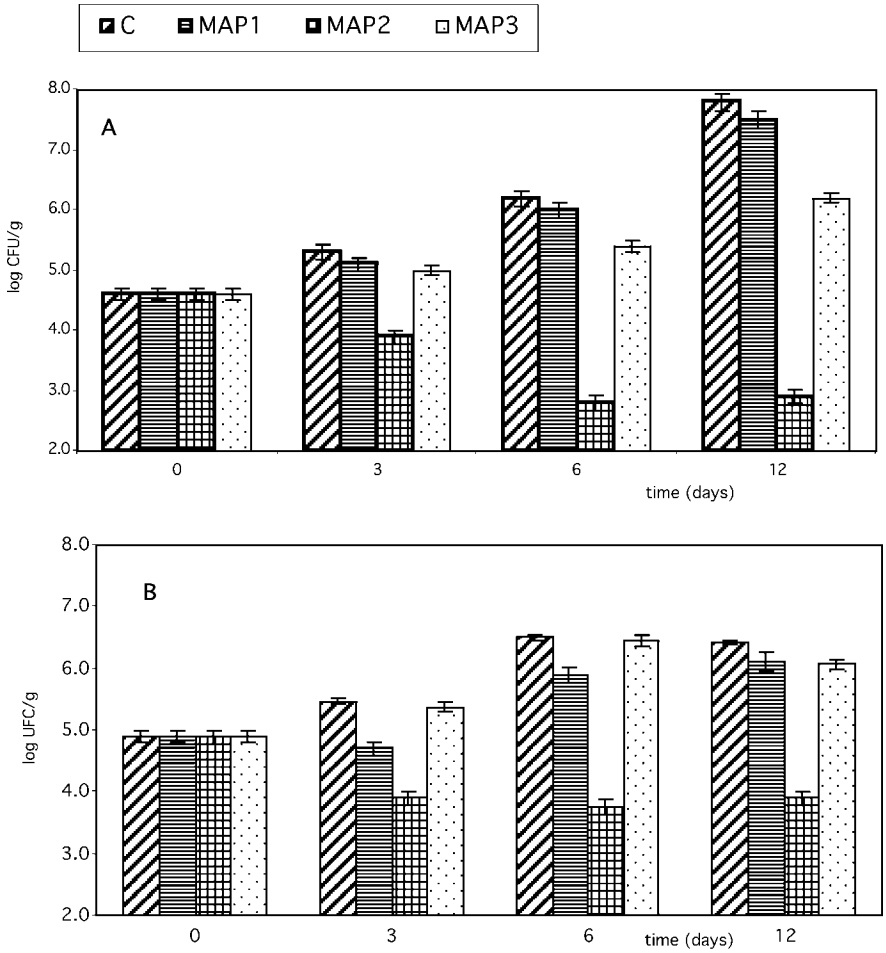

FIGURE 2. *Counts of total mesophilic bacteria (A) and yeasts (B) in fresh sausages during storage under different MAP conditions. The results are expressed as the mean of the determinations performed on three different samples for each batch. MAP1, 20% CO_2, 70% O_2, and 10% N_2; MAP2, 40% CO_2 and 60% O_2; MAP3, 40% CO_2, 30% O_2, and 30% N_2; C, control.*

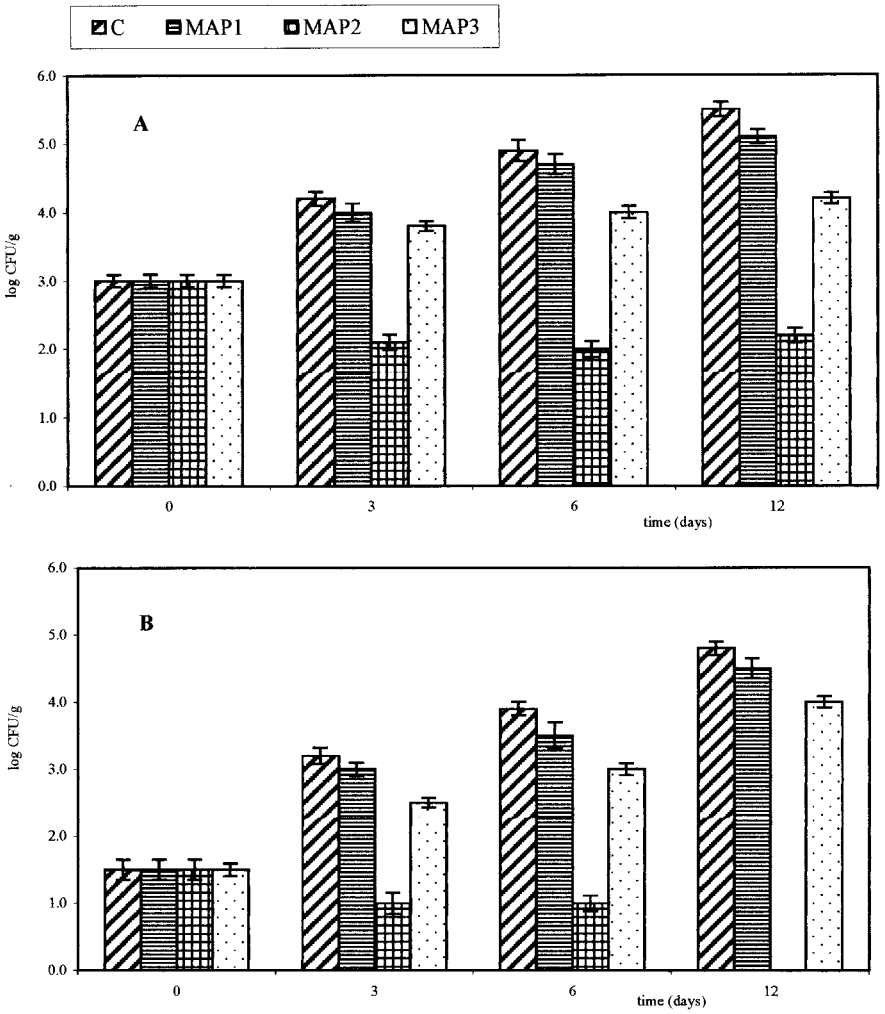

FIGURE 3. *Counts of total coliforms (A) and fecal coliforms (B) in fresh sausages during storage under different MAP conditions. The results are expressed as the mean of the determinations performed on three different samples for each batch. MAP1, 20% CO_2, 70% O_2, and 10% N_2; MAP2, 40% CO_2 and 60% O_2; MAP3, 40% CO_2, 30% O_2, and 30% N_2; C, control.*

type and percentage of gases used should be determined. The present study was conducted to compare three different modified atmospheres containing different gas mixtures.

MATERIALS AND METHODS

Use of MAP. A mix of triturated lean pork, 10% fat, 2.5% NaCl, and 0.15% black pepper was used to fill natural casings. Two hundred forty sausages (about 150 g each) were produced, divided into four batches (60 sausages per batch), and packaged with a vacuum pump CS30 (Vacuum Pump S.p.A., Breverate di Brivio, Italy) in laminated bags (one sausage per bag) 20 by 22 cm (Cryovac BB4L, Passirana di Rho, Milan, Italy). Diffusion coefficients were 150 $cm^3/24\ h \cdot m^2 \cdot bar$ for CO_2, 35 $cm^3/24\ h \cdot m^2 \cdot bar$ for O_2, and 1.4 $cm^3/24\ h \cdot m^2 \cdot bar$ for N_2. Vacuum was created before the injection of the different gas mixtures. The first batch, MAP1, was packaged in a modified atmosphere of 20% CO_2, 70% O_2, and 10% N_2; the second, MAP2, was packaged in a modified atmosphere of 40% CO_2 and 60% O_2, and the third, MAP3, was packaged in a modified atmosphere of 40% CO_2, 30% O_2, and 30% N_2. A fourth batch was used as a control (C) and was packaged without gas. Each batch was stored at 4°C for 12 days.

pH measurement. Potentiometric measurement of pH was performed at 0, 3, 6, and 12 days of storage by inserting a pin electrode of a pH meter (Crison 2001, Crison, Modena, Italy) directly into each sample. The results were expressed as the mean of four determinations performed on different parts of three samples for each batch.

Color determination. Color was determined on the surface of sausages after removal of the casing. Measurements were performed at 0, 3, 6, and 12 days of storage using the Hunter L*, a*, b* system *(1)* with a reflectance spectrophotometer (Minolta CR300b, Suita-shi, Osaka, Japan). Values were determined 30 min after each pack was opened to allow the color to stabilize on exposure to air *(5)*. The results were expressed as the mean of three determinations performed on different parts of six samples for each batch.

Microbiological analyses. Ten grams of product from three samples for each batch was aseptically withdrawn immediately after filling and after 3, 6, and 12 days of storage and was homogenized with 90 ml of sterile quarter-strength Ringer's solution in a lab blender (stomacher, Seward Medical, London, UK). Subsequent serial dilutions were inoculated into appropriate media. Lactic acid bacteria were enumerated on deMan Rogosa Sharpe agar (Oxoid, Milan, Italy) after anaerobic incubation (Gas Pack Anaerobic System, BBL, Becton Dickinson, Sparks, Md.) at 30°C for 72 h. *Micrococcaceae* were enumerated on mannitol salt agar (Oxoid) after incubation at 30°C for 36 h. Enterococci were enumerated on Slanetz and Bartley medium (Oxoid) after incubation at 37°C for 48 h. Fecal and total coliforms were counted on violet red bile lactose agar (Oxoid) after incubation for 48 h at 44 and 37°C, respectively. Total mesophilic bacteria were determined on

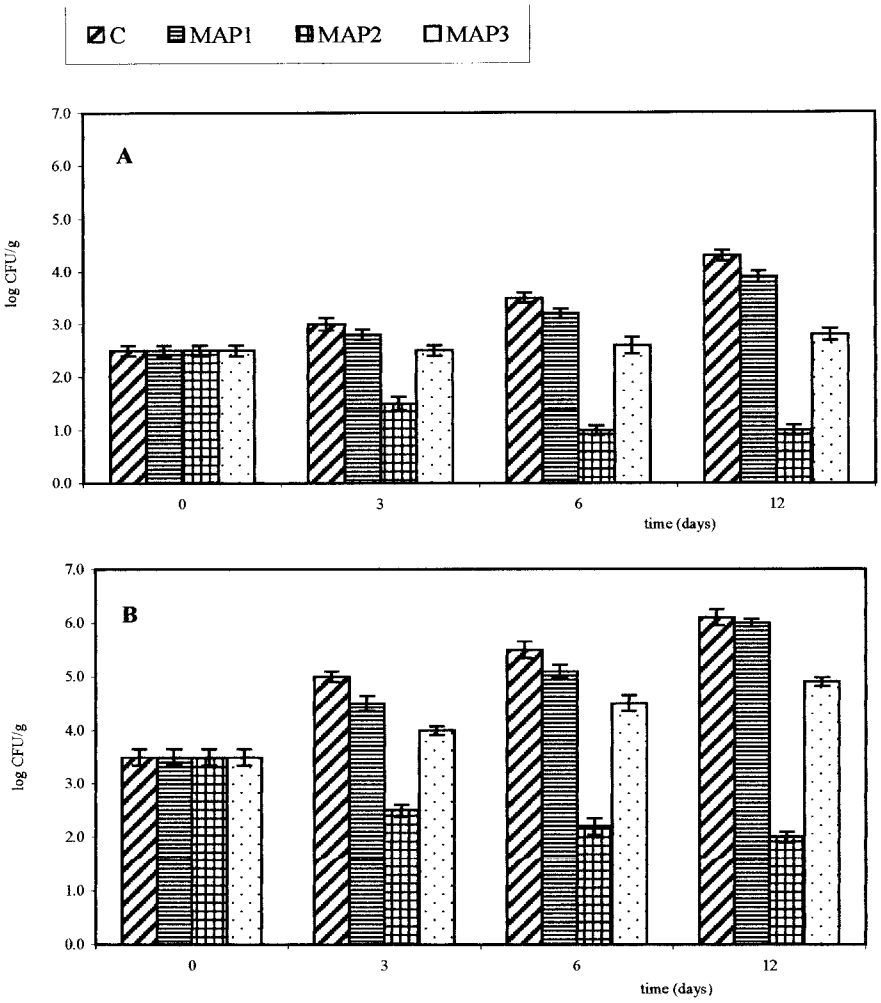

FIGURE 4. *Counts of* Brochothrix thermosphacta *(A) and* Pseudomonas *spp. (B) in fresh sausages during storage under different MAP conditions. The results are expressed as the mean of the determinations performed on three different samples for each batch. MAP1, 20% CO_2, 70% O_2, and 10% N_2; MAP2, 40% CO_2 and 60% O_2; MAP3, 40% CO_2, 30% O_2, and 30% N_2; C, control.*

plate count agar (Oxoid) after incubation at 28°C for 48 h. Yeasts and molds were counted on YPD agar (20 g/liter peptone, 20 g/liter dextrose, 10 g/liter yeast extract, and 20 g/liter agar) after incubation at 30°C for 72 h. *B. thermosphacta* was enumerated on streptomycin thallous acetate actidione agar base (Oxoid) with streptomycin thallous acetate selective supplement (Oxoid) after incubation at 37°C for 48 h. *Pseudomonas* spp. were counted on *Pseudomonas* agar (Oxoid) with SR102E supplement (Oxoid) after incubation at 22°C for 24 h.

WHC, cooking loss, and shear force analyses. Water holding capacity (WHC) was measured with the procedure described by Monetti *(18),* and values were expressed as water expulsed from meat. At 0, 3, 6, and 12 days of storage, 300 mg of sausage with the covering removed was collected from three samples for each batch and placed between two Whatman paper filters (55-mm diameter; Whatman, Clifton, N.J.). The samples were pressed for 5 min with a 1-kg metal cylinder. The dry area on the filter paper was measured with a planimeter (model 317E, Gebruder Haff GMBH, Pfronten, Germany) and recorded as a percentage of the total paper filter area.

For cooking loss determination, three sausages for each batch were weighed and cooked at 177°C in a convection oven to an internal temperature of 74°C. The temperature was measured with a thermocouple probe inserted into the center of the sample. After cooling at room temperature, sausages were weighed, and cooking loss was determined as a percentage of the uncooked weight.

For Warner-Bratzler shear force determination, cooked sausages were cooled, wrapped in freezer paper, and held overnight at 4°C before shear values were obtained from four 1.27-cm-diameter cores. Each core was cut three times at a crosshead speed of 230 mm/min. Mean shear values for each sample were calculated on the basis of 12 measurements.

RESULTS

pH detection. Figure 1 shows the pH values for fresh sausages stored for 12 days under different MAP conditions. Values are reported as the mean of four determinations performed on different parts of three samples for each batch. Samples from MAP1, MAP3, and C increased in pH during storage, whereas those of MAP2 were characterized by stable pH. The highest increase in pH was detected in MAP1 sausages after 12 days.

Color values. Commission Internationale de l'clairage L*, a*, and b* values are given in Table 1. Lightness measurement (L*) revealed slight changes for batches MAP1 and MAP2 over the entire storage period, but a marked decrease was observed in MAP3 and control samples. Red color was preserved in sausages from batch MAP2 until the 12th day of storage ($a^* > 13$), whereas a marked decrease in this value was observed in the other batches after 3 days of storage. An increase in yellowness (b*) characterized the sausages from batches MAP1, MAP3, and control. Contrary to previous results, MAP2 samples were characterized

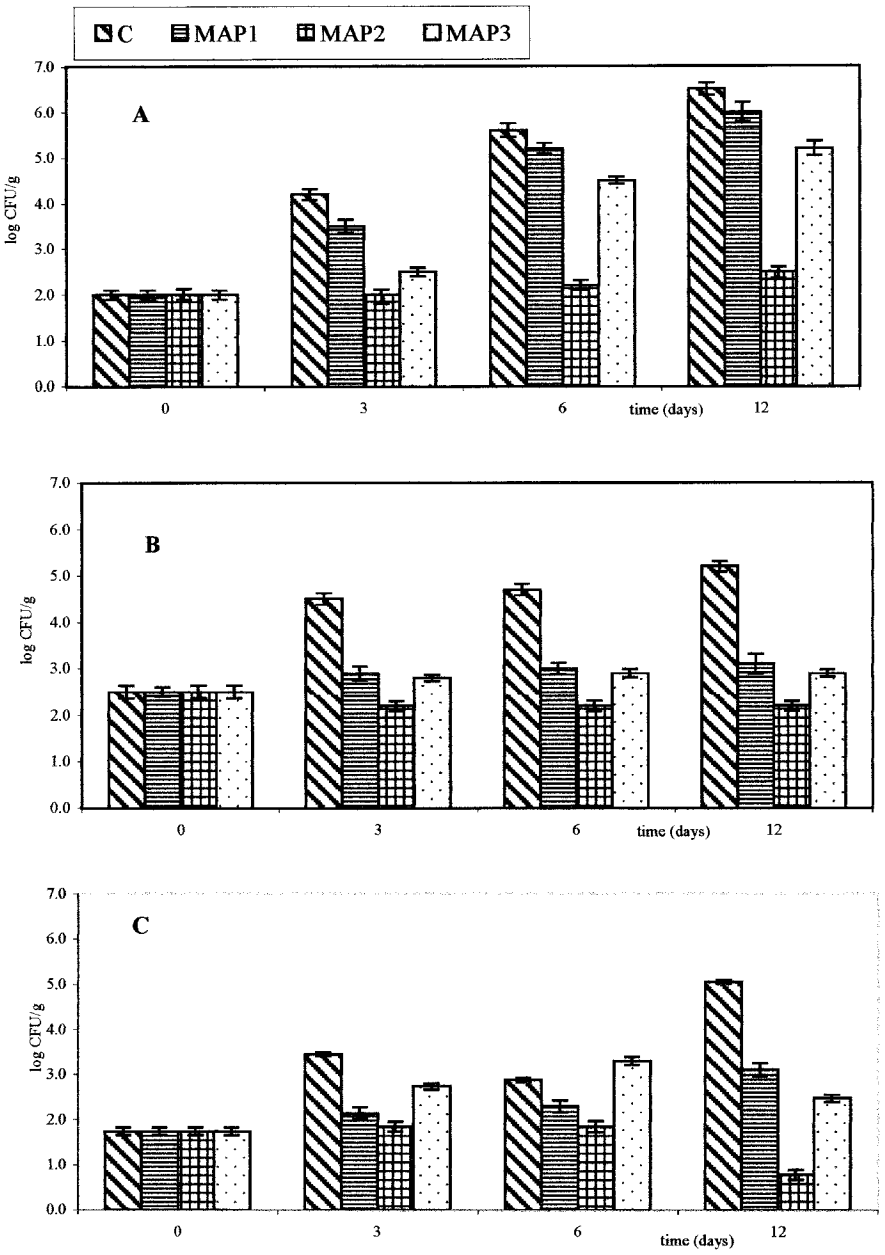

FIGURE 5. *Counts of lactic acid bacteria (A),* Micrococcaceae *(B), and enterococci (C) in fresh sausages during storage under different MAP conditions. The results are expressed as the mean of the determinations performed on three different samples for each batch. MAP1, 20% CO_2, 70% O_2, and 10% N_2; MAP2, 40% CO_2 and 60% O_2; MAP3, 40% CO_2, 30% O_2, and 30% N_2; C, control.*

by a decrease in b* value (from 5.52 to 4.20) during the storage period.

Microbial population. The growth of total mesophilic bacteria and yeasts is shown in Figure 2. Total mesophilic bacteria increased in the samples from the control, MAP1, and MAP3 batches (Fig. 2A). The counts for the control and MAP1 samples had a 3.0-log increase during the storage period, whereas a marked decrease in counts characterized batch MAP2. During the first 3 days of storage, yeasts (Fig. 2B) decreased in MAP2 samples and then did not change much through the end of the storage. A constant increase in yeasts was detected in the other batches. Total and fecal coliform counts are reported in Figure 3A and 3B. Total coliforms increased in the control and MAP1 batches from an initial value of 3.0 log CFU/g at the start of the storage to ca. 5.0 to 5.5 log CFU/g at the end of the storage. Coliform counts in MAP3 samples did not increase as much as did those in MAP1 or control samples. Samples from batch MAP2 were characterized by a decrease in total coliforms during the first 3 days of storage, but from the third day until the end of storage the counts remained stable. Similar results were obtained for fecal coliforms, except that MAP2 samples were characterized by a reduction in fecal coliforms to <10 CFU/g by 12 days of storage. *B. thermosphacta* counts (Fig. 4A) decreased in MAP2 samples during storage, whereas those for MAP3 samples were constant. *B. thermosphacta* counts in both MAP1 and control samples increased by 1.5 to 2.0 log CFU/g. *Pseudomonas* counts (Fig. 4B) were initially about 3.5 log CFU/g. At the end of the storage, counts of about 6.0 log CFU/g were detected in sausages from the control and MAP1 batches, whereas in MAP3 samples counts were ca. 4.8 log CFU/g. A constant decrease in *Pseudomonas* counts was found in sausages from batch MAP2. Counts of lactic acid bacteria (Fig. 5A) were essentially constant during the entire period of storage in MAP2 sausages, but a constant

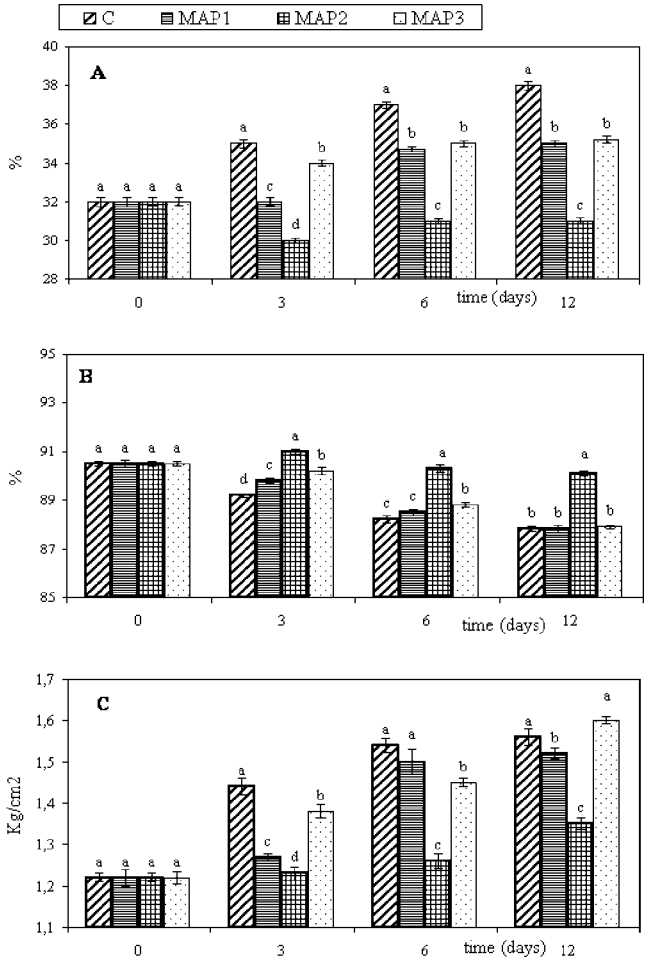

FIGURE 6. *Changes in weight loss from cooking (A), water holding capacity (B), and shear force (C) of sausages during storage under different MAP conditions. The results are expressed as the mean of the determinations performed on three different samples for each batch. Different letters at each time indicate significant differences (*$P \leq 0.01$*). MAP1, 20% CO_2, 70% O_2, and 10% N_2; MAP2, 40% CO_2 and 60% O_2; MAP3, 40% CO_2, 30% O_2, and 30% N_2; C, control.*

increase was observed in the other batches. Counts performed on mannitol salt agar (Fig. 5B) revealed a high concentration of *Micrococcaceae* in control samples during the first days of storage, but counts in batches MAP1 and MAP3 were stable over the storage period. MAP2 samples had slightly decreasing *Micrococcaceae* counts. Enterococci counts (Fig. 5C) were stable during the storage period in sausages from batch MAP2, but in the other batches, enterococci increased from about 2.0 log CFU/g to 3.0 to 3.5 log CFU/g. Control samples had a more marked increase in enterococci to a final value of about 5.0 log CFU/g.

WHC, cooking loss, and shear force analyses. During the storage period, loss of sausage weight after cooking (Fig. 6A) moderately increased and was inversely proportional to the WHC (Fig. 6B). Samples from MAP2 had less cooking loss for all storage times compared with samples from other batches. The lowest value (30%) was recorded on the third day of storage and corresponded to the highest WHC (91%). The cooking loss for the control samples was higher than those for MAP1 and MAP3 samples. Shear force of sausages increased (i.e., sausages were harder to cut) with storage time for all batches (Fig. 6C). The highest shear force was detected in control sausages, and the lowest was found in MAP2 sausages.

DISCUSSION

From the results obtained in the microbiological, physical, and rheological analyses, it was possible to understand the role of the different atmosphere compositions on microbial growth and sausage quality during storage. Packaging without a modified atmosphere resulted in an increase in microorganisms, thereby producing undesirable alterations in the product. The samples from the control batch had a marked increase in psychrotrophic bacteria, such as *Pseudomonas* spp., which are able to degrade proteins and produce off flavors and free amines.

B. thermosphacta has been described *(10, 19)* as an important and undesirable microorganism in fresh meat products. This species occurs commonly in products packaged without a modified atmosphere. In the present study, *B. thermosphacta* counts increased in control samples during storage. An increase in counts of all other microbial groups evaluated also was observed in the control samples.

Marked inhibition of microbial growth was observed in samples packaged with a modified atmosphere including higher CO_2 concentrations, as has been previously reported *(4)*. However, the effect and extent of the antimicrobial activity were attributable to both CO_2 concentration and the presence of other gases. Batches treated with 20% CO_2 (MAP1) had microbial counts very similar to those of controls. The most desirable result for the fresh sausages was obtained with 40% CO_2, but this concentration had different effects on microbial growth when combined with various O_2 concentrations. The mixture of 40% CO_2 and 60% O_2 (MAP2) resulted in a significant inhibitory effect on microbial growth, but no inhibitory action was detected in samples packaged with 40% CO_2 and 30% O_2 (MAP3). This combination had no effect on microbial counts, but there was an increase in ''altering'' microorganisms, even though their concentrations in the MAP3 batch were slightly lower than those in the MAP1 and control batches. MAP1 conditions (20% CO_2) did not seem to have any inhibitory effect on spoilage bacteria. These results confirm that microorganisms are CO_2 sensitive in the presence of high O_2 concentrations but are not CO_2 sensitive at low O_2 concentrations *(17)*. The combination of 40% CO_2 and 60% O_2 (MAP2) resulted in inhibition of both gram-negative bacteria (e.g., *Pseudomonas* spp. and *B. thermosphacta*) and gram-positive bacteria (e.g., lactic acid bacteria and *Micrococcaceae*).

MAP2 conditions also had a positive effect on the definition and maintenance of the color red, the most important parameter for consumer choice. In samples from the MAP2 batch, the high O_2 concentration maintained myoglobin in the oxidative state (which is responsible for the color red) while the high CO_2 concentration assured the microbiological stability of the product. The above phenomena were not seen in the samples from the other batches.

Higher WHC was found in sausages from batch MAP2, with consequently lower cooking loss. These sausages also had a lower shear value.

The gas mixture used in the MAP2 treatment produced an inhibitory effect on the microorganisms, delaying spoilage and positively affecting some rheological parameters.

REFERENCES

1. Commission Internationale de l'clairage. 1978. International Commission on Illumination recommendations on uniform color spaces, color difference equations, psychometric color terms. Supplement 2 to C.I.E. publication 15 (E-1.3.1) 1971/(TC-1.3). Bureau Central de la Commission Internationale de l'clairage, Paris.
2. Coventry, M. J., M. W. Hickey, R. Mawson, P. Drew, J. Wan, D. Krause, and V. Boghossian. 1998. The comparative effects of nitrogen and oxygen on the microflora of beef steaks in carbon dioxide–containing modified atmosphere vacuum skin–packaging (MA-VSP) systems. *Lett. Appl. Microbiol.* 26:427–431.
3. Djenane, D., A. Sánchez-Escalante, J. A. Beltrán, and P. Roncalés. 2002. Ability of α-tocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere. *Food Chem.* 76:407–415.
4. Djenane, D., A. Sánchez-Escalante, J. A. Beltrán, and P. Roncalés. 2003. The shelf-life of beef steaks treated with DL-lactic acid and antioxidants and stored under modified atmospheres. *Food Microbiol.* 20:1–7.
5. Djenane, D., A. Sánchez-Escalante, J. A. Beltrán, and P. Roncalés. 2003. Extension of the shelf life of beef steaks packaged in a modified atmosphere by treatment with rosemary and displayed under UV-free lighting. *Meat Sci.* 64:417–426.
6. Faustman, C., and R. C. Cassens. 1990. The biochemical basis for discoloration in fresh meat: a review. *J. Muscle Foods* 1:217–243.
7. Faustman, C., R. C. Cassens, D. M. Schaefer, D. R. Beuge, S. N. Williams, and K. K. Scheller. 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *J. Food Sci.* 54:858–862.
8. Garcia de Fernando, G. D., G. J. E. Nychas, M. W. Peck, and J. A. Ordonez. 1995. Growth/survival of psychrotrophic pathogens on meat packaged under modified atmospheres. *Int. J. Food Microbiol.* 28:221–232.
9. Gill, C. O., and L. E. Jeremiah. 1991. The storage life of non-muscle offals packaged under vacuum or carbon dioxide. *Food Microbiol.* 8:339–353.
10. Grau, F. H. 1980. Inhibition of the anaerobic growth of *Brochothrix thermosphacta* by lactic acid. *Appl. Environ. Microbiol.* 40:433–436.
11. Hugas, M., M. Garriga, and J. M. Monfort. 2002. New mild technologies in meat processing: high pressure as a model technology. *Meat Sci.* 62:359–371.
12. Jayasingh, P., D. P. Cornforth, C. E. Carpenter, and D. Whittier. 2001. Evaluation of carbon monoxide treatment in modified atmosphere packaging or vacuum packaging to increase color stability of fresh beef. *Meat Sci.* 59:317–324.
13. Jeremiah, L. E., Z. L. Carpenter, and G. C. Smith. 1972. Beef color as related to consumer acceptance and palatability. *J. Food Sci.* 37: 476–479.
14. Ledward, D. A. 1970. Metamyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. *J. Food Sci.* 35:33–37.
15. Ledward, D. A. 1984. Haemoproteins in meat and meat products, p. 33–64. *In* B. J. F. Hudson (ed.), Developments in food proteins, vol. 3. Applied Science Publishers, London.
16. Luno, M., P. Roncalés, D. Djenane, and J. A. Beltrán. 2000. Beef shelf life in low O_2 and high CO_2 atmospheres containing different low CO concentrations. *Meat Sci.* 55:413–419.
17. Mano, S. B., J. A. Ordonez, and G. D. Garcia de Fernando. 2000. Growth survival of natural flora and *Aeromonas hydrophyla* on refrigerated uncooked pork and turkey packaged in modified atmospheres. *Food Microbiol.* 17:183–191.
18. Monetti, P. G. 1997. Appunti di suinicoltura. Giraldi Edizioni, Bologna, Italy.
19. Nissen, H., O. Sorheim, and R. Dainty. 1996. Effects of vacuum, modified atmospheres and storage temperature on the microbial flora of packaged beef. *Food Microbiol.* 13:183–191.
20. Sánchez-Escalante, A., D. Djenane, G. Torrescano, J. A. Beltrán, and P. Roncales. 2001. The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. *Meat Sci.* 58:421–429.
21. Skandamis, P. N., and G. J. E. Nychas. 2002. Preservation of fresh meat with active and modified atmosphere packaging conditions. *Int. J. Food Microbiol.* 79:35–45.
22. Sorheim, O., T. Aune, and T. Nesbakken. 1997. Technological, hygienic, and toxicological aspects of carbon monoxide used in modified atmosphere packaging of meat. *Trends Food Sci. Technol.* 8:307–312.
23. Tsigarida, E., P. N. Skandamis, and G. J. E. Nychas. 2000. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5°C. *J. Appl. Microbiol.* 89:901–909.
24. Zambonelli, C., F. Papa, P. Romano, G. Suzzi, and L. Grazia. 1992. Microbiologia dei salumi. Edagricole, Bologna, Italy.
25. Zhang, Y. M., J. G. Jia, Z. Q. Zhou, R. Q. Yan, and H. Wang. 1996. Development of preservatives for fresh pork. *Meat Hyg.* 8:1–4.