Meat Science 96 (2014) 554–561

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Stability of lamb loin stored under refrigeration and packed in different modified atmosphere packaging systems

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ARTICLE INFO

Article history:
Received 13 February 2012
Received in revised form 4 July 2013
Accepted 8 August 2013

Keywords:
Lamb
Vacuum packaging
Gas composition
Stability
Sensory analysis

ABSTRACT

The aim of the present study was to evaluate the effect of different modified atmosphere packaging (MAP) systems (vacuum, 75% O_2 + 25% CO_2 and 100% CO_2) on the stability of lamb loins stored at 1 ± 1 °C for 28 days. Microbiological (counts of aerobic and anaerobic psychrotrophic microorganisms, coliform at 45 °C, coagulase-positive staphylococci and lactic acid bacteria and presence of *Salmonella*), physical and chemical (thiobarbituric acid reactive substances [TBARS], objective color, pH, water loss from cooking [WLC] and shear force), sensory (acceptance testing using a 9-point hedonic scale) and gas composition analyses were performed. Lamb meat remained stable with respect to the majority of the evaluated physical and chemical indexes and within the standards established by Brazilian legislation for pathogenic microorganisms throughout the storage period in all three packaging systems. However, with respect to psychrotrophic microorganisms, 100% CO_2 packaging system provided increased stability despite presenting lower appearance preference.

1. Introduction

In recent years, Brazil has significantly increased the production and supply of lamb meat, which is almost exclusively commercialized as frozen cuts due to its long shelf life. According to Fernandes, Venanzi, Guerra, Kamimura, et al. (2010), Fernandes, Venanzi, Guerra, Trindade, et al. (2010), vacuum packaged lamb loin lasts 12 months, when stored at −18 °C, with physical chemical, microbial and sensory quality parameters well preserved.

However, as a result of an increasing demand for fresh and ready-to-use products, a need has emerged for further studies involving the possibility of extending shelf life of the refrigerated meat. It is quite known that this conservation technology, when applied to perishable food such as fresh meat, results in a shorter shelf life. Fernandes, Freire, Guerra, Balieiro, and Trindade (2012), evaluated the stability of vacuum packaged lamb loin, stored at 4 °C for 28 days. In spite of maintenance of the main physical and chemical quality parameters during the evaluated period, the authors noticed an important increase of spoiling microorganisms' counts from the 7th to 14th day of storage, limiting the product shelf life.

In this context, the modified atmosphere packaging (MAP) in combination with low temperatures has been considered an important technology with respect to maintaining quality standards and extending the shelf life of fresh meat (Baracat, Luchiari Filho, Pereira, Silva, & Cesar, 2005; Parry, 1993). In addition to delaying the development of spoilage microorganisms, this packing technique also contributes to reducing lipid oxidation, which can result in a rancid odor and flavor. It is important to emphasize that a proper gaseous atmosphere must be applied in order to reach a longer shelf life of highly perishable foods.

According to Devlieghere, Debevere, and Impe (1998), CO_2 is one of the most widely used compounds in meat packing because it exhibits antimicrobial activity, and it is partially soluble in water and fat within the food until the solubility equilibrium is reached. The gas O_2 is responsible for the desirable bright red color (oxymyoglobin) at the time of purchase. However, O_2 can promote lipid and myoglobin oxidation rather quickly, resulting in discoloration, i.e., the formation of metmyoglobin (Bórnez, Linares, & Vergara, 2010; Jakobsen & Bertelsen, 2000; Mancini, Hunt, Hachmeister, Kropf, & Johnson, 2005). A gas composition of 20 to 30% CO_2 and 70 to 80% O_2 is generally used for meat packaging.

Although there is quite available research on modified atmosphere technology applied for red meat, studies involving lamb meat, particularly for Santa Inês breed, typically from Brazil, are scarce. In addition, it is important to consider the current scenario in this country, which shows a continuous request for high quality lamb meat products. Today, a great part of the market is subjected to frozen cuts imported from other countries, mainly from Uruguay. For this reason it is imperative to develop and apply technologies to attend to the outstanding national demand for premium cuts of refrigerated lamb meat. Considering

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http://dx.doi.org/10.1016/j.meatsci.2013.08.005

the arguments above the aim of the present study was to apply MAP technology using different gas compositions (vacuum, pure CO_2 or CO_2 in combination with O_2) to increase lamb meat stability when stored under refrigeration.

2. Materials and methods

2.1. Raw materials and modified atmosphere packaging

The samples were obtained from the loins of 105 intact male lambs from crosses of Santa Inês × Dorper. The lambs were aged between five and six months with a live weight between 35 and 40 kg. The animals were slaughtered in a commercial slaughterhouse according to the standards for animal welfare and good manufacturing practices established by Brazil's Ministry of Agriculture, Livestock and Food Supply. The carcasses were refrigerated (4 °C) for 24 h, suspended by the Achilles tendon. The samples were then obtained from cuts between the first and sixth lumbar vertebrae (*Longissimus lumborum*) on both sides of the carcasses.

All of the samples were packed in perforated Drylock expanded polystyrene trays (140 × 178 × 25 mm) and contained absorbent for liquid drainage (21P LinStar, LINPAC). The trays were individually placed in 180 × 370 mm 7 layer EVA/PVDC plastic bags; 48 a 62 μm thickness; O_2 permeability of 25 cm^3/m^2.day (1 atm/23 °C/0% RU) and water vapor permeability of 10 g H_2O m^2 day (1 atm/38 °C/90% RU) (BB2800, CRYOVAC). Three treatments were performed: 1) vacuum packaging; 2) MAP containing 100% CO_2; and 3) MAP constituting of 75% O_2 + 25% CO_2. Five volumes of gas were injected, as recommended by the manufacturer, considering the proportion between the weight of the meat package volume The packaged meat for all the treatments was refrigerated (1 ± 1 °C) for 28 days.

2.2. Evaluation of the lamb loins

A completely randomized 3 × 5 factorial design was used to analyze the microbiological, physical, chemical and sensory parameters. The three treatments were assessed with respect to these parameters at five storage intervals (0, 7, 14, 21 and 28 days). Three replicates were performed for each treatment at each of the storage time intervals.

Except for the microbiological analysis, all of the results were analyzed using analysis of variance (ANOVA) with SAS (Statistical Analysis Software) version 9.1.3. The means were compared using Tukey's test at 5% significance level. The experimental results were fit using regression models to examine the variations that occurred throughout the storage period and/or among treatments.

2.2.1. Analysis of headspace gas composition

The gas composition inside the package was determined in each evaluated interval, using a portable gas analyzer (Dansensor, Check-Point O_2/CO_2). An electrochemical sensor was used to measure O_2 levels, and an infrared sensor was used to measure CO_2 levels by means of puncture of packaging, according to the method described in Sarantópoulos et al. (2002).

2.2.2. Chemical composition analysis

To analyze the chemical composition of the samples, the official methodology by Horwitz and Latimer (2007) was used to measure moisture (950.46), fixed mineral residue (or ash) (920.153) and protein (981.10). The lipid content was determined using the method described by Bligh and Dyer (1959).

2.2.3. Physical and chemical analysis

A portable colorimeter (HunterLab, MiniScan XE) was used for the objective color analyses using the L^*, a^* and b^* scales of the CIE-L*a*b* evaluation system (CIE, 1976), with L^* indicating brightness, a^* the red–green range and b^* the blue–yellow range. A D_{65} illuminant was used at an observation angle of 10° and with a cell opening of 30 mm. Equipment was calibrated using an white standard ($L^* = 93.80$, $a^* = -0.89$, $b^* = 0.95$) and black ($L^* = 1.19$, $a^* = 1.27$, $b^* = 1.92$) and the readings were obtained at three different points 30 min following the exposure of the sample surface to the atmosphere. The average of the readings was taken as final measure.

The pH was measured using a pH meter (Hanna, HI 99163) with a combined electrode for performing readings in triplicate with perforation of the meat. The same samples used for color and pH analyses were subjected to an evaluation of the extent of lipid oxidation using the thiobarbituric acid reactive substance (TBARS) assay according to Vyncke's (1970) methodology. The results of the assay were expressed as milligrams of malonaldehyde per kilogram of the sample.

The water loss from cooking (WLC) was assessed according to the methodology described by Koohmaraie (1996). The initial weights of samples were obtained using a semi-analytical balance (Marconi, AS 5500C). Samples were then cooked in an electric oven (Eco, Gran Master Gourmet) at 180 °C until the internal (geometric center) temperature reached 72 °C, and were inverted when internal temperature reached 36 °C. Individual temperature sample control was carried out using thermopairs (Exacta) connected to a temperature indicator (Gulton). When at room temperature, samples were weighed again and the WLC was determined using the following equation: WLC = [(initial weight − final weight) / initial weight)] × 100. The results were expressed as percentages.

After cooking, the samples were cut individually and parallel to the muscle fibers as ten pieces measuring 2 × 1 × 1 cm, and the shear forces of these cuts were determined using a *Warner Bratzler* texturometer (Salter Brecknell, 235 6×) and the results were expressed in kilograms (AMSA, 1995).

2.2.4. Microbiological analysis

For sequential decimal dilutions, 25 g small slice samples were ascetically taken from several superficial points of meat samples. This material was placed in sterile bags (Nasco Whirl-Pak®) added with 225 mL sterilized peptone water solution at 0.1% (w/v) (Merck) and homogenized in a Stomaker (Marconi) for 2 min at 100 rpm at room temperature.

The total counts of aerobic and anaerobic psychrotrophic microorganisms were performed according to the methodology described by Johnston and Tompkin (1992), where Petri plates (J. Prolab, 90 × 15 mm) containing Plate Count Agar (PCA, Oxoid) were incubated at 20 °C for 72 h, after sample inoculation. For anaerobic psychrotrophic microorganisms, upside down plates were placed in jars (Probac®, Acrilic − 2.5 L) containing an anaerobiosis generating system (Probac®, Anaerobac − 90 mm).

The presence of *Salmonella* was determined using a rapid pre-enrichment method (A.O.A.C. 2003.09), employing BAX® System (Dupont/Qualicon), an automated analysis equipment which works by polymerase chain reaction (PCR), with pre-enrichment of sample performed from the incubation of the 10^{-1} dilution for 18 to 24 h at 35 °C.

Coagulase-positive staphylococci were identified following the A.O.A.C. 2003.11 method, using Petrifilm™ Staph Express plates (3M Company), with samples incubated at temperatures between 35 and 37 °C for 24 ± 2 h. Confirmation was carried out with Petrifilm™ Staph Express disk (3M Company) when there were formation of different types of colonies.

Thermotolerant coliforms were identified using the A.O.A.C. 989.10 method (Horwitz and Latimer, 2007), using Petrifilm™ Staph Express plates (3M Company). After inoculation, samples were kept at 44 ± 1 °C, for 24 ± 2 h in order to verify the formation of specific colonies.

The lactic acid bacteria were identified using the methods described by Hall, Ledenbach, and Flowers (2001), Kennedy, Buckley, and Kerry (2004), and Lauzurica et al. (2005). The method employed deep

inoculation, where 1 ml of diluted sample was placed in sterile and empty Petri plates (J. Prolab, 90 × 15 mm) and covered with a layer of Man, Rogosa and Sharpe Agar (MRS, Oxoid). Plates were placed upside down in jars (Probac®, Acrilic — 2.5 L) containing generating anaerobiosis system and incubated at 36 ± 1 °C during 48 ± 3 h.

Except for the *Salmonella*, all the results were expressed in Log CFU/g of sample after the identification and enumeration of microorganisms evaluated.

2.2.5. Sensory analyses

Sensory analyses were conducted only under previous microbial innocuity evaluation of samples, according to Brazilian regulation (BRAZIL, 2001), related to pathogen microorganisms and/or visible presence of colonies indicative of microbial deterioration.

Fifty consumers were recruited among the University's students, staff and faculty; enjoying lamb meat was the only selection criterion. Among the selected participants, 57% were female, and 78% were aged between 20 and 40 years. The recruited consumers were given a free and informed consent form to be read and signed prior to performing the tests. Two different affective tests were performed to evaluate the sensory stability of the samples during refrigerated storage: 1) an acceptance test using a 9-point hedonic scale for the consumption of cooked samples and 2) a preference-ranking test to assess the appearance of the samples while still in the packaging. Both of the tests were conducted in individual booths that were illuminated by white light, according to the methodology described by Meilgaard, Civille, and Carr (1991). For the acceptance tests, the samples were cooked in a similar manner to that described for the evaluation of WLC and were stored in an oven at 60 °C for a maximum of 30 min. A randomized complete block design was used, and the samples were served to the participants individually inside disposable plastic cups that were coded by three-digit numbers. The panelists assessed aroma, texture, juiciness, flavor and overall quality.

The visual preference-ranking test consisted of the simultaneous observation of samples from the three treatments, in their packaging, presented in a random order and with different codes. The panelists were asked to rank the samples in increasing order of preference with respect to their overall appearance.

3. Results and discussion

3.1. Analysis of headspace gas composition

The residual concentration of CO_2 and O_2 in the headspace of the packages was assessed for each of the three treatments at seven-day intervals. A constant increase in the percentage of CO_2 and a simultaneous reduction in the O_2 concentration were noted for the vacuum treatment (Fig. 1), an environment that enabled the development of anaerobic microorganisms. This behavior occurred likely due to the consumption of O_2 by muscle respiration, which is higher in the first days following the slaughter and which decreases during the storage period and also due to microbial metabolism, which increases during storage (McMullen & Stiles, 1991). A similar pattern was noted by Nishi (2008) when studying beef stored at temperatures of 0, 2, 4, 7 and 10 °C along 63 days. Conversely, Sørheim, Kropf, Hunt, Karwoski, and Warren (1996) observed that CO_2 levels in pork loins stored for 22 days decreased over time.

An increase in CO_2 and a progressive decrease in O_2 levels were also noted for the 75% O_2 + 25% CO_2 treatment (Fig. 2). A decrease in CO_2 levels would be expected throughout the storage period due to the high solubility of this gas in refrigerated meat. However, the observed increase in the CO_2 concentration can be explained by the reduction in the O_2 concentration due to its consumption by the muscle respiration and due to the growing of aerobic bacteria in the meat producing CO_2 (Jeremiah, 2001). Similar to the present study, Sørheim, Nissen, and Nesbakken (1999) examined different atmospheres (0.4% CO + 60% CO_2 + 40% N_2 with and without an O_2 absorbent, 70% O_2 + 30% CO_2 and vacuum) for the storage of pork meat. In these experiments, the meat was stored at 4 and 8 °C for up to 21 days. The authors reported that the O_2 concentration in the packages contained high O_2 concentrations that were decreased throughout the study period and reached levels between 60 and 65%.

For lamb meat packed under 100% CO_2, oxygen and carbon dioxide concentrations kept constant along the 28 days of storage time, with values close to zero and 95%, respectively.

3.2. Determination of the chemical composition of the samples

With respect to the chemical composition analyses for moisture, ash, lipids and proteins, the samples exhibited concentrations of 75.50 ± 0.54, 1.05 ± 0.03, 2.48 ± 0.42 and 21.20 ± 0.97%, respectively. These values are consistent with several reports in the literature (Hoffman, Muller, Cloete, & Schmidt, 2003; Jardim et al., 2007; Madruga et al., 2006) in which the reported moisture percentages ranged from 74 to 76%, those of ash ranged from 0.98 to 1.2%, those of lipids ranged from 2 to 4% and those of proteins ranged from 20 to 23%. It is worth noting that the samples used in the present study consisted exclusively of lean meat, without visible fat or connective tissue.

3.3. Chemical and physical stability

The results obtained from the descriptive statistical analysis for each variable and treatment (vacuum, 75% O_2 + 25% CO_2 and 100% CO_2) are shown in Table 1.

Regarding the objective color analyses, no significant difference was observed in the L* values between treatments ($P > 0.05$) or throughout the storage period ($P > 0.05$). No interactions were observed between the treatments and storage periods ($P > 0.05$). The vacuum, 75% O_2 + 25% CO_2 and 100% CO_2 treatments resulted in mean L* values of 36.95 ± 1.05, 38.47 ± 1.05 and 36.32 ± 1.05, respectively. The a* values significantly varied ($P < 0.05$) among treatments, and less redness was noted in the 100% CO_2 treatment (13.01 ± 0.54) than in the

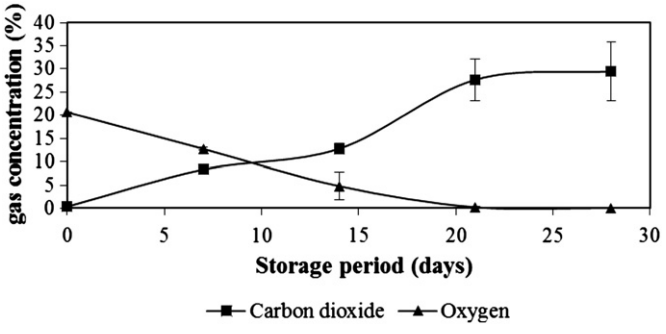

Fig. 1. O_2 and CO_2 concentrations inside the packages in the vacuum treatment group (n = 09 packages by storage period).

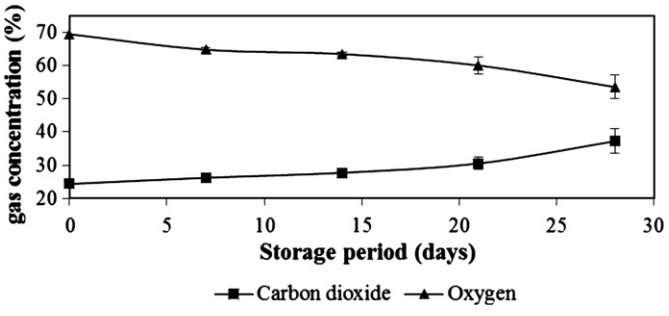

Fig. 2. O_2 and CO_2 concentrations inside the packages in the 75% O_2 + 25% CO_2 treatment group (n = 09 packages by storage period).

Table 1
Analysis of variance according to treatment during the storage of loin samples for 28 days at 1 °C.

Variable	Treatment		
	Vacuum	75% O_2 + 25% CO_2	100% CO_2
	Mean ± SE[1]	Mean ± SE	Mean ± SE
Tenderness (kg)	3.95 ± 0.39[a]	4.31 ± 0.39[a]	4.64 ± 0.39[a]
WLC[2] (%)	17.42 ± 0.78[a]	13.62 ± 0.78[b]	17.48 ± 0.78[a]
pH	5.77 ± 0.08[a]	5.95 ± 0.08[a]	5.86 ± 0.08[a]
TBARS[3]	0.33 ± 0.11[a]	0.45 ± 0.11[a]	0.32 ± 0.10[a]
L*	36.95 ± 1.05[a]	38.47 ± 1.05[a]	36.32 ± 1.05[a]
a*	15.35 ± 0.54[a]	16.47 ± 0.54[a]	13.01 ± 0.54[b]
b*	12.43 ± 0.58[b]	14.36 ± 0.58[a]	10.95 ± 0.58[b]

*a, b: Different letters in the same row indicate significant variations ($P < 0.05$) in the analyzed variable during the evaluated storage period.
n = 15 samples by treatment.
[1] Standard error.
[2] Water loss from cooking.
[3] Thiobarbituric acid reactive substances.

other two treatments. The overall mean a* values for the vacuum and 75% O_2 + 25% CO_2 treatments were 15.35 ± 0.54 and 16.47 ± 0.54, respectively, suggesting probable superficial discoloration (Jeremiah, 2001). There was also a significant variation in b* ($P > 0.05$) among treatments. Yellowness was greater in the 75% O_2 + 25% CO_2 group (14.36 ± 0.58) than in the other groups; the overall means in the b* values for the vacuum and the 100% CO_2 treatments were 12.43 ± 0.58 and 10.95 ± 0.58, respectively. The high degree of yellowness in the 75% O_2 + 25% CO_2 group was likely due to the high O_2 content and consequently higher oxymyoglobin content in this treatment. Unlike the results obtained in the present study, Vergara and Gallego (2001) noted that in lamb, the L* value of *Longissimus dorsi* muscles significantly increased over time in samples stored in either 20% CO_2 + 10% O_2 + 70% N_2 or 80% CO_2 + 20% O_2, with values ranging from 45.32 to 48.92. This observation was likely due to protein denaturation, which increased its dispersion of light (Insausti et al., 2001). Furthermore, a* values decreased significantly in these atmospheres, with values between 11.99 and 16.55. The reported b* values were greater than in oxygen-free packages, ranging from 7.72 to 11.85. The L* in the present study was similar to that observed by Luciano et al. (2009) when assessing the color stability of the *Semimembranosus* muscle of the same species stored in 80% O_2 + 20% CO_2 for 14 days at 4 °C. Furthermore, Berruga, Vergara, and Gallego (2005) noted that vacuum-packed samples exhibited greater color stability, noting that L* and b* values were higher in the treatment containing high O_2 levels, whereas the a* values were lower.

Myoglobin oxygenation occurs in approximately the upper 5 mm of the meat cut. The color at the surface depends on the chemical state of this pigment and the balance between the availability of surface O_2 and tissue respiration (Jose, Pethick, Jacob, & Gardner, 2009). High O_2 levels favor oxygenation of the meat pigment and thus prolong the color of the meat before metmyoglobin becomes visible on the surface (Mancini & Hunt, 2005). In the present study, the 100% CO_2 packaging system resulted in greater discoloration and a gray appearance (Fig. 6). The presence of low levels of residual O_2 (above 0.1%) is known to favor myoglobin oxidation, resulting in the formation of metmyoglobin and the consequent darkening of the meat during storage (Gill, 1996; Insausti et al., 2001; Jeremiah, 2001; O'Keeffe & Hood, 1980). CO_2 concentrations above 30% increase the degree of discoloration in red meat (Luño, Beltrán, & Roncalés, 1998).

No significant variation in pH was noted among treatments ($P > 0.05$) or throughout the storage period ($P > 0.05$), and no interaction ($P > 0.05$) was observed between the treatments and the storage periods. The mean pH values for the samples packed under vacuum, 75% CO_2 + 25% O_2 and 100% CO_2 throughout the storage period were 5.77 ± 0.08, 5.95 ± 0.08 and 5.86 ± 0.08, respectively. These values were slightly above the normal range (5.5 to 5.8) for lamb meat

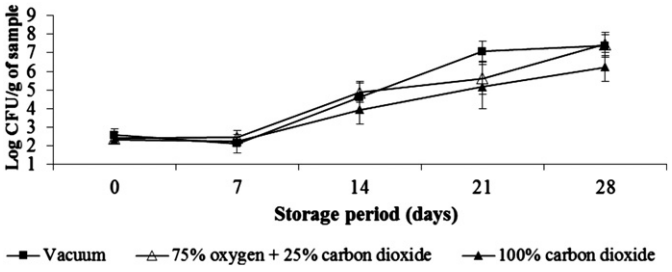

Fig. 3. Aerobic psychrotrophic microorganism counts throughout the refrigeration period (1 °C) (n = 03 samples by treatment–storage period combination).

reported by Sobrinho, Purchas, Kadim, and Yamamoto (2005). A decrease in pH values over time was expected due to the increasing growth of lactic acid bacteria (Cayré, Vignolo, & Garro, 2003) in all three treatments. Moreover, CO_2 dissolution in the muscle could have contributed to this reduction in pH in the two gas injection treatments given that CO_2 dissolution results in carbonic acid formation (Gill, 1988; Jakobsen & Bertelsen, 2004). The absence of an observed reduction in pH in any treatment was likely due to the depletion of glycogen reserves in the muscle at the beginning of storage (Lawrie & Ledward, 2006). However, lamb meat already has a slightly higher pH than other meats (e.g., beef), a factor that can favor more rapid microbial growth and result in a lower durability of the meat (Terlouw, 2005). The pH values obtained in the present study are also higher than those observed by Bórnez et al. (2010), who reported values between 5.57 and 5.62. However, in agreement with our results, the authors' study demonstrated no significant difference in pH values throughout the evaluated storage period. In contrast, Linares, Bórnez, and Vergara (2008) noted that lamb meat samples (*Longissimus dorsi* muscle) packed under different packaging conditions (A: 70% O_2 + 30% CO_2, B: 69.3% N_2 + 30% CO_2 + 0.7% CO or C: 60% N_2 + 40% CO_2) and stored at 2 °C for 21 days exhibited reduced pH values between 14 and 21 days for treatments A and B; however, there was no significant variation among treatments.

With respect to the lipid oxidation of refrigerated lamb loin, which was measured using the TBARS assay (milligrams malonaldehyde per kilogram of the sample), no significant variations were detected among treatments ($P > 0.05$), and there were no interactions ($P > 0.05$) between the analyzed treatments and storage periods. However, there was a significant effect ($P < 0.06$) throughout the 28-day storage period. Regression analyses indicated a significant quadratic behavior ($P < 0.06$). According to the derived equation, the maximum TBARS index occurred at 17.78 days of storage at 1 °C, with a value of 0.54 mg malonaldehyde/kg of sample. This was followed by a decrease in the TBARS index at the end of the evaluated storage period. It is worth noting that the TBARS values observed throughout the study period remained low, with an overall mean of 0.35 mg malonaldehyde/kg of sample. This likely occurred due to the samples' low percentages of lipids (approximately 2.48%) that contained high levels of saturated fatty acids, making the samples less susceptible to lipid oxidation (Ellis & Bertol, 2002).

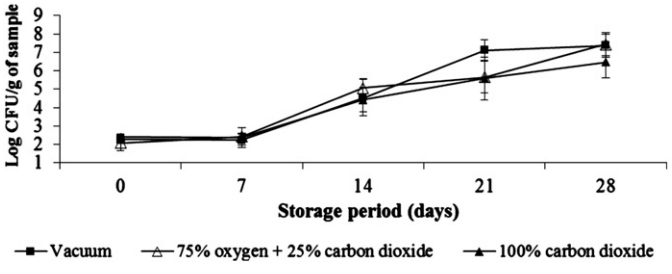

Fig. 4. Anaerobic psychrotrophic microorganism counts throughout the refrigeration period (1 °C) (n = 03 samples by treatment–storage period combination).

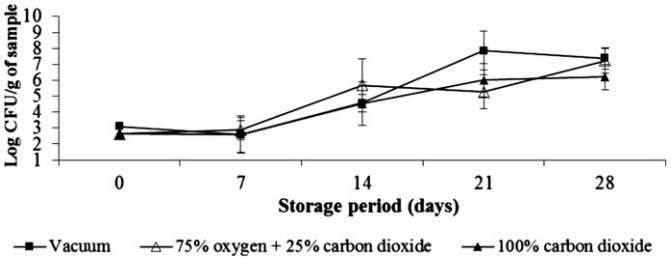

Fig. 5. Lactic acid bacteria counts throughout the refrigeration period (1 °C) (n = 03 samples by treatment–storage period combination).

The occurrence of lipid oxidation should be prevented by anaerobic conditions (Jeremiah, 2001), whereas a greater degree of lipid oxidation is observed in meat stored under high levels of O_2 (Jakobsen & Bertelsen, 2000). Given that high levels of O_2 have been shown to induce lipid oxidation (Raines & Hunt, 2010), a higher degree of oxidation was expected for the 75% O_2 + 25% CO_2 atmosphere treatment. However, this result was not observed.

According to the results obtained for WLC in the present study, a significant effect ($P < 0.05$) was only noted among treatments, with no significant differences over time ($P > 0.05$) or interactions ($P > 0.05$) between the treatments and storage periods. The packages containing 75% O_2 + 25% CO_2 resulted in lower mean WLC values (13.62 ± 0.78%) than did vacuum packaging (17.42 ± 0.78%) or the packages containing 100% CO_2 (17.47 ± 0.78%). In contrast, Vergara and Gallego (2001) observed a tendency toward greater WLC values for samples containing low CO_2 levels. Warner et al. (2005) also observed an increase in WLC over time, and vacuum-packed samples of lamb *Longissimus thoracis et lumborum* and *Gluteus medius* muscles stored under refrigeration exhibited greater losses on the fifth day than on the first day of storage.

There was no significant difference in tenderness ($P > 0.05$) of the lamb loins between the vacuum-packed, 75% O_2 + 25% CO_2 and 100% CO_2 treatments. Additionally, there were no differences ($P > 0.05$) in this metric throughout the storage period. Furthermore, no interaction ($P > 0.05$) was observed between the storage times and the treatment groups. The three treatments exhibited similar behavior throughout the study period, and there were no significant changes in shear force. A reduction in shear force would be expected due to the maturation of the meat during refrigerated storage; however, this was not detected. A similar pattern was reported by Vergara and Gallego (2001), who observed no significant differences in shear force over 16 days of storage at 2 °C for lamb *Longissimus dorsi* muscles packed in different modified atmospheres (A: 20% CO_2 + 10% O_2 + 70% N_2, B: 40% CO_2 + 60% N_2, C: 80% CO_2 + 20% O_2 and D: 80% CO_2 + 20% N_2). In contrast, Bórnez et al. (2010) assessed lamb *Longissimus dorsi* muscles after 7, 14 and 21 days of storage at 2 °C in two types of atmospheres (A: 70% O_2 + 30% CO_2 and B: 69.3% N_2 + 30% CO_2 + 0.7% CO). They reported that the O_2 and CO_2 atmosphere resulted in a significant decrease in shear force throughout the evaluated storage period (from 54.1 to 36.1 N/cm^2).

3.4. Microbiological stability

No coagulase-positive staphylococci microorganisms (<10 CFU/g of sample) or *Salmonella* were detected throughout the study period in any of the treatments. Only a sporadic count of thermotolerant coliforms (5.02 × 10^3 CFU/g of sample) was detected at 21 days of storage for the vacuum treatment, which was likely due to possible contamination during sample manipulation (deboning at the slaughterhouse).

Therefore, lamb loin meat packaged under vacuum or with the gas compositions used in the present study and stored at 1 °C for 28 days remained within the acceptable limits established by Brazilian legislation (BRAZIL, 2001). The maximum acceptable counts for vacuum-packed meat, not matured, are 10^4 for thermotolerant coliforms, 3 × 10^3 for coagulase-positive staphylococci and zero *Salmonella* in a 25 g sample.

The obtained counts of aerobic and anaerobic psychrotrophic microorganisms and lactic acid bacteria are presented in Figs. 3, 4 and 5.

The results obtained revealed that the counts of aerobic and anaerobic psychrotrophic microorganisms and lactic acid bacteria remained stable from the beginning to the seventh day of storage for all the three packaging systems. A more rapid growth was noted for these groups from seventh to 14th day of storage. A similar pattern was observed for all treatments. After 21 days of storage at 1 °C, there was less microbial growth in the treatments submitted to 100% CO_2 or 75% O_2/25% CO_2 than in vacuum-packed samples, demonstrating the effective action of CO_2 on the microbiological stability in lamb meat. This information is consistent with the literature, where concentrations of both CO_2 and O_2 equal to or greater than 10% can inhibit aerobic and anaerobic bacterial growth, respectively (Livingston, Brewer, Killifer, Bidner, & McKeith, 2004). Gill (1996) states that a 50% inhibition of psychrotrophic microorganism growth can be achieved in systems containing atmospheres with 20% CO_2.

After 28 days of refrigerated storage, the lamb loin samples submitted to 100% CO_2 exhibited lower microorganism counts on the order of one logarithmic cycle (between 10^6 and 10^7 CFU/g of sample) than the other two treatments (between 10^7 and 10^8 CFU/g of sample). Therefore, the most effective MA treatment for reducing microbial growth was that composed of pure CO_2 due to its bacteriostatic action (Gill & Harrison, 1989). Mano, Pereda, and Fernando (2002) also confirmed the effectiveness of high CO_2 levels in inhibiting microbial growth in pork meat during refrigerated storage. Similar results were reported by Kennedy, Buckley, and Kerry (2005), who microbiologically evaluated cuts from lamb *Longissimus dorsi* muscles stored at 4 °C for eight days under different gas compositions (A: 100% CO_2 + 0% N_2, B: 90% CO_2 + 10% N_2 and C: 80% CO_2 + 20% N_2).

3.5. Sensory stability

The sensory evaluations of the three different MAP systems were only performed up to 21 days of storage due to excessive microbial deterioration, with visible colonies and slime, in the vacuum and 75% O_2 + 25% CO_2 treatments at 28 days of storage. The 100% CO_2

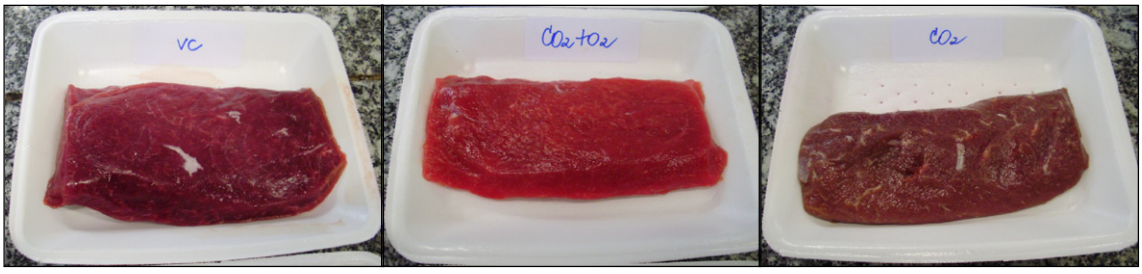

Fig. 6. From left to right, meat samples packed under the vacuum, 75% O_2 + 25% CO_2 and 100% CO_2 conditions, respectively.

Table 2
Analysis of variance according to treatment throughout the 21-day storage of loin samples at 1 °C, after cooking of the samples.

Variable	Treatment		
	Vacuum	75% O_2 + 25% CO_2	100% CO_2
	Mean ± SE[1]	Mean ± SE	Mean ± SE
Aroma	5.65 ± 0.13[b]	5.92 ± 0.13[a]	5.75 ± 0.13[ab]
Juiciness	7.16 ± 0.11[a]	6.91 ± 0.11[b]	7.21 ± 0.11[a]

*a, b: Different letters in the same row indicate significant variations ($P < 0.05$) in the analyzed variable throughout the evaluated storage period.
n = 200 consumers by treatment.
[1] Standard error.

treatment remained suitable for consumption even at 28 days, and this group was subjected, in isolation, to sensory evaluations at this time.

3.5.1. Acceptance test

For the three different MAP systems under evaluation, the mean values given by the panel for all of the attributes were generally close to 7 ("liked moderately"), except for aroma, for which the average was 5.77 (near to "liked slightly"). This lower acceptance in terms of aroma was likely due to the lack of seasoning on the meat. No seasoning was added to avoid the masking of potential differences between the different experimental groups.

The aroma and juiciness attributes only varied ($P < 0.05$) among treatments (Table 2). With respect to aroma, the vacuum-packed samples were less acceptable than those packed with 75% O_2 + 25% CO_2. A lower acceptance of juiciness was noted for the 75% O_2 + 25% CO_2 treatment. There was no decrease ($P > 0.05$) in the acceptance of these attributes (aroma and juiciness) when comparing the samples throughout the 21 days of storage, demonstrating that untrained consumers were unable to perceive changes in these attributes over the storage period. Similar to the results obtained in the present study, Clausen, Jakobsen, Ertbierg, and Madsen (2009), noted reduced juiciness in the samples packed with high O_2 levels (80% O_2 + 20% N_2) when conducting sensorial evaluations of beef samples (*Longissimus dorsi* muscle) stored at 2 °C for 23 days.

With respect to the texture, flavor and overall quality of the samples, a linear decrease was noted ($P < 0.05$) in the acceptance of the 75% O_2 + 25% CO_2 group throughout the 21 days of storage. The remaining two treatments remained stable with respect to these metrics and exhibited good acceptance throughout the storage period (Figs. 7, 8 and 9). These results were likely obtained because high O_2 levels influence texture. This effect is due to the possible formation of protein complexes in a high O_2 environment and the consequent loss of enzymatic function, negatively affecting tenderness (Zakrys, Hogan, O'Sullivan, Allen, & Kerry, 2008). The samples that were packed with 100% CO_2 were expected to exhibit greater acceptance throughout refrigerated storage due to increased microbial inhibition.

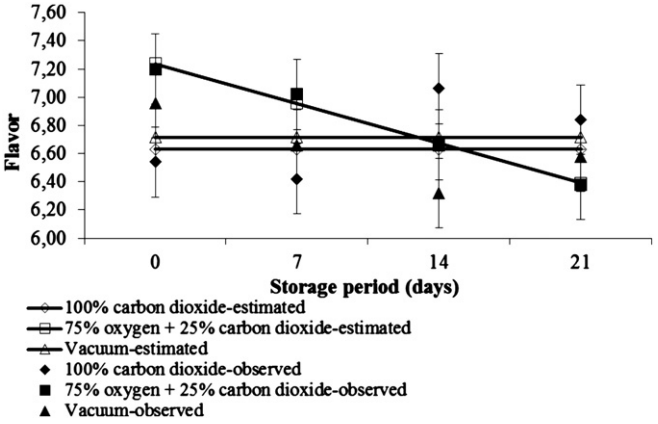

Fig. 8. Sensory acceptance with respect to flavor for each treatment throughout the storage period (n = 50 consumers by treatment–storage period combination).

Conversely, when conducting sensory evaluations of vacuum-packed samples of lamb *Longissimus lumborum* muscles stored for 1, 2, 4, 8 and 16 days at 3 ± 1 °C using quantitative descriptive analysis, Martínez-Cerezo, Sañudo, Medel, and Olleta (2005) demonstrated a progressive increase in the tenderness and juiciness for all breeds and slaughter weights under assessment, suggesting good acceptance. Zakrys, O'Sullivan, Allen, and Kerry (2009), however, observed lower acceptance of texture and juiciness for samples packed with O_2 concentrations of 50% or more when assessing steer *Longissimus dorsi* muscles stored for 12 days at 4 °C at O_2 concentrations ranging from 40 to 80% in the presence of CO_2. This result was likely due to decreased myofibrillar fragmentation or proteolysis, confirming the results obtained in the present study.

3.5.2. Preference ranking test

The results obtained from the preference ranking tests are presented in Table 3. The consumer attitude toward the appearance of the loins (packaged meat) was the same for all four storage time intervals under assessment. The vacuum-packed samples and those stored in an atmosphere containing 75% O_2 + 25% CO_2 were preferred over samples subjected to the injection of 100% CO_2, as presented in Table 3. The lower sensory acceptance of the 100% CO_2 treatment may be associated with the low a* intensity observed throughout the study period for this group. Low a* values indicate a worsening appearance because this metric measures the redness of meat. The a* value is,

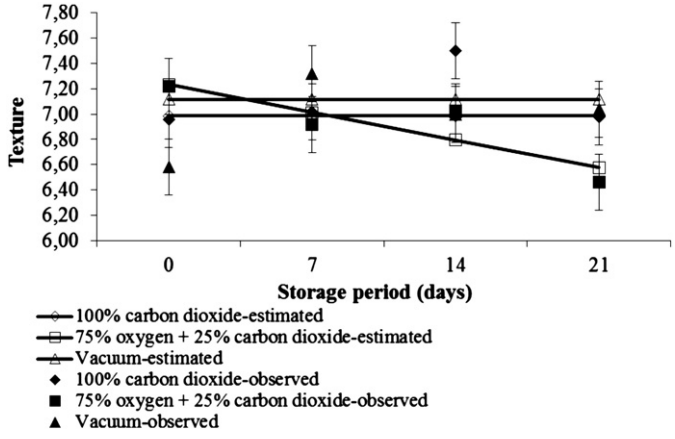

Fig. 7. Sensory acceptance with respect to texture for each treatment throughout the storage period (n = 50 consumers by treatment–storage period combination).

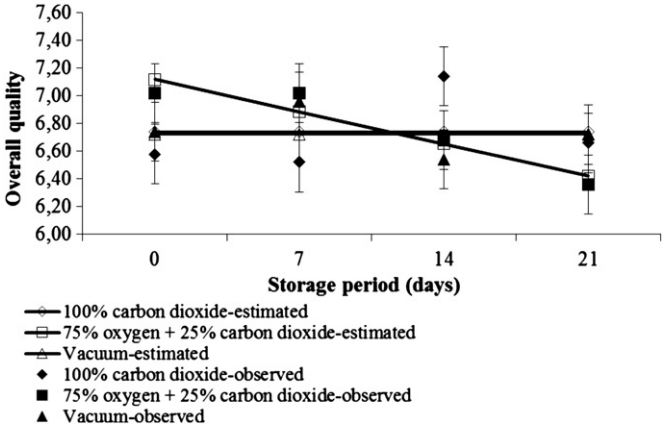

Fig. 9. Sensory acceptance with respect to the overall quality for each treatment throughout the storage period (n = 50 consumers by treatment–storage period combination).

Table 3
Results of the rank sums test using Newell and MacFarlane's table.

Treatment	Storage period (days)			
	0	7	14	21
Vacuum	121[a]	120[a]	124[a]	128[a]
75% O_2 + 25% CO_2	120[a]	124[a]	121[a]	107[a]
100% CO_2	59[b]	56[b]	55[b]	65[b]

a, b: Different letters in the same row indicate a significant variation ($P < 0.05$) throughout the evaluated storage period.
n = 50 consumers by treatment-storage period combination.

therefore, a reasonable indicator of acceptance (Behrends, Mikel, Armstrong, & Newman, 2003). Alternatively, the food service industry can benefit from the extension of shelf life provided by packaging with 100% CO_2. This is because food safety is more relevant to this market than the color of packed meat, and the end consumer will only observe the meat after it has been prepared.

This result demonstrates that the majority of consumers generally assess the appearance of the muscle prior to purchase, primarily associating the meat's color with the degree of freshness (Jeremiah, 2001; Liu et al., 2003). Carpenter, Cornforth, and Whittier (2001) evaluated consumer preferences with respect to beef color and packaging type and concluded that, despite the advanced technology that is now available, acceptance is better when the bright red color is maintained and when the meat is packed in PVC wrapping. This is true even if the flavor does not match the initial perception of the fresh product during consumption. It is important to mention that the color should remain stable for at least 21 days, the period that determines the ideal packaging system for fresh meat (Jayasingh, Cornforth, Carpenter, & Whittier, 2001).

Scholtz, Jordaan, Kruger, Nortje, and Naude (1992) conducted sensory analyses of pork meat samples (*Longissimus thoracis*) packed under different conditions (A: 25% CO_2 and 75% O_2, B: vacuum and C: 100% CO_2) with respect to color, odor and acceptability. This group found that trained panelists classified vacuum-packed meat as being the darkest, as having an unpleasant odor and as being less acceptable than the meats subjected to the other treatments. In this previous study, the samples stored in packages containing 100% CO_2 were determined to have a more pleasant odor and a lighter color and to be more acceptable following a 21-day storage period at 0 °C when compared to other packaging systems. Also in this previous study, the samples remained closed for the color and acceptability analyses, whereas odor was assessed immediately after opening the packaging systems.

3.5.3. Sensory stability of the 100% CO_2 treatment

Given the positive results obtained with respect to the analyzed microbiological metrics (i.e., the absence of pathogens) and sensory stability up to 21 days for the 100% CO_2 treatment, only this group was subjected to sensory evaluation at 28 days.

According to the average scores given by the panel, the samples did not suffer significant changes ($P < 0.05$) with respect to the examined attributes at any point during the storage period, demonstrating the stability of meat stored in a packaging system containing 100% CO_2 for up to 28 days at 1 °C (Table 4).

Atmospheres containing CO_2 effectively increase the storage period of meat products by reducing microbial growth (O'Keeffe & Hood, 1980). Given this fact and in agreement with the present study, Scholtz et al. (1992) examined the shelf life of pork *Longissimus thoracis* muscles stored for 21 days at 0 °C in different packaging systems (A: 25% CO_2 and 75% O_2, B: vacuum and C: 100% CO_2). This group reported that treatment C was rated the highest in terms of odor, and the shelf life was longer than for the other systems.

4. Conclusions

According to the results of the microbiological, physical and chemical evaluations, as well as the sensory acceptance of cooked meat, we conclude that the samples stored in packages containing 100% CO_2 for 28 days at 1 °C have greater stability and better shelf life than vacuum-packed samples and those packaged in a 75% O_2 + 25% CO_2 atmosphere for 21 days. However, the 100% CO_2 treatment was the least preferred in terms of the appearance of the raw meat while still packed when compared to the other MAP systems under evaluation. Therefore, despite presenting greater stability, further studies should be performed on the packaging of lamb meat at high concentrations of CO_2. Other gases in combination with CO_2 may enable the maintenance of a more acceptable color of the packaged meat, especially when it is intended for retail sales.

Acknowledgements

The authors would like to thank the São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo [FAPESP]) for financial support (Process nº. 2009/15555-2), Cryovac for donating the plastic films, LINPAC for donating the expanded polystyrene trays and the Brazilian Federal Agency for the Improvement of Higher Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior [CAPES]) for granting scholarships.

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Table 4
Analysis of variance with respect to 100% CO_2 treatment during the 28-day storage of loin samples at 1 °C, after cooking of the samples.

Variable	Storage period (days)				
	0	7	14	21	28
	Mean ± SE[1]	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Aroma	5.78 ± 0.25[a]	5.80 ± 0.25[a]	5.90 ± 0.25[a]	5.52 ± 0.25[a]	5.32 ± 0.25[a]
Texture	6.96 ± 0.21[a]	7.02 ± 0.21[a]	7.50 ± 0.21[a]	6.98 ± 0.21[a]	6.68 ± 0.21[a]
Juiciness	7.28 ± 0.20[a]	7.00 ± 0.20[a]	7.52 ± 0.20[a]	7.04 ± 0.20[a]	6.92 ± 0.20[a]
Flavor	6.54 ± 0.24[a]	6.42 ± 0.24[a]	7.06 ± 0.24[a]	6.84 ± 0.24[a]	6.62 ± 0.24[a]
Overall quality	6.58 ± 0.21[a]	6.52 ± 0.21[a]	7.14 ± 0.21[a]	6.66 ± 0.21[a]	6.74 ± 0.21[a]

*a, b: Different letters in the same row indicate significant variations ($P < 0.05$) in the analyzed variable throughout the evaluated storage period.
n = 50 consumers by storage period (days).
[1] Standard error.

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