Optimization of the shelf life of lamb forelegs packed in different multilayer polymeric materials and modified atmospheres

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Abstract Lamb forelegs were packed in three different gas mixtures (Treatment N₂: 70% N₂–30% CO₂; Treatment O₂: 70% O₂–30% CO₂; Treatment Ar: 70% Ar–30% CO₂) and two types of polymeric materials (B₁, a polyamide-polypropylene bag and B₂, a bag made of bio-oriented polyamide, aluminium and polyethylene). Physical, chemical and microbiological parameters were determined, initially and after 7, 14 and 21 days of storage (3 ± 1 °C). Colour and oxidative stability decreased and the number of microorganisms increased significantly during the storage period. Treatment O₂ increased a* and TBARs. Despite the different permeability to O₂, shown by the polymeric material (< 6.0 vs. < 0.5 cm³/m²/24 h bar), the different types of packaging did not significantly affect any of the studied parameters. Regarding the atmosphere treatment or type of packaging, these factors did not significantly affect *mesophile* or *Enterobacteriaceae* counts either.

Keywords Lamb forelegs · Shelf life · Modified atmosphere · Polymeric material

Introduction

Lamb meat industry is of high social and economic importance in Europe. The production of lamb meat in Spain is distinguished by the short age of slaughtering,

which can be ascribed to the Spanish consumer's demands, who traditionally prefer this kind of meat, and by the economical need to separate suckling lambs at early ages from mothers in order to get milk. Such lamb meat reaches the highest market prices, due to its high quality, pale pink colour, reduced amount of fat and subtle flavour (Gutiérrez et al. 2011).

The lamb meat export profits have increased during the latest years. In 2016, lamb meat exports were worth 136.741 Euros (MAPAMA 2017). The rise in exports leads to the necessity of using and improving preserving systems, in order to guarantee the safeness and quality of the stored meat.

According to Karabagias et al. (2011), shelf life of lamb meat was up to 7 days for air packaged samples on the basis of sensory analysis (odour) and microbiological data. Other authors also concluded that shelf life of lamb meat currently commercialised in refrigeration and packaged conditions, could reach up to 6–10 days (McMillin 2008). For such a reason, modified atmosphere packaging (MAP) in combination with refrigeration, are currently used to promote a longer shelf life of meat (Gutiérrez et al. 2011; Bellés et al. 2017).

Some parameters indicating the quality of fresh lamb meat are colour, flavour, texture and microbial counts, and they depend on storage conditions and atmospheric composition of packaging. Gases used for MAP are usually O₂, CO₂ and N₂. The use of gases such as oxygen allows the maintenance of the red colour on the surface of meat whereas carbon dioxide inhibits the growth of microorganisms (Šćetar et al. 2010; McMillin 2017). Modified atmosphere packaging (MAP) with 60–80% O₂ and 40–20% CO₂, is the most usual gas mixture used in red raw meat (Linares and Vergara 2009). McMillin (2008) reported that the inclusion of 20–30% CO₂ prolongs the shelf

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life by inhibiting bacterial growth and Ščetar et al. (2010) stated that CO₂ proportion should not be higher than 20% in order to achieve a maximal inhibition of aerobic bacteria. High concentrations of oxygen are used to enhance oxymyoglobin formation, which promotes the bright red colour of meat (Rubio et al. 2016). However, high oxygen concentrations also promote some negative effects in lamb meat, such as the development of oxidative reactions in the fat, which is known as rancidity (Rubio et al. 2016).

A single type of plastic polymer does not usually meet the permeability requirements for an optimal fresh meat packaging and hence, lamination, coating or coextrusion is normally used to manufacture the plastic with the required properties (McMillin 2008). Consequently, most meat packaging films are multilayer films which incorporate a variety of polymeric resins. The materials used for meat packaging in MAP should provide a barrier for diffusion of gases from the atmosphere to the headspace and from this to the atmosphere. Storage life can be increased up to a 10–15% by using films with oxygen permeabilities less than 2 cm³/m²/24 h/bar at 23 °C and 0% RH (Ščetar et al. 2010). Despite the growing number of investigations dealing with packaging of lamb meat in modified atmospheres so far, most of these studies have been focused on lamb cuts and lamb minced meat to this date (Gutiérrez et al. 2011; Karabagias et al. 2011; Andrés et al. 2017). There is scarce scientific literature dealing with lamb forelegs meat, which is highly appreciated for being fleshy and succulent and it is considered a gourmet, delicatessen product for its organoleptic characteristics. Shelf life of lamb forelegs was not higher than 13 days (Rubio et al. 2016; Bellés et al. 2017) because microbial growth exceed the limit for acceptable quality meat (7 log CFU g⁻¹) stated by ICMSF (2004). Whereas the use of polymeric bags for vacuum packaging in lamb meat has been approached in previous studies (Kennedy et al. 2005; Soldatou et al. 2009; Rubio et al. 2016) there is no scientific works dealing with the aptitude of these polymeric bags to extend shelf life of lamb forelegs in modified atmosphere packaging.

Therefore, the aim of the current study was to prolong the shelf life of large cuts of Merino lamb forelegs in modified atmospheres by using different polymeric materials. Physical and chemical, as well as microbiological parameters were determined, initially (day 0) and after 7, 14 and 21 days of storage in refrigeration conditions (3 ± 1 °C).

Materials and methods

Animals

30 lambs randomly selected were used in this study. Lambs were fattened up until slaughter weight, up to 30–35 kg. Carcasses were stored for 24 h under refrigeration conditions (2 ± 1 °C) after slaughtering. Then, the two front forelegs of each lamb were cut and randomly assigned to the different treatments. *Triceps brachii* muscle was used to carry out the corresponding analysis.

Materials

The used bags consisted on flexible polymeric materials, as shown in Table 1. The different used bags (B₁ and B₂) showed different permeability to oxygen, carbon dioxide and water vapor. Both of them also showed a high physical resistance in order to adapt to the irregular shape of forelegs, in which some stinging bone surface could also be exposed.

Gas mixtures consisting on N₂, O₂ and CO₂, were chosen because they are the most used gases in meat packaging industries. Ar has also been included in the experiment since it is increasingly used for lamb retail cuts packaging in Spain and it could have some improvements on lamb meat preservation (Bellés 2017).

Treatments

6 lamb forelegs were used as control treatment and were immediately analyzed (day 0). The remaining 54 forelegs were packed in two different types of plastic pouches (B₁ and B₂), provided by SP Group (Table 1), with three different gas mixtures (Treatment N₂: 70% N₂–30% CO₂; Treatment O₂: 70% O₂–30% CO₂; Treatment Ar: 70% Ar–30% CO₂). The gas volume to meat ratio was 2:1. 27 lamb forelegs were packed within each type of bag (B₁, consisting in polyamide and propylene complex and B₂, consisting in bio-oriented polyamide, aluminium and polyethylene). Finally, lamb forelegs were stored under refrigeration at 3 ± 1 °C during 21 days. Samples were taken after 0, 7, 14 and 21 days in order to carry out the analysis.

Analysis

The *triceps brachii* from lamb forelegs was analyzed in this research.

Table 1 Characteristics of the bags used for the experiment

Technical characteristic	Types of bags	
	B ₁	B ₂
Thickness (μm)	204 ± 10%	161 ± 10%
Density (g/cm ³)	1.03 ± 10%	1.04 ± 10%
Unit weight (g/m ²)	209.4 ± 10%	168.0 ± 10%
Welding start temperature (°C)	130–140	120–130
Operating temperature (°C)	+ 2– + 121	– 18– + 100
Permeability to Oxygen (cm ³ /m ² /24 h) to 23 °C, 50%, RH	< 6.0	< 0.5
Water vapor permeability (cm ³ /m ² /24 h) to 38 °C, 90%, RH	< 2.5	< 0.5
Permeability to Carbon Dioxide to 23 °C, 50%, RH	< 24.0	< 0.5
Permeability to Nitrogen (cm ³ /m ² /24 h) to 23 °C, 50%, RH	< 1.5	< 0.5

B₁: PA + PP COPO 4: complex of polyamide and polypropyleneB₂: OPA + ALU + PEMD: complex of biaxially oriented polyamide, aluminum and polyethylene

Analysis of physical and chemical general parameters

Meat pH was measured with a pH puncture electrode (Hanna, model HI 99163, USA). For the study of the water holding capacity (WHC) as a percentage of free water, the method by Grau and Hamm (1953) was followed and the results was expressed in weight. Dry matter (DM) is moisture-free meat and was determined from the difference between the weight of the sample dried with a heater at 105 °C to a constant weight and the initial weight of the sample, according to the AOAC (2000). Moisture (M) was calculated from the dry matter results, following the following calculation: % M = 100–DM.

Analysis of gas composition within the headspace of the bags

In order to monitor headspace of packages and the presence of leakages, gas composition of the headspace was analysed before opening packages using a headspace analyser (Checkpoint, PBI Dansensor, Denmark). A septum was placed in the package and a gas aliquot was withdrawn for analysis of relative oxygen and carbon dioxide content.

Instrumental colour

Colour measurements were taken in the sample surface immediately after opening the package (in order to prevent colour degradation due to light and oxygen), in accordance with the recommendation on colour determination from the American Meat Science Association (AMSA 2011). The following colour coordinates were determined: lightness (L*), redness (a*, red–green) and yellowness (b*, yellow–blue). The colour parameters were determined using a Minolta CR-300 colorimeter reflectance spectrophotometer

(Minolta Camera Co., Osaka, Japan) (Illuminant D65/0 standard observer and 0.8 cm port/viewing area). Three measurements were made in different areas of the samples.

Heme pigment content

The concentration of heme pigments was assayed from the total content of heme according to Hornsey (1956).

Analysis of the oxidative stability of lipids and proteins

The extent of lipid oxidation was estimated as TBARS (thiobarbituric acid reactive substances) by the extraction method described by Jorgensen and Sorensen (1996). Absorbance at 532 nm (A₅₃₂) was measured on three replicates of each sample on a Shimadzu UV-1201 spectrophotometer (Unicam, Mod.Helios). Correction for sample turbidity was made by subtracting the absorbance at 600 nm (A₆₀₀) from the absorbance at 532 nm (A₅₃₂). TBARS were expressed as mg of malondialdehyde (MDA) per kg of muscle in samples using tetraethoxypropane (TEP) as a standard.

Protein oxidation was determined by the quantification of carbonyls using 2,4-dinitrophenylhydrazin according to Oliver et al. (1987). Protein content was calculated with absorbance at 280 nm and carbonyls at 370 nm, measured with a Shimadzu UV-1201 spectrophotometer (Unicam, Mod. Helios) and it was expressed in carbonyl nmoles per protein milligrams.

Microbiological analysis

The samples were taken aseptically and homogenized with 90 mL of peptone water (Merck, 1.07043) in a laboratory blender (Stomacher® 400 Circulator). Serial decimal

dilutions were made in sterile peptone water and 1 mL samples of appropriate dilutions were poured or spread onto total count and selective agar plates. Total aerobic mesophilic counts were performed using a standard Plate Count Agar (PCA) (Merck, Darmstadt, Germany), which were incubated at 30 °C for 72 h and 16 °C for 5 days, respectively. To determine the aerobic mesophiles was used the ISO 21528–2. *Enterobacteriaceae* were enumerated by the pour-overlay method using Violet Red Bile Glucose (VRBG) agar (Merck, Darmstadt, Germany) and incubated at 37 °C for 24–48 h; *Enterobacteriaceae* counts were determined by the ISO method 21528-2. The microorganism values were expressed as log UFC cm⁻². The limits, qualification and normative regulating *mesophile* and *enterobacteriaceae* counts in ovine carcasses are those indicated by the Commission Regulation (EC) (2007).

Statistic analysis

Data were analyzed using the statistical software package IBM SPSS Statistics version 20.0.0. Descriptive analysis was performed in order to calculate means and standard error of the means of the measurements for each parameter. Data were analyzed using a three-way analysis of variance, with storage, atmosphere and type of packaging as main effects. Significance was defined as $P < 0.05$. In cases where the effect of some of these independent variables was significant, the means were compared using the Tukey test ($P < 0.05$).

Results and discussion

pH, WHC, dry matter and moisture

Table 2 shows the values of pH, WHC, DM and moisture content in *Triceps brachii* muscle from lamb forelegs.

pH values were around 6.0–6.2, higher than the values obtained by Gutiérrez et al. (2011) which ranged from 5.10 to 5.45, but similar to the numbers reported by Andrés et al. (2017). In the present research, pH remained constant during storage ($P > 0.05$) which is in agreement with data reported by Gutiérrez et al. (2011) and Rubio et al. (2016). On the contrary, significant decreases in pH were observed during storage of packed lamb meat in other studies carried out by Vergara and Gallego (2001) and Kennedy et al. (2005). However, a substantially higher concentration of CO₂ (80%, 90% and 100%) was used in the mentioned studies, which can be the explanation for the differences with the present results, since CO₂ is easily dissolved in muscle tissue, fat and water, hence leading to a decrease in pH values (Vergara and Gallego 2001). In this sense, there

Table 2 Mean values and effect of storage time, atmosphere and packaging on pH, water holding capacity% (WHC), dry matter% (DM) and moisture content% (M) in lamb forelegs stored during 21 days

	pH	WHC	DM	M
Effect storage time (T)				
0 day	6.1	26.1 ^{ab}	23.8	76.2
7 days	6.1	27.2 ^{ab}	23.7	76.3
14 days	6.2	28.6 ^b	23.5	76.5
21 days	6.0	25.0 ^a	23.9	76.1
*SEM	0.1	0.4	0.1	0.1
P values	0.053	0.006	0.875	0.875
Effect atmosphere (A)				
N ₂	6.1	27.2	23.9	76.1
O ₂	6.1	27.1	23.5	76.5
Ar	6.0	26.6	23.7	76.3
*SEM	0.0	0.4	0.1	0.1
P values	0.067	0.645	0.487	0.488
Packing effect (P)				
B ₁	6.1	26.6	23.9	76.1
B ₂	6.1	27.3	23.5	76.5
*SEM	0.0	0.4	0.1	0.1
P values	0.592	0.369	0.271	0.271
Interactions P values				
T*A	0.214	0.063	0.071	0.071
T*P	0.724	0.919	0.956	0.956
A*P	0.048	0.167	0.423	0.422
T*A*P	0.157	0.509	0.088	0.087

*SEM: Standard error of mean; statistical significant differences $P < 0.05$

^{a, b}: Different letters indicate differences among averages

B₁: Complex of polyamide and polypropylene; B₂: Polyamide bion-oriented complex, aluminium and polyethylene

N₂: 70% N₂ + 30% CO₂; O₂: 70% O₂ + 30% CO₂; Ar: 70% Ar + 30% CO₂

were no significant pH changes as a result of the different gas mixtures used in the present experiment ($P > 0.05$) which is not unexpected, considering the similar percentage of CO₂ within the headspace of the packs, initially (30% CO₂) and all throughout storage ($P > 0.05$) (Table 3). The different permeability to oxygen, carbon dioxide and water vapour of packaging material did not affect pH values either ($P > 0.05$) since, as can be observed in Table 2, pH was 6.1 in lamb meat packed in B₁ and B₂ type of bags.

Water holding capacity (WHC), as a percentage of free water, is related to pH, ionic strength and myofibrillar protein oxidation (Huff-Loneragan and Lonergan 2005). Among these parameters, the final pH values affect WHC

Table 3 Mean values and effect of the storage time, atmosphere and packaging on the percentages of O₂ and CO₂ within the headspace of the packs with lamb forelegs stored during 21 days

	O ₂	CO ₂
Effect storage time (T)		
0 days	—	—
7 days	25.3 ^b	21.8
14 days	21.1 ^a	21.2
21 days	26.0 ^c	21.8
*SEM	4.2	0.9
<i>P</i> values	0.000	0.226
Atmosphere effect (A)		
N ₂	0.3 ^a	20.4 ^a
O ₂	70.5 ^b	21.9 ^c
Ar	0.1 ^a	21.4 ^b
*SEM	4.8	0.2
<i>P</i> values	0.000	0.087
Packing effect (P)		
B ₁	22.7	21.6
B ₂	25.6	21.6
*SEM	4.8	0.2
<i>P</i> values	0.794	0.717
Interactions <i>P</i> values		
T*A	0.000	0.000
T*P	0.179	0.005
A*P	0.906	0.860
T*A*P	0.248	0.030

*SEM: Standard error of mean; statistical significant differences $P < 0.05$

^a, ^b, ^c: Different letters indicate differences among averages

B₁: Complex of polyamide and polypropylene; B₂: Polyamide bioriented complex, aluminum and polyethylene

N₂: 70% N₂ + 30% CO₂; O₂: 70% O₂ + 30% CO₂; Ar: 70% Ar + 30% CO₂

up to a 80%, due to the fact that the water holding capacity is minimum when pH value is close to the protein isoelectric point (pH = 5.5). Water holding capacity values ranging between 25.0 and 28.6% were obtained in this study (Table 2), with important variations during storage and minimal values after 21 days (25.0%) ($P < 0.05$). This minimal value was reached in meat with a pH value of 6.0, which, in addition, was the lowest pH value during storage. In fact, a significant Pearson correlation coefficient was calculated between pH and WHC ($R^2 = 0.689$, $P < 0.05$), thus suggesting a positive relationship between these parameters.

The CO₂ promotes exudation of meat as a consequence of the decrease in WHC values. In this experiment, there was not a significant variation of water holding capacity due to an atmospheric effect ($P > 0.05$), which can be a result of the fact that initial CO₂ levels were equal for every pack (30%) and were similar all throughout storage ($P > 0.05$, Table 3). As far as the effect of packing on WHC is concerned, this was not statistically significant in the present experiment ($P > 0.05$), which is not unexpected considering that the type of bag did not affect pH values either and the already mentioned role of pH values on WHC.

Moisture content in *Triceps brachii* muscle samples ranged between 76.1 and 76.5%, which was similar to data reported by Gutiérrez et al. (2011) for Merino lamb meat and other breeds of lamb (Fernandes et al., 2014). There was not a significant variation for moisture content during storage ($P > 0.05$), which is in agreement with results obtained by Gutiérrez et al. (2011). The loss of moisture as a result of water exudation from the meat can affect the price of meat per kilogram and its appearance, which does not seem to be this case, since the loss of moisture content in this scientific work was not significant during storage up to 21 days (Table 2). The different gas mixtures and packing material did not have significant effects on moisture content either ($P > 0.05$). This could be in relation to the similar pH of N₂, O₂ and Ar treatments ($P > 0.05$) and B₁ and B₂ treatments ($P > 0.05$), considering that the change in pH values has been previously shown to be related to moisture values in meat (Lambertini et al., 2006)

Colour

Colour coordinates L*, a* and b* are shown in Table 4. Average values of L* evolved during the storing period from 40.8 initially to 43.5 after 21 days under refrigeration conditions. However, this variation did not turn out to be significant ($P > 0.05$). Values obtained were similar to those reported by Gutiérrez et al. (2011).

Regarding the effect of the mixture of gases on L*, this was not significant ($P > 0.05$). The type of packaging material did not significantly affect L* values ($P > 0.05$) either.

Red colour intensity (a*) was significantly reduced during the storage period ($P < 0.05$) from 16.4 to 11.5 (Table 4). The loss of intensity of red colour of fresh lamb meat during storage was observed by many authors such as Vergara and Gallego (2001), Kennedy et al. (2005), Gutiérrez et al. (2011) and Bañón et al. (2012). Other authors such as Karabagias et al. (2011) and Rubio et al. (2016), reported constant a* values during storage of MAP lamb meat (60% CO₂/40% N₂ and 20% CO₂/70% O₂/10% N₂, respectively) though in those reports, time of storage

Table 4 Mean values and effect of storage time, atmosphere and packaging on colour parameters L*, a*, b* and myoglobin content (mg Mb g⁻¹ sample) in lamb forelegs stored during 21 days

	L*	a*	b*	Mb
Effect storage time (T)				
0 day	40.8	16.4 ^b	5.0	4.3 ^b
7 days	42.5	13.0 ^a	5.3	4.5 ^b
14 days	43.9	12.8 ^a	5.0	3.0 ^a
21 days	43.5	11.5 ^a	4.7	2.8 ^a
*SEM	0.4	0.4	0.2	0.2
P values	0.346	0.001	0.784	0.008
Atmosphere effect (A)				
N ₂	43.4	11.8a	4.6a	3.7
O ₂	43.9	13.0b	6.4b	3.1
Ar	42.6	12.7b	4.7a	3.5
*SEM	0.5	0.4	0.3	0.2
P values	0.314	0.046	0.000	0.554
Packing effect (P)				
B ₁	44.1	12.2	5.1	3.7
B ₂	42.5	12.8	5.0	3.2
*SEM	0.5	0.4	0.3	0.2
P values	0.051	0.392	0.879	0.301
Interactions P values				
T*A	0.141	0.278	0.186	0.950
T*P	0.754	0.013	0.055	0.539
A*P	0.104	0.071	0.060	0.582
T*A*P	0.851	0.207	0.332	0.809

*SEM: Standard error of mean; statistical significant differences $P < 0.05$

^{a, b}: Different letters indicate differences among averages

B₁: Complex of polyamide and polypropylene; B₂: Polyamide bioriented complex, aluminium and polyethylene

N₂: 70% N₂ + 30% CO₂; O₂: 70% O₂ + 30% CO₂; Ar: 70% Ar + 30% CO₂

was substantially shorter than in the present experiment. In relation to the effect of different gas mixtures on a* in this study, the use of high amounts of oxygen (70% O₂, O₂ treatment) promoted significantly higher values for a* in comparison with the rest of treatments ($P < 0.05$), which is consistent with the higher percentage of oxygen in O₂ treatment (70.5%) (Table 3) Fernandes et al. (2014) and Rubio et al. (2016) reported high a* values in lamb loins and suckling lamb forelegs respectively, packed with high oxygen quantities. When meat is exposed to oxygen, deoxymyoglobin becomes oxygenated to oxymyoglobin which is responsible for the bright cherry red colour (Mancini and Hunt 2005). However, after several hours up to several days, oxymyoglobin is further oxidized to metmyoglobin, the pigment responsible for the brown

discoloration associated with a rejectable meat. In this sense, Bañón et al. (2012) pointed out a decrease in a* values in lamb meat packed in high oxygen concentration atmospheres as a result of metmyoglobin formation during 7 days of storage.

Among plastics, there are structural polymers such as polypropylene and polyethylene, which provide mechanical and barrier properties against the transfer of gases, flavors and odors (Raheem 2013). In order to preserve oxidation and meat colour stability, the packaging film used in retail meat packaging should show an oxygen permeability value lower than 5 cm³/m²/24 h bar) at 23 °C and 0% RH (Jeremiah 2001). According to Ščetar et al. (2010), storage life of meat can be increased up to a 10–15% by using films with oxygen permeabilities lower than 2 cm³/m²/24 h/bar at 23 °C and 0% RH. In the present report, despite the different permeability to O₂ shown by the polymeric material (< 6.0 vs. < 0.5 , Table 1), the different types of packaging used (B₁ vs. B₂) did not significantly affect shelf life of lamb forelegs in terms of colour, since there were no significant differences for a* ($P > 0.05$).

Myoglobin content

The concentration of myoglobin found in meat ranged from 4.3 in day 0 to 2.8 mg g⁻¹ after 21 days (Table 4). Myoglobin values in lambs may vary according to the type of animal feeding supplied, from 2 mg g⁻¹ in case of suckling lambs, to values around 5 mg g⁻¹ in the case of grown up lambs which have been previously fattened up (Osés et al. 2013). In the current study, a decrease in myoglobin content was reported during storage (0, 7, 14, 21 days), in all probability as a result of myoglobin denaturalization. Kennedy et al. (2005) reported a decrease in redness of stored lamb meat due to oxidation of myoglobin and formation of metmyoglobin.

Different gas mixtures did not affect myoglobin content ($P > 0.05$) in the ongoing experiment, in spite of the different oxygen content among treatments (Table 3). This can be ascribed to the fact that total heme pigment, not individual chemical species, has been determined in this manuscript. The use of different types of polymeric bags did not have a significant effect either, despite the different permeabilities of the plastic bags to O₂.

Oxidative stability of lipids and proteins

Lipid oxidation is one of the main chemical reactions which can limit the shelf life of meat and meat products packaged in modified atmospheres (Jeremiah, 2001).

As can be observed in Table 5, lipid oxidation values varied between 0.1 and 1.0 mg MDA Kg⁻¹, showing an

Table 5 Mean values and effect of storage time, atmosphere and packaging on TBARs (mg MDA Kg⁻¹) and carbonyl content (nmol carbonyls mg⁻¹ protein) in lamb forelegs stored during 21 days

	TBARs	Carbonyls
Effect storage time (T)		
0 day	0.1	2.1 ^b
7 days	0.7	1.3 ^a
14 days	0.7	2.5 ^b
21 days	1.0	2.0 ^b
*SEM	0.1	0.1 ^b
P values	0.075	0.000
Atmosphere effect (A)		
N ₂	0.5 ^a	1.9
O ₂	1.5 ^b	2.0
Ar	0.5 ^a	2.0
*SEM	0.1	0.1
P values	0.000	0.069
Packing effect (P)		
B ₁	0.8	1.8
B ₂	0.9	2.0
*SEM	0.1	0.2
P values	0.770	0.055
Interactions P values		
T*A	0.066	0.001
T*P	0.110	0.818
A*P	0.827	0.224
T*A*P	0.180	0.271

*SEM: Standard error of mean; statistical significant differences $P < 0.05$

^a, ^b: Different letters indicate differences among averages

B₁: Complex of polyamide and polypropylene; B₂: Polyamide bioriented complex, aluminum and polyethylene

N₂: 70% N₂ + 30% CO₂; O₂: 70% O₂ + 30% CO₂; Ar: 70% Ar + 30% CO₂

increasing trend during lamb forelegs storage. Insausti et al. (2001) established a limit concentration of 5 mg MDA/kg beef meat, as a detectable concentration for humans. In other studies, a concentration value of 4.4 mg MDA/kg was taken to mark the initiation of lipid oxidation/rancidity of lamb meat samples (Soldatou et al. 2009). However, the effect of storage time did not turn out to be statistically significant ($P > 0.05$). Such an uprising tendency of MDA content during the storage was also observed by many authors (Linares and Vergara 2012; Gutiérrez et al. 2011). Oxidation of meat is a normal reaction during storage, especially in the case of high concentrations of oxygen in the headspace of the pack. In fact, the atmosphere treatment had a significant effect on TBA values in the present research ($P < 0.001$), O₂

treatment showing higher values of TBA (1.5 mg MDA Kg⁻¹) than the rest of the treatments, which is consistent with the highest O₂ content of this batch (70.5%, Table 3). These results are in line with the values obtained by several authors (Gutiérrez et al. 2011; Rubio et al. 2016) who observed a more intense lipid oxidation in meat packed with oxygen rich atmospheres in comparison with atmospheres with high proportions of nitrogen.

Finally, in the current study, lipid oxidation was not significantly affected by the type of bag used, despite of the different permeabilities to O₂ (Table 5), which is consistent with the lack of differences in oxygen concentration within the headspace of B₁ and B₂ treatments ($P > 0.05$) (Table 3).

The number of carbonyls is a clear signal of oxidative damage on proteins (Estévez 2011). In the present study, protein oxidative stability decreased significantly ($P < 0.05$) during storage (0, 7, 14, 21 days) showing the highest values of carbonyls after 14 days. This is due to the fact that meat protein carbonylation takes place during storage under refrigeration (Estévez 2011). Other authors also reported results indicating that protein oxidation phenomena occurred and increased progressively during storage of lamb meat (Andrés et al. 2017).

A higher protein oxidation has been reported in meat stored in atmospheres with 80% oxygen (Zakrys et al. 2008). On the contrary, no significant changes in protein oxidation were reported as a consequence of the different atmosphere treatment in the ongoing research ($P > 0.05$). The type of packaging did not significantly affect protein oxidation either ($P > 0.05$) despite the different gas permeabilities of both B₁ and B₂ bags, which did not promote different oxygen contents within the headspace of the packs.

Microbiological analysis

The evolution of counts (log CFU cm⁻²) of total aerobic microorganisms, *Mesophiles* and *Enterobacteriaceae* in lamb forelegs are shown in Table 6. These microorganisms are naturally present in sheep meat (Gutierrez et al. 2011). Results showed the absence of pathogens (*L. monocytogenes* and *Salmonella spp.* and *Staphylococcus* in 25 g of meat) analyzed at the beginning of storage, so they were not again analyzed at the following days of storage.

The initial number of *Mesophiles* was 1.0 log CFU cm⁻² and it increased significantly ($P < 0.05$) during storage (0, 7, 14, 21 days), reaching the highest value, 2.5 log CFU cm⁻², after 14 days. These values are below the microbiological limits established by the ICMSF (International Commission for Microbial Specifications in Food) in fresh meat (ICMSF 2004), which were set at 7 log₁₀ cfu g⁻¹. In other study, Osés et al. (2013) reported a shorter shelf life of 11 days for lamb meat packed in atmospheres

Table 6 Mean values and effect of the storage time, atmosphere and packaging on *Mesophile* and *Enterobacteriaceae* counts (log UFC cm⁻²) in lamb forelegs stored during 21 days

	Mesophiles	Enterobacteriaceae
Effect storage time (T)		
0 day	1.0 ^a	0.0 ^a
7 days	2.0 ^b	0.4 ^a
14 days	2.5 ^b	2.0 ^b
21 days	2.2 ^b	1.9 ^b
*SEM	0.1	0.1
P values	0.001	0.000
Atmosphere effect (A)		
N ₂	2.2	1.4
O ₂	2.5	1.6
Ar	2.2	1.5
*SEM	0.1	0.1
P values	0.896	0.672
Packing effect (P)		
B ₁	2.1	1.5
B ₂	2.4	1.6
*SEM	0.1	0.1
P values	0.299	0.952
Interactions P values		
T*A	0.012	0.308
T*P	0.159	0.622
A*P	0.163	0.035
T*A*P	0.649	0.995

*SEM: Standard error of mean; statistical significant differences $P < 0.05$

^{a, b}: Different letters indicate differences among averages

B₁: Complex of polyamide and polypropylene; B₂: Polyamide biori-entented complex, aluminum and polyethylene

N₂: 70% N₂ + 30% CO₂; O₂: 70% O₂ + 30% CO₂; Ar: 70% Ar + 30% CO₂

consisting in 70% O₂/30% CO₂ in comparison with the present experiment. Regarding the atmosphere treatment or type of packaging, these factors did not significantly affect *mesophile* counts ($P > 0.05$, Table 6), which is consistent with the similar concentration of CO₂ in N₂, O₂ and Ar treatments and B₁ and B₂ treatments (Table 3), considering the bacteriostatic effect of CO₂.

Enterobacteriaceae values (Table 6) ranged from 0.0 to 2.0 log CFU cm⁻² but did not exceed 2.5 log CFU cm⁻², therefore, being acceptable values according to the regulations above mentioned (ICMSF 2004). The *Enterobacteriaceae* counts increased significantly ($P < 0.05$) during storage. Such results agree with those obtained by other authors such as Soldatou et al. (2009), Karabagias et al. (2011) and Rubio et al. (2016). Finally, the effect of

atmosphere and different types of bags on *Enterobacteriaceae* counts was not significant in this study ($P > 0.05$), which was consistent with the similar percentage of CO₂ measured in all the treatments.

Conclusion

Lamb forelegs can be stored for up to 21 days in the storing conditions used in the current experiment since quality is maintained. This is a significantly longer storage period than earlier studies carried out in our laboratory. Both packaging polymers were suitable for maintaining the desired gas mixtures all throughout storage. A gas mixture consisting in 70% O₂ and 30% CO₂ is however recommended, since lamb meat showed the reddest colour and oxidation levels were below the accepted limits.

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