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Influence of packaging conditions on microbial and lipid oxidation in lamb meat

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Abstract

The effect of vacuum and three different modified atmospheres (MA) (A: 40% CO_2/60% N_2; B: 80% CO_2/20% O_2 and C: 80% CO_2/20% N_2) on rancidity, colour as CIE L^* (lightness), a^* (redness) and b^* (yellowness) values, sensorial colour, sensorial odour and microbial content was studied in lamb meat. *Longissimus dorsi* samples were examined at 7-day intervals during storage at 2 °C. Rancidity increased with storage time in all four groups, but was more pronounced in B (with O_2), and less in vacuum. Vacuum packaging conditions maintained a relatively optimum colour stability, while group B showed the highest L^* and b^*, and the lowest a^* values. Sensory evaluation showed colour deterioration during storage in all groups, but was more marked in C. In MA treatments, lipid oxidation was significantly negative and positively correlated with a^* and b^* values, respectively. Unacceptable off-odours were first detected by panellists in B packs. Microbial growth was faster in vacuum packs, but after 28 days was similar in all treatments. *Brochothrix thermosphacta* was the predominant microbial group in MA, and lactic acid bacteria in vacuum.

Keywords: Lamb meat; Lipid oxidation; Microbiology; Modified atmosphere; Vacuum

1. Introduction

Modified atmospheres (MAs) are commonly used for preserving fresh meat (Jeremiah, 2001). Consumers often select packed meat for its bright red colour that suggests freshness and superior quality at the time of purchase, but they cannot assess other characteristics in unopened packs, such as odour, rancidity degree or microbiological quality. Many of these factors are essential for extending meat shelf-life.

It has been reported that optimum colour stability in red meats is obtained by using gas mixtures containing elevated concentrations of oxygen joined with low proportions of carbon dioxide (Jeremiah, 2001). However, lamb meat packed under high proportions of carbon dioxide, with low or no oxygen, maintains suitable colour and odour (Vergara and Gallego, 2001). Gas mixtures enriched in carbon dioxide could

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be considered as an alternative for packing lamb meat.

On the other hand, modified atmosphere packaging at refrigeration temperatures has been widely shown to delay the growth of spoilage aerobic bacteria and considerably prolong the shelf-life of red meats (Jeremiah, 2001). The use of carbon dioxide as a bacteriostatic increases this shelf-life (Jeremiah, 2001; Jayas and Jeyamkondan, 2002). Several studies have also shown that meat preservation under vacuum is a good and commonly used method (Jeremiah, 2001; Jayas and Jeyamkondan, 2002).

In general, studies on rancidity have been carried out in processed meats or antioxidant supplements into feed (Gatellier et al., 2001; Smiddy et al., 2002); although information about the effect of extended periods of vacuum or MAs on the lipid oxidation of raw lamb meat is sparse (Kerry et al., 2000). Rancidity causes meat quality deterioration during refrigerated or frozen storage (Cifuni et al., 2001). Lipid composition constitutes the major determinant for susceptibility to oxidative changes and rancidity development leading to warmed-over flavours (Jeremiah, 2001), and the presence of oxygen in packages could enhance lipid oxidation of meat (Smiddy et al., 2002), an effect that may be favoured by fatty acid composition of lamb meat (Cifuni et al., 2001). It is also postulated that interrelations exist between oxidation of lipids and oxymyoglobin in muscle tissues (Yin and Fautsman, 1993; O'Grady et al., 2001), which could have some influence on colour evolution.

A previous work (Vergara and Gallego, 2001) showed that some enriched carbon dioxide atmospheres could be appropriate to extend the shelf-life of fresh lamb meat. The aim of this study is to complement this preceding work by providing more data on the shelf-life of lamb meat with regards to rancidity, colour, sensory quality and microbial development, as well as the relationships among them. Another objective is to compare these MAs with the vacuum packaging, which is a commonly used technique for meat storage.

2. Material and methods

2.1. Animals

Twenty-four lambs of Manchega breed from the Castilla-La Mancha University Experimental Farm at Albacete (Spain) were slaughtered at an average weight of 25 kg in a commercial abattoir using standard commercial procedures. After slaughter, the carcasses were chilled at 4 °C for 24 h in a conventional chiller.

2.2. Sample preparation and experimental design

Twenty-four hour post-mortem, the *Longissimus dorsi* muscle (a total of 24 samples) was removed from both sides of each carcass and cut into 17 portions of similar size for each lamb. One portion was assigned to initial measurements (day 0). The 16 remaining portions were assigned to each treatment and packaged individually for assessment at 7-day intervals. Samples were chilled at 2 °C for up to 29 days post-slaughter (i.e. 7, 14, 21 and 28 post-packing).

Vacuum packaging was carried out with a vacuum packaging machine (Model Swissvac 380, Berkshire, UK), using Cryovac barrier bags with an oxygen transmission rate of 30 $cm^{-3}\,m^{-2}\,day^{-1}\,atm^{-1}$ at 23 °C.

A thermoforming atmosphere packaging machine (Model Tiropac 1000, Contel, Brescia, Italy) was used to obtain the three gas atmospheres as in Vergara and Gallego (2001), these being:

- atmosphere A: 40% CO_2/60% N_2;
- atmosphere B: 80% CO_2/20% O_2;
- atmosphere C: 80% CO_2/20% N_2.

The samples were packaged in clear semi-rigid trays (Ecoform V 600 W, having an oxygen permeability (OP) rate of 0.4 $cm^3\,m^{-2}\,day^{-1}$ at 1 atm, and 20 °C and a cover film with an OP of 30–40 $cm^3\,m^{-2}\,day^{-1}$ at 1 atm and 20 °C) with the corresponding gas mixture.

Twenty-four samples were examined per atmosphere type (A–C) and vacuum and per sampling time to determine instrumental and sensorial characteristics.

2.3. Microbiological analysis

Loin samples for microbiological examination were randomly selected from eight carcasses for each storage treatment at each storage interval. Samples were aseptically collected from the packages with a sterile aluminium template (10 cm^2) and by removing by excision a 2–3 mm thick layer with a scalpel. Each sample was homogenised in a Seward Medical Stomacher 80 (London, UK) with a 100 ml of 0.1% peptone water (w/v) for 60 s. Additional serial 10-fold dilutions of

homogenates were made in peptone water and appropriate dilutions were plated in the following manner: total viable counts (TVC), on plate count agar (PCA) (Merck, 1.05463) at 32 °C for 48 h; lactic acid bacteria (LAB) were enumerated using the medium of Man, Rogosa and Sharpe (MRS) agar (Merck, 1.10660), pH 5.7, and incubated at 32 °C for 48 h; *Brochothrix thermosphacta*, on streptomycin thallous acetate actidione agar (STAA) (Oxoid, CM 881, SR 151) incubated at 25 °C for 72 h; and Enterobacteriaceae, using pour plates of Violet Red Bile Dextrose agar (VRBD) (Merck, 1.10275) and incubated at 32 °C for 24 h. All microbial counts were expressed as base-10 logarithms of colony forming units per cm^2 of surface area (log CFU cm^{-2}).

2.4. Instrumental analysis

Colour as L^* (lightness), a^* (redness) and b^* (yellowness) values were determined using a Minolta CR 200 colorimeter (Osaka, Japan) calibrated against a standard white tile. Measurements were taken in duplicate directly on the meat surface as in Vergara and Gallego (2001).

Sample rancidity was determined in duplicate by the thiobarbituric acid assay (TBA) as described by Botsoglou et al. (1994). Absorbencies were measured with a spectrophotometer Perkin Elmer Lambda 20 (Norkwalk, USA) at 532 nm. Results were expressed as mg malondialdehyde kg^{-1} meat.

2.5. Sensory evaluation

Immediately after opening packages, each sample which underwent microbiological and instrumental analysis was also assessed for off-odours by a group of five experts from the laboratory. Colour was assessed 15 min after opening. Odour evaluation was performed as in Vergara and Gallego (2001), where samples were categorised as: 1 = not acceptable (strong off-odour); 2 = acceptable (slight off-odour) or 3 = very acceptable (no off-odour). Colour was scored on a five point hedonic scale where 1 corresponds to bad colour, 2 = poor colour but customer would buy if cheaper, 3 = good colour, 4 = very good colour, and 5 = excellent colour. Samples with two or more points were considered acceptable for colour.

2.6. Data analysis

The data obtained from meat cuts under modified atmospheres and vacuum were analysed using analysis of variance. When the differences among types of modified atmosphere were significant ($P < 0.05$), Tukey's test was carried out to determine the differences between pairs of groups. Pearson correlation coefficients between rancidity and meat colour (L^*, a^* and b^*) were estimated to assess the influence of lipid oxidation on these meat parameters. Statistical analyses were performed using SPSS 10.0.6 Statistical Software (1999).

3. Results and discussion

3.1. Rancidity

None of the four treatments assayed were sufficient to avoid rancidity (Fig. 1), since lipid oxidation increased with storage time in all groups ($P < 0.05$). There were significant differences among groups at all storage times ($P < 0.001$): atmosphere B (with O_2) showed the highest rancidity rate ($P < 0.05$) and vacuum the lowest ($P < 0.05$). Groups A and C also showed higher rancidity rates than vacuum (Fig. 1). Previously, Kerry et al. (2000) have reported that vacuum is very effective in controlling lipid oxidation of lamb patties. Our re-

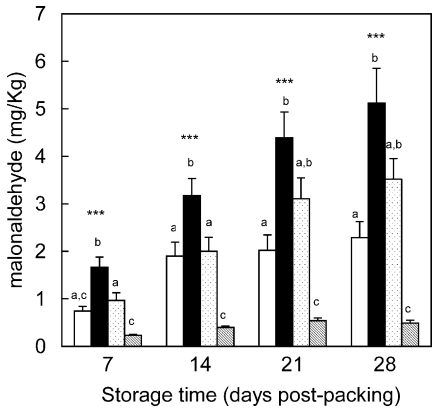

Fig. 1. Rancidity levels (TBA test; mg malondialdehyde kg^{-1} meat) in lamb loin cuts stored at 2 °C in A: 40% CO_2/60% N_2 (white bars); B: 80% CO_2/20% O_2 (black bars); C: 80% CO_2/20% N_2 (pointed bars); or under vacuum packs (cross bars). [$n = 24$; a, b, c: values in the same storage day with different letters are significantly different ($P < 0.05$)].

sults showed that the presence of oxygen favours lipid oxidation, in agreement with other authors (O'Grady et al., 2000; Insausti et al., 2001; Jeremiah, 2001). However, groups A and C gas mixtures and vacuum also seemed to be insufficient to limit rancidity. Low levels of residual oxygen could explain lipid oxidation reactions. Residual oxygen was not measured in the packs of this study, but according to other studies (Smith et al., 1983; Rousset and Renerre, 1990; Doherty et al., 1996; Smiddy et al., 2002), vacuum or no-oxygen MA (as in A or C) do not always completely remove oxygen, since oxygen could remain within 0–2%, which could be enough to cause lipid oxidation (Smiddy et al., 2002). The higher malondialdehyde levels in groups A and C than in vacuum may consequently be due to greater levels of residual oxygen, in agreement with Smiddy et al. (2002).

Products of lipid oxidation have been associated with off-flavours and off-odours (Insausti et al., 2001; Jeremiah, 2001). Starting from the third week of storage, samples packaged in atmosphere B showed TBA values equal to or greater than 5 mg malondialdehyde kg^{-1} meat reported by Insausti et al. (2001) as a detectable concentration for humans (Fig. 1). These levels coincided with slight off-odours detected by the sensory panellists at the final storage times (Table 3).

3.2. Instrumental colour and relationship with rancidity

Initial L^*, a^* and b^* mean values of samples (24 h post-slaughter) were 47.15 ± 0.33, 15.57 ± 0.17 and 6.82 ± 0.13, respectively. These results agree with Vergara and Gallego (2001) in the same breed. Table 1 shows L^*, a^* and b^* mean values during storage of samples under the four treatments. Group A and vacuum showed the lowest L^* values from the second week onward, which were significantly different in comparison with group B ($P < 0.01$). In all times analysed, a^* and b^* values were significantly different between treatments ($P < 0.001$). Redness (a^*) decreased significantly in groups A–C ($P < 0.05$), and this reduction was more pronounced in treatment B. This parameter was more stable in vacuum. The increase in b^* values was significant ($P < 0.05$) in groups B and C (with higher CO_2) and b^* values were higher in group B for all the times, as observed in Vergara and Gallego (2001).

The decreases in a^* and the increases in b^* values have frequently been associated with the gradual for-

Table 1
Effect of packaging on meat colour (coordinates L^*, a^*, b^*) of Manchego lamb

Coordinate	Modified atmosphere[a]					
	Storage time (days)[b]	Type A (n = 24)	Type B (n = 24)	Type C (n = 24)	Vacuum (n = 24)	ANOVA
L^*	7	49.9 ± 0.69 y	50.34 ± 0.67 y	49.55 ± 0.57 y	47.96 ± 0.64 xy	NS[c]
	14	49.56 ± 0.67 ab, xy	51.15 ± 0.61 a, yz	50.81 ± 0.59 a, y	48.10 ± 0.49 b, xy	**
	21	49.25 ± 0.64 a, xy	52.91 ± 0.57 b, zk	50.43 ± 0.59 a, y	49.30 ± 0.43 a, xy	***
	28	49.09 ± 0.65 a, xy	53.77 ± 0.75 b, k	50.61 ± 0.45 a, y	49.88 ± 0.59 a, y	***
a^*	7	14.85 ± 0.48 ab, x	15.16 ± 0.32 a, xy	13.45 ± 0.37 b, y	17.57 ± 0.35 c, x	***
	14	12.34 ± 0.57 a, y	12.98 ± 0.72 a, y	11.65 ± 0.55 a, z	18.01 ± 0.38 b, x	***
	21	12.20 ± 0.70 a, y	8.95 ± 0.74 b, z	10.18 ± 0.51 ab, z	17.25 ± 0.39 c, x	***
	28	12.29 ± 0.84 a, y	7.73 ± 0.60 b, z	10.03 ± 0.65 ab, z	16.78 ± 0.36 c, xy	***
b^*	7	9.08 ± 0.37 a, y	11.58 ± 0.37 b, y	9.78 ± 0.43 a, y	6.57 ± 0.24 c, x	***
	14	10.61 ± 0.50 a, y	12.72 ± 0.38 b, y	11.45 ± 0.42 ab, z	6.94 ± 0.33 c, xy	***
	21	10.63 ± 0.58 a, y	14.17 ± 0.38 b, z	11.97 ± 0.57 a, zk	6.95 ± 0.21 c, xy	***
	28	10.47 ± 0.62 a, y	15.18 ± 0.51 b, z	13.46 ± 0.59 b, k	7.66 ± 0.23 c, y	***

Values in the same row with different letters (a, b, c) are significantly different ($P < 0.05$); values in the same column with different letters (x, y, z, k) are significantly different ($P < 0.05$).

[a] Type A corresponds to a gas mixture of 40% CO_2/60% N_2; type B: 80% CO_2/20% O_2; type C: 80% CO_2/20% N_2.

[b] Days post-packing.

[c] Not significant.

** Significance levels at 0.01.

*** Significance levels at 0.001.

mation of metmyoglobin and consequently with a meat discolouration result (Insausti et al., 2001; Jeremiah, 2001). According to this fact, the evolution of L^*, a^* and b^* values suggests that vacuum, followed by atmosphere A, maintained a more stable colour than groups B and C. Smith et al. (1983) studied the evolution of colour in fresh lamb meat packed under MA and vacuum, and found a more desirable appearance of lamb meat packed in vacuum than in MA. The development of metmyoglobin is associated with the occurrence of residual oxygen, which produces colour deterioration (Rousset and Renerre, 1990; Penny and Bell, 1993; Jeremiah, 2001). Very low oxygen concentrations in packs (≥0.15%) have been reported to be responsible for the discolouration of fresh beef and lamb meat (Rousset and Renerre, 1990; Penny and Bell, 1993). Although the residual oxygen composition has not been measured, the colour results suggest the presence of residual oxygen. This fact could be avoided by using any O_2-scavenger protection to maintain better colour stability, as recommended by Rousset and Renerre (1990).

Overall, significant negative correlations (Table 2) were observed between TBA and a^* values on MAs during storage. TBA values were also correlated with b^* values, but positively (Table 2). In addition, the highest b^* values found in B treatments (compared with A and C) corresponded to the highest TBA values (Table 1 and Fig. 1). The relation between rancidity and changes in colour have been described previously in beef by others (O'Grady et al., 2000; Gatellier et al., 2001; Insausti et al., 2001) in atmospheres containing oxygen. Several researchers have suggested that lipid oxidation promotes oxymyoglobin oxidation (Yin and Fautsman, 1993; Gatellier et al., 2001; O'Grady et al., 2001), while others (Baron et al., 2002) indicated that myoglobins are able to initiate lipid oxidation.

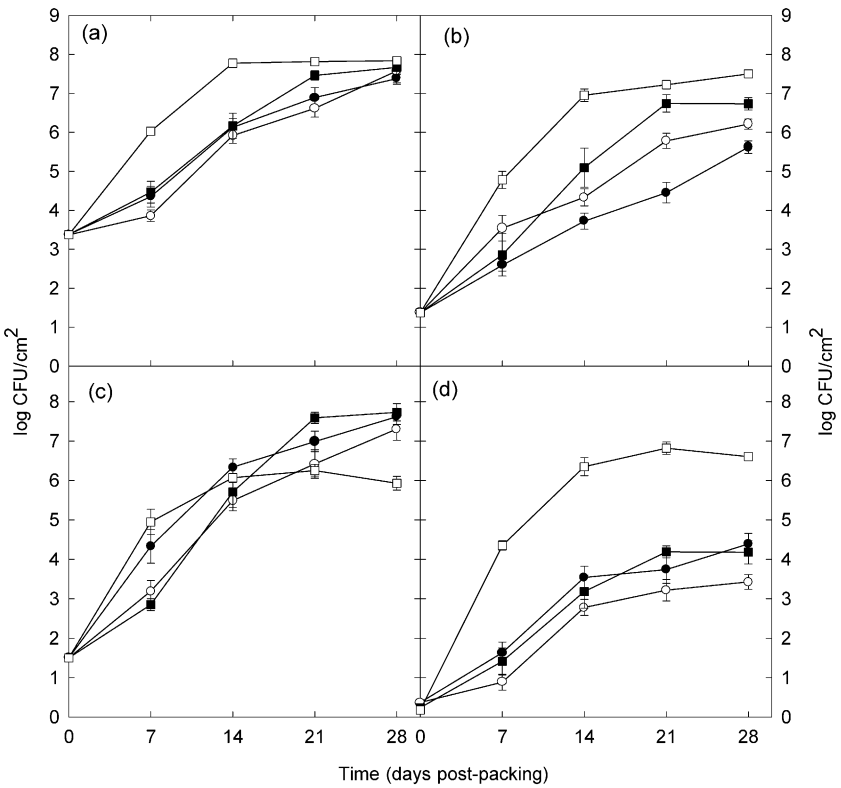

Fig. 2. Growth (mean values and standard error, $n = 8$) of total viable counts (a), lactic acid bacteria (b), *B. thermosphacta* (c) and Enterobacteriaceae (d) counts for lamb loin cuts stored at 2 °C in A: 40% CO_2/60% N_2 (●), B: 80% CO_2/20% O_2 (○), C: 80% CO_2/20% N_2 (■), or under vacuum packs (□).

Table 2
Correlation coefficients between TBA values (thiobarbituric acid) and meat colour (L^*, a^*, b^*) during storage

	Time (days)[a]	Colour		
		L^*	a^*	b^*
Type A	7	0.09	−0.02	0.25
	14	−0.06	−0.74**	0.67**
	21	0.09	−0.68**	0.83**
	28	0.11	−0.72**	0.85**
Type B	7	−0.02	0.09	0.56**
	14	0.11	−0.63**	0.26
	21	0.25	−0.54**	0.60**
	28	0.10	−0.35	0.43*
Type C	7	0.18	−0.57**	0.61**
	14	−0.12	−0.51*	0.68**
	21	0.07	−0.66**	0.63**
	28	−0.17	−0.38	0.68**
Vacuum	7	−0.12	0.14	0.18
	14	−0.18	−0.38	−0.38
	21	0.01	−0.06	0.12
	28	−0.24	−0.38	0.06

Type A corresponds to gas mixture of 40% CO_2/60% N_2; type B: 80% CO_2/20% O_2; type C: 80% CO_2/20% N_2; and type V: vacuum.
[a] Days post-packing.
* $P < 0.05$.
** $P < 0.01$.

3.3. Microbial growth

Fig. 2 shows bacterial growth during storage. Initial microbial levels (3.3 log CFU/cm^2) were inferior to the ICMSF (International Commission for Microbial Specifications in Food) recommended microbiological limits for fresh meat (ICMSF, 1986). In general, growth in vacuum package was significantly greater for all the bacteria groups studied, except *B. thermosphacta*. Larger bacterial numbers were also observed by Sheridan et al. (1997) in vacuum packed lamb at 0 °C, when compared with atmospheres that contain CO_2.

The total viable counts (TVC) increased faster in vacuum packaged meat samples, in comparison with the other three groups (Fig. 2a). However, after 28 days TVC were not significantly different among treatments. Some authors reported that microbial spoilage of meat occurs with TVC at levels of 7–8 log CFU/cm^2 or g (Insausti et al., 2001; Jeremiah, 2001). In our study this number was reached in vacuum packed meat after two storage weeks but without unacceptable off-odours

Table 3
Mean percentage (%) of packs considered acceptable

	Storage time (days)[a]	Modified atmosphere[b]			Vacuum
		Type A	Type B	Type C	
Colour[c]	0	100	100	100	100
	7	100	100	96	100
	14	100	92	92	100
	21	96	84	80	100
	28	80	78	58	100
Odour[d]	0	100	100	100	100
	7	100	100	100	100
	14	100	96	100	100
	21	92	92	88	100
	28	84	78	88	96

[a] Days post-packing.
[b] Type A corresponds to gas mixture of 40% CO_2/60% N_2; type B: 80% CO_2/20% O_2; type C: 80% CO_2/20% N_2.
[c] Samples were categorised as: 1 = bad colour (not acceptable), 2 = poor colour but customer would buy if cheaper (acceptable), 3 = good colour, 4 = very good colour, or 5 = excellent colour.
[d] Samples were categorised as: 1 = strong off-odour (not acceptable), 2 = slight off-odour (acceptable), or 3 = no off-odour (very acceptable).

or colours for panellists (Table 3). The remaining treatments reached 7 log CFU/cm^2 after 3 or 4 weeks.

Lactic acid bacteria (LAB) grew faster in vacuum than in modified atmospheres (Fig. 2b). After 28 days LAB counts in atmosphere A were one order of magnitude lower than B and C, and two orders lower than vacuum. Faster LAB growth under vacuum than under CO_2 atmospheres agrees with other authors (Garout et al., 1989; Gill and Harrison, 1989; Taylor et al., 1990).

B. thermosphacta represented the dominant flora in MA groups (Fig. 2c). This species grew quickly in the early stages of storage under all treatments. After 2 weeks the fast growth continued, with exception of vacuum where growth was retarded. High carbon dioxide has been reported to restrict the growth of *B. thermosphacta* (Garout et al., 1989; Sheridan et al., 1997). However, our results showed an important growth of this Gram-positive bacteria in CO_2 atmospheres as other authors previously observed in pork and beef (Gill and Harrison, 1989; Taylor et al., 1990; Skandamis and Nychas, 2002). For *B. thermosphacta*, the inhibitory effect under anaerobic conditions also depends on the combination of several intrinsic (pH, L-lactate, water activity, fat content) and extrinsic (residual oxygen, temperature) factors (Grau, 1981, 1983;

Gill and Harrison, 1989; Jeremiah, 2001), which could possibly favour its growth.

Numbers of Enterobacteriaceae increased to 3–4 log CFU/cm^2 at the end of storage in atmospheres A–C (Fig. 2d). This behaviour was different in vacuum packs where levels of ca. 6 log CFU/cm^2 were attained after 14 days. After this time, the Enterobacteriaceae growth tended to plateau and was quite similar to growth of *B. thermosphacta* in vacuum. Sheridan et al. (1997) observed parallel growth of *B. thermosphacta* and Enterobacteriaceae under vacuum conditions. As in this study, the presence of CO_2 levels over 40% in packs limits the growth of Enterobacteriaceae in contrast to vacuum (Garout et al., 1989; Gill and Harrison, 1989; Sheridan et al., 1997).

3.4. Sensory evaluation

Although colour acceptability decreased with time, panellists found that all vacuum packs had acceptable colour (≥2 points) in all storage times (Table 3). By contrast, the acceptability decreased in the three MA treatments, more so in group C with 42% of packs considered unacceptable after 28 days. Lamb packaged under vacuum maintained desirable appearance better than atmospheres that contained carbon dioxide, particularly if concentrations are higher, in agreement with Smith et al. (1983).

Also, off-odours developed faster in several samples of MA groups (Table 3) than in vacuum, probably caused by the growth of *B. thermosphacta* (Fig. 2c) and the rancidity levels (Fig. 1) at these times. Low oxygen levels allow *B. thermosphacta* to produce off-odours due to the aerobic fermentation of meat substrates (Jeremiah, 2001). As well, Enterobacteriaceae growth could contribute to putrid odours (Gill and Harrison, 1989) if oxygen is present in high proportions, as in group B.

4. Conclusions

Results revealed that under the conditions of this study vacuum packaging was superior to the modified atmospheres tested (A: 40% CO_2/60% N_2; B: 80% CO_2/20% O_2 and C: 80% CO_2/20% N_2) with respect to rancidity, colour and odour stability in lamb meat. However, the total viable counts, lactic acid bacteria and Enterobacteriaceae growth were faster in vacuum.

On the other hand, lipid oxidation and instrumental colour (a^* and b^* values) were highly correlated in modified atmospheres which contain carbon dioxide, a fact which may be used to predict the rancidity degree of packed lamb meat by colour measurements. However, further work is necessary to establish correct relationships according to the gas mixture used.

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