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Evolution of the fatty acid composition and oxidative stability of Merino lamb meat stored under different modified atmospheres

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The effect of four different gas mixtures on the evolution of the fatty acid composition of neutral lipids and polar lipids, and oxidative stability of Merino fresh lamb meat was studied. Merino fresh lamb meat was packed under four different atmospheres (Atmosphere 1: Air; Atmosphere 2: 70% O_2 + 30% CO_2; Atmosphere 3: 80% O_2 + 20% CO_2; and Atmosphere 4: 30% CO_2 + 69.6% Ar + 0.4% CO) and stored under refrigeration (3±1 °C) for 12 days. Time of storage only affected the proportions of saturated fatty acids of neutral lipids ($P<0.05$). There were no significant differences among gas mixtures for the fatty acid profile of neutral and polar lipids during storage ($P>0.05$). Malondialdehyde and hexanal concentrations were higher for the atmospheres with the highest proportion of oxygen (Atmospheres 2 and 3) indicating lower oxidative stability. The atmosphere consisting of 30% CO_2 + 69.6% Ar + 0.4% CO is recommended, due to a higher oxidative stability of meat during refrigerated storage.

Keywords: Fatty acid; lamb meat; modified atmosphere; oxidation

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Introduction

The Merino is an autochthonous breed from the Iberian Peninsula. Approximately 75% of national production is reared in the western area of the Peninsula, called Extremadura (MAGRAMA 2012). Merino lambs are slaughtered at a young age, after weaning (between 30 and 60 days) and after a short period of fattening based on Spanish consumer preferences (pink colour, reduced amount of fat and subtle flavor) (Beriain *et al*. 2000; Castro *et al*. 2005), resulting in low weight and high-quality carcasses. Merino lamb meat is currently commercialised in refrigeration conditions, so that shelf life normally ranges from 6 to 10 days (Williams 1987). However, there is an increasing trend to centralise cutting and modified atmosphere packaging (MAP) of meat which would provide longer storage times.

Modified atmosphere packaging is commonly used for preserving fresh meat (Jeremiah 2001). However, the presence of high proportions of oxygen in packaged fresh meat, necessary to preserve the bright red colour of meat, can accelerate the oxidation of lipids (Smiddy *et al*. 2002; Linares *et al.* 2007; Gutiérrez *et al*. 2011). The oxidation of lipids is an important issue because it determines the flavour and acceptability of meat (Nawar 1996; Toldrá and Flores 1998). In many countries, consumers are concerned about the rancid flavour of meat packed in modified atmospheres (MA) which are rich in oxygen (Knut and Guy 2006). The oxidative stability of meat packed in atmospheres without oxygen is higher (Insausti *et al*. 2001; Jeremiah 2001), but some authors have found intense discolorations on the surface of meat when packed in an oxygen-free atmosphere (Gill 1990; Luño, Beltrán and Roncalés 1998), thus inducing rejection by consumers. The use of limited amounts of carbon monoxide (CO) in the package headspace could alleviate this problem, since this molecule binds strongly to the muscle pigment myoglobin and creates a stable bright red colour (Krause *et al.* 2003). The use of CO is not allowed in the EU since it is not included in the list of permitted food additives (Directive 2001/5/EC). However CO is considered as GRAS (Generally Recognized as Safe) by United States Food and Drug Administration (USFDA). Moreover, the European Scientific Committee on Food (2001) concluded that there is no health concern associated with the use of 0.3%–0.5% CO in a gas mixture with CO_2 and N_2 as a MAP gas for fresh meat provided the temperature during storage and transport does not exceed 4 °C.

As well as altering flavour and colour, lipid oxidation can also reduce the nutritional value and safety of meat (Lynch *et al.* 1999). The oxidative stability of lipid fractions depends to a great extent, on their fatty acids (Monahan *et al*. 1992). Polar lipids (PL) have a high content of unsaturated fatty acids and are therefore more susceptible to oxidation than neutral lipids (NL), rich in saturated fatty acids (SFA) (Pikul *et al.* 1984). Because the susceptibility of unsaturated fatty acids to oxidation is related to the degree of unsaturation, polyunsaturated fatty acids (PUFA) are more prone to oxidation than monounsaturated fatty acids (MUFA) (Mottram 1998).

Gutiérrez *et al*. (2011) recently evaluated some of the traits directly related to quality of Merino lamb meat packed in MA such as instrumental colour, sensory traits and microbiological counts. To date, no studies have been reported on the effect of MAP on Merino lamb meat composition during storage. Therefore, the purpose of this research was to report the modifications in fatty acid composition and its effect on oxidative stability

on Merino lamb meat during storage in different gas atmospheres.

Materials and Methods

Animals

Data were obtained from 32 Merino lambs reared with their dams and weaned at 14 kg live weigh (at approximately 38 days of age). They then had *ad libitum* access to concentrate [g/kg, 500 barley, 210 soya, 170 corn, and 120 others (wheat, calcium carbonate, sodium chloride, and mineral-vitamin mix)], with 150 g/kg crude protein dry matter basis, and cereal straw *ad libitum* using a confinement feeding system. They were slaughtered in a licensed abattoir when they reached the predetermined weight of 24 kg (after approximately 45 days confinement). After slaughter, the carcasses were cooled at 4 °C for 24 h according to the normal working practice in the abattoir. They were then cut longitudinally and the left side of each carcass was divided into standard commercial cuts: leg, shoulder, rib and loin, breast, and neck. Legs were transported under refrigeration conditions to the facilities of the School of Engineering.

Samples

Left side lamb legs (a total of 32 samples), with bone included, were cut into 16 homogeneous slices 1 cm thick for each lamb and randomly assigned to one of the following treatments: one group of samples was simply placed in trays of polypropylene without plastic covering (Atmosphere 1) so that the composition of the headspace was always ambient air (21% O_2 + 0.04% CO_2), in order to mimic the conditions of the local butcher shop. The rest of the samples were packed in MA with the following gas mixtures:

Atmosphere 2: 70% O_2 + 30% CO_2.
Atmosphere 3: 80% O_2 + 20% CO_2.
Atmosphere 4: 30% CO_2 + 69.6% Ar + 0.4% CO.

Samples in atmospheres 2, 3 and 4 were placed individually into rigid trays of polypropylene with an oxygen permeability of 2.5 $cm^3/m^2/24$ h/at STP (Hinagal S.L., Badajoz, Spain) sealed with a plastic material (polyamide/polyethylene) 70 μm thick with an oxygen permeability of 45 $cm^3/m^2/24$ h at STP (Hinagal S.L., Badajoz, Spain). A thermosealing machine (Lavezzini Mod. VG600, Fiorenzuola d'Arda, Italy) was used for packing.

The gas volume to meat ratio was 2:1 in each pack. The gas composition of the headspace was analysed before opening the packages using a headspace analyser (LS212, Abiss, Witten, Germany). A septum was placed in the package and a 6 ml gas aliquot was withdrawn for analysis of relative oxygen (± 1%) and carbon dioxide (± 2%) content. Meat samples were stored under refrigeration at 3±1 °C for twelve days in darkness. Sampling (n=8/atmosphere) was carried out after 1, 4, 7 and 12 days post-packaging. Samples were packed under vacuum and maintained at –80 °C until chemical analysis. The muscle used for analysis was *Biceps femoris* (BF). No data from atmosphere 4 on day 12 were available, since leaks were detected in these samples.

Lipid analysis

Lipids were extracted with chloroform/methanol (2:1) according to the method of Folch, Lees and Sloane-Stanley (1957) and dried under nitrogen. Neutral and polar lipid fractions from intramuscular fat were separated using amino-propyl 500 mg minicolumns (Phenomenex, Torrance, USA), following the method described by Ruiz *et al.* (2004). Fatty acid methyl esters (FAME) of two fractions were prepared by acidic-trans-esterification in the presence of sulphuric acid (0.9M) in methanol (Sandler and Karo 1992). Fatty acids were

analysed by gas chromatography using a Hewlett Packard HP-4890 series II gas chromatograph, equipped with a flame ionisation detector. Separation was carried out on a polyethylene glycol–terephthalic acid modified fused silica semicapillary column (30 m long, 0.53 mm i.d., 1 μm film thickness, Sigma-Aldrich, Química S.L., Madrid, Spain). The oven temperature was maintained constant at 225 °C. Injector and detector temperature set points were stated at 230 °C. Carrier gas was nitrogen at a flow rate of 1.8 ml min^{-1}. Individual FAME peaks were identified by comparing their retention times with those of reference compounds (Sigma Chemical Co., St. Louis, Missouri, USA). Finally, the fatty acids of the different fractions were expressed as percentages of total peak area of the FAME identified on the chromatograms.

Lipid oxidation

The extent of lipid oxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) using the method described by Jørgensen and Sørensen (1996). The TBARS values were expressed as mg malondialdehyde kg^{-1} muscle. Lipid oxidation was measured in duplicate for each sample homogenised in trichloroacetic acid after 1, 4, 7, and 12 days. Homogenates were filtered and the absorbance was measured at 530 nm on a spectrophotometer (Unicam, model Helios, Thermoscientific, Madrid, Spain). Malondialdehyde (MDA) concentration was calculated from a standard curve in triplicate, using solutions of 1,1,3,3-tetraethoxypropane (TEP) (Merck, Darmstad, Germany).

Hexanal content was quantified by headspace-solid phase microextraction (SPME) and GC/MS (gas chromatograph Hewlett-Packard 5890 series II, coupled to a mass selective detector Hewlett-Packard HP-5791 A), according to the method by Ruiz *et al*. (1998). A minced sample (0.5 g) and 1.5 ml of distilled water were thoroughly mixed in a vial, which was then closed with a teflon/silicone septum. An SPME fibre (75 mm carboxen-polydimethylsiloxane coating) was inserted through the septum and exposed to the headspace of the vial. Extraction was carried out at 40 °C for 30 min with stirring in a water bath. After extraction, the SPME fibre was immediately transferred to the injector of the chromatograph which was in splitless mode at 280 °C. The separation of hexanal was performed on a 5% phenyl-methyl silicone (HP-5)-bonded phase fused silica capillary column (Hewlett-Packard, 30 m long, 0.32 mm i.d., 1.05 μm film thickness), operating at 6 psi of column head pressure. The oven temperature programme was: 35 °C for 5 min, 4 °C min^{-1} to 150 °C, 2 °C min^{-1} to 250 °C and held at 250 °C for 5 min. The transfer line to the mass spectrometer was maintained at 280 °C. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V and collecting data at a rate of 1 scan s^{-1} over the m/z range 30–500. Hexanal was tentatively identified by comparing mass spectra and retention time with that of a standard (Sigma, St. Louis, USA) injected under the same conditions. Hexanal content was calculated using a standard curve with solutions of hexanal (Sigma, St. Louis, USA) in triplicate, extracted and analysed under exactly the same conditions as the samples. Hexanal content was expressed as mg hexanal kg^{-1} muscle.

Statistical analysis

An analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the SPSS package (SPSS 19.0) was performed for all variables considered in the study, considering two factors and their interaction. When main effects

(time or atmosphere) or their interaction were significant ($P<0.05$), comparisons of means were performed using the Tukey's test at the 5% level. Pearson's correlation coefficient between variables was also calculated using the GLM procedure of SPSS (SPSS 19.0).

Results and Discussion

Fatty acid composition

The average intramuscular fat content for the samples was 2.55 ± 0.11 g 100 g^{-1}. The fatty acid composition of the NL fraction in refrigerated lamb meat is shown in Table 1. Oleic acid (C18:1 n-9) showed the highest percentage, followed by palmitic (C16:0), stearic (C18:0) and linoleic (C18:2 n-6) acids at day 1. The NL fraction profile was initially 40.94–43.76% SFA, 47.82–50.62% MUFA and 6.91–9.86% PUFA, with no significant differences between the experimental atmospheres. Fatty acid composition of NL was similar to the composition of total lipids found by other authors in Merino lamb meat (Tejeda, Peña and Andrés 2008), because the NL represented at least 81–82% of total lipids (Jerónimo *et al*. 2009).

Compared to day 1, the proportion of SFA was higher ($P<0.05$) after 12 days of storage. These differences for SFA in the NL fraction during storage were mainly due to the least abundant SFA (C13:0, C14:0, C15:0 and C17:0), since most abundant fatty acids (C16:0 and C18:0) did not change during storage ($P>0.05$). In a previous study carried out in Manchego lamb meat (Camo, Beltrán and Roncalés 2008), SFA increased throughout a storage period that was substantially larger (28 days) than the present study. In contrast, Alvarez *et al*. (2009) did not find a significant effect of the time of storage on the proportions of SFA in NL. The percentages of MUFA and PUFA in NL fraction did not significantly change during chilled storage ($P>0.05$). The storage period significantly affected the proportion of linolenic acid (C18:3 n-3) in samples packaged in atmosphere 1 (ambient air), with a decrease after 4 and 7 days of refrigeration storage, and an increase after 12 days of storage. According to Alvarez *et al*. (2009) in meat from lambs fed on diets which had not been supplemented with vitamin E, C18:3 n-3 significantly decreased throughout 28 days of storage in MA. However, Díaz *et al*. (2011) did not find significant changes in linolenic acid after 7 days of refrigeration storage in ambient air.

Concerning the effect of the different gas atmospheres on the fatty acid composition of the NL fraction, differences were significant for the total SFA after 4 and 7 days of refrigerated storage ($P<0.05$ and $P<0.01$, respectively), the samples packaged in 70% O_2 and 30% CO_2 (atmosphere 2) showing the highest proportions of SFA. As well as for the effect of storage, the minority fatty acids of the SFA fraction were responsible for these differences. An interaction between time and atmosphere of packaging can be observed in Table 1 for SFA suggesting a different evolution with time depending on the type of packaging.

The fatty acid composition of the PL fraction in refrigerated lamb meat is shown in Table 2. The C18:2 n-6, C18:1 n-9, C16:0 and C18:0 were the majority fatty acids in raw lamb meat stored under refrigeration at day 1. No differences between the experimental groups were observed for total SFA, MUFA and PUFA in the PL fraction.

A number of papers describe the importance of oxidative and hydrolytic processes of PL during refrigeration of meat (Alasnier and Gandemer 1998; Alasnier *et al*. 2000; Sklan, Tenne and Budowski 1983). On the one hand, lipolysis can be responsible for a decrease in PL content,

Table 1. Effect of gas mixture composition and storage time on the fatty acid composition of the neutral lipid fraction of Merino lamb meat packed in MAP.

Day	Atmosphere	C12:0	C14:0	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1 n-9	C18:2 n-6	C18:3 n-3	C20:0	C20:1	C20:4 n-6	SFA	MUFA	**PUFA**
	1	0.39	3.83^{axy}	0.52^{xy}	23.12	2.84	1.98^{xy}	1.14^{xy}	12.55	44.98	4.55	0.73^{ax}	0.68	0.37	0.92	43.76^{xy}	49.32	6.91
	2	1.06	1.97^{by}	0.35^{y}	22.14	3.02	1.36^{y}	1.01	11.92	46.03	5.98	0.36^{b}	1.23	0.56^{x}	1.49	40.94^{y}	50.62	8.44
1	3	0.60	3.20^{ab}	0.46	22.65	2.74	1.58^{y}	1.09	12.11	43.62	6.70	0.69^{ab}	0.93	0.37	1.77	42.32^{y}	47.82	9.86
	4	0.34	3.29^{ab}	0.49	23.68	2.98	1.63	1.11	11.88	44.83	5.24	0.60^{ab}	1.36^{x}	0.43	1.19	42.94	49.35	7.71
	$P_{Atmosphere}$		**									*						
	1	0.36	2.84^{bz}	0.55^{xy}	21.75	2.9	2.91^{x}	1.38^{x}	11.81	44.85	6.55	0.47^{y}	0.38	0.34	1.42	41.47^{bz}	49.46	9.07
	2	0.36	3.69^{abx}	0.68^{x}	23.66	2.73	2.31^{xy}	1.26	13.14	42.61	5.32	0.56	0.35	0.26^{y}	1.26	45.14^{ax}	46.87	7.99
4	3	0.39	3.89^{a}	0.53	23.63	2.92	1.96^{xy}	1.20	12.26	44.93	4.48	0.65	0.35	0.33	0.82	44.03^{abxy}	49.38	6.58
	4	0.28	2.96^{b}	0.47	21.79	2.76	2.12	1.12	12.75	45.87	5.72	0.56	0.39^{y}	0.41	1.07	41.75^{b}	50.15	8.09
	$P_{Atmosphere}$		**		**											*		
	1	0.29	3.04^{yz}	0.44^{by}	23.2	2.98	1.43^{by}	1.05^{y}	11.47	43.63	6.95	0.45^{y}	0.43	0.38	2.10	41.89^{byz}	48.03	10.08
	2	0.72	3.52^{x}	0.69^{ax}	23.76	2.59	2.52^{axy}	1.38	14.04	41.15	5.23	0.62	0.63	0.35^{xy}	1.13	46.72^{ax}	45.46	7.82
7	3	0.52	3.26	0.57^{ab}	22.11	3.04	1.76^{abxy}	1.29	11.94	42.49	6.64	0.77	0.36	0.31	2.51	42.27^{by}	47.13	10.60
	4	0.31	3.67	0.58^{ab}	23.42	2.88	1.86^{ab}	1.24	12.05	42.54	5.88	0.66	0.51^{y}	0.31	1.80	43.89^{ab}	46.97	9.13
	$P_{Atmosphere}$			**			*									**		
	1	0.39^{b}	3.99^{ax}	0.61^{x}	22.92	2.86	2.25^{xy}	1.18^{xy}	12.01	42.14	6.11	0.80^{x}	0.64	0.32	1.74	44.13^{x}	46.49	9.38
	2	1.09^{a}	2.59^{bxy}	0.54^{xy}	21.33	2.36	3.11^{x}	1.33	12.52	41.90	5.70	0.78	1.07	0.34^{xy}	1.13	45.72^{x}	45.93	8.35
12	3	0.37^{b}	3.51^{ab}	0.61	23.17	2.74	2.78^{x}	1.20	12.27	42.43	5.21	0.83	0.69	0.33	0.96	45.64^{x}	46.69	7.66
	$P_{Atmosphere}$	**	*															
SEM		0.04	0.09	0.01	0.26	0.03	0.09	0.02	0.14	0.29	0.21	0.02	0.05	0.01	0.11	0.53	0.68	0.74
	1		**	*			*	*				**				**		
P_{day}	2		*	*			*							*		**		
	3						*									*		
	4												*					
$P_{day\ x\ atmosphere}$			***	**	**		*							*		***		

MAP = modified atmosphere packaging; SEM = pooled standard error of the mean.
Atmosphere 1 = ambient air; Atmosphere 2 = 70% O_2 + 30% CO_2; Atmosphere 3 = 80% O_2 + 20% CO_2; Atmosphere 4 = 69.6% Ar + 30% CO_2 + 0.4% CO.
[abc] Different letters within the same day denote significant statistical differences among atmospheres ($P<0.05$).
[xyz] Different letters within the same treatment denote significant statistical differences among days of storage ($P<0.05$).

Table 2. Effect of gas mixture composition and storage time on the fatty acid composition of the polar lipid fraction of Merino lamb meat packed in MAP.

Day	Atmosphere	C14:0	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1 n-9	C18:2 n-6	C18:3 n-3	C20:0	C20:1	C20:4 n-6	C20:5 n-3	C22:5 n-3	SFA	MUFA	PUFA
	1	0.31	0.16^{y}	26.01	0.90	1.10	1.14	7.41^{y}	24.60	19.53	0.32	0.14	0.17	8.19	2.91	3.79	35.38	26.82	37.80
	2	0.13	0.19	18.95	0.84	1.00	0.89	9.04	20.66	26.18	0.11^{y}	0.10	0.18	12.18	3.77	2.17	29.50	22.57^{x}	47.94^{x}
1	3	0.30	0.21^{y}	24.58	0.78	1.03^{y}	0.55	12.41	20.54	20.34	0.22	0.16	0.21	10.20	2.31	2.96	38.88	22.09	39.04
	4	0.34	0.22	17.53	0.70	1.10	0.75	10.79	18.01	23.30	0.60	0.10	0.16	15.33	4.34	2.78	30.44	19.62	49.94
	$P_{atmosphere}$																		
	1	0.24	0.21^{xy}	25.00	0.73	1.45	0.80	9.64^{xy}	19.46	20.14	0.27	0.13	0.17	11.44	2.97	3.52	36.96	21.16	41.88
	2	0.41	0.23	24.08	0.72	1.24	0.51	10.67	17.22	20.94	0.17^{y}	0.11	0.15	11.62	3.80	3.57	36.91	18.60^{y}	44.49^{x}
4	3	0.15	0.21^{y}	26.32	0.72	0.91^{y}	0.59	8.48	18.96	24.66	0.27	0.08	0.18	10.68	3.18	1.33	36.28	20.44	43.28
	4	0.37	0.21	21.16	0.88	1.13	0.55	9.96	23.61	19.05	0.26	0.12	0.16	10.71	3.45	4.46	33.29	25.19	41.52
	$P_{atmosphere}$																		
	1	0.36	0.22^{xy}	22.35	0.92	1.31	0.96	10.85^{x}	19.80	20.02	0.17	0.14	0.25	11.62	2.70	4.18	35.75	21.94	42.31
	2	0.20	0.26	23.29	0.80	1.25	0.99	9.19	21.34	23.11	0.23^{y}	0.16	0.19	7.25	3.15	5.07	34.59	23.32^{x}	42.09^{xy}
7	3	0.22	0.26^{y}	18.08	0.98	1.19^{y}	0.82	11.39	20.41	19.90	0.41	0.11	0.18	14.48	2.08	5.64	31.68	22.39	45.93
	4	0.25	0.14	31.54	0.54	1.00	0.71	9.29	18.00	16.90	0.42	0.09	0.17	11.07	4.38	2.11	42.51	19.42	38.07
	$P_{atmosphere}$																		
	1	0.23	0.28^{x}	21.90	1.00	1.14^{b}	0.74	10.84^{bx}	20.88	18.14^{a}	0.36	0.20	0.21	11.39	2.71^{ab}	6.18	34.96	22.82	42.22
	2	0.39	0.35	21.47	0.85	1.83^{a}	2.83	16.17^{a}	21.72	13.98^{b}	0.46^{x}	0.19	0.19	5.30	1.29^{b}	9.27	40.79	25.59^{x}	33.62^{y}
12	3	0.23	0.32^{x}	19.74	0.89	1.54^{abx}	0.85	11.42^{b}	21.31	20.65^{a}	0.28	0.10	0.22	9.75	3.83^{a}	4.53	33.55	23.28	43.17
	$P_{atmosphere}$					*		*		**					**				
SEM		0.02	0.00	0.96	0.03	0.05	0.07	0.32	0.51	0.57	0.09	0.01	0.00	0.37	0.11	0.42	0.75	0.51	0.76
	1		*					*											
P_{day}	2										*							**	*
	3		**			**													
	4																		
$P_{day\ x\ atmosphere}$			***			*		*		**		*			*	*	**		**

See footnotes to Table 1.

releasing free fatty acids. Higher susceptibility of PL to oxidation as compared with NL has been demonstrated previously (Igene, Pearson and Gray 1981) and is mainly due to their high content in PUFA, which are more susceptible to oxidation, and due to their location in cell membranes, close to heme pigments and other prooxidant systems (Asghar *et al*. 1988). A decline in PL content could not be demonstrated during storage in this study, since absolute quantification of fatty acids was not carried out. However, it can be observed that the profile of SFA, MUFA and PUFA in the PL fraction remained constant during storage at refrigeration ($P>0.05$), which may suggest that the processes of enzymatic hydrolysis affected the three families in the PL fraction to the same extent. Similarly, the time of storage did not significantly affect the proportions of fatty acids in PL, as observed by Alvarez *et al*. (2009). However, when results are expressed as fatty acid content (mg/100 g of muscle), Díaz *et al*. (2011) reported substantial changes in the SFA, MUFA and PUFA content of the PL fraction after 7 days of refrigeration storage, caused by enzymatic hydrolysis of muscle lipids (Alasnier *et al*. 2000) and oxidation changes (Alvarez *et al*. 2009). Regarding the effect of different atmospheres on the fatty acid profiles of PL fraction, there were no significant differences during storage ($P>0.05$).

Lipid oxidation

Figure 1 (a, b) shows the evolution of MDA content and hexanal content during chill storage of raw lamb meat (*Biceps femoris* muscle) packaged in different MA.

The TBARS values increased significantly from the first day of storage in the present report ($P<0.01$), (Figure 1a). The TBARS values were higher in samples with the highest oxygen content in the headspace of the pack (70 and 80% oxygen, atmospheres 2 and 3, respectively). After 7 days storage, samples packed in atmospheres 2 and 3 showed TBARS values equal to or higher than 5 mg MDA kg^{-1} meat, reported by Insausti *et al*. (2001) as a detectable concentration for humans. Differences in TBARS values among gas mixtures were statistically significant after 1, 4, 7 and 12 days of storage ($P<0.01$). The highest MDA content was exhibited by the atmosphere with 80% O_2 and 20% CO_2 (atmosphere 3), the differences being statistically significant after 7 days ($P<0.001$) and 12 days ($P<0.01$). Likewise, numerous studies have found that the fresh meat packaged in MA with high proportions of oxygen show a more intense lipid oxidation in beef (O'Grady *et al.* 2000; Insausti *et al*. 2001) and in lamb meat (Berruga, Vergara and Gallego 2005; Linares *et al*. 2007). In this sense, atmosphere 4, oxygen-free packaging, had the lowest concentrations of MDA during storage ($P<0.001$). Linares *et al*. (2007) also found lower levels of MDA in lamb packaged in MA with no oxygen included. However, atmosphere 4 seemed to be insufficient to limit TBARS values. Low levels of residual oxygen could explain lipid oxidation reactions. According to other studies (Smiddy *et al*. 2002; Berruga *et al*. 2005) vacuum or no-oxygen MA 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).

A significant and positive Pearson's correlation coefficient was found between TBARS and SFA of the NL fraction ($R=0.345$, $P<0.01$) and negative between TBARS and MUFA of the NL fraction ($R=-0.256$ and $R=0.151$, $P<0.05$). This suggests that lipid oxidation is mainly related to the MUFA of the NL fraction oxidation rather than the SFA fraction.

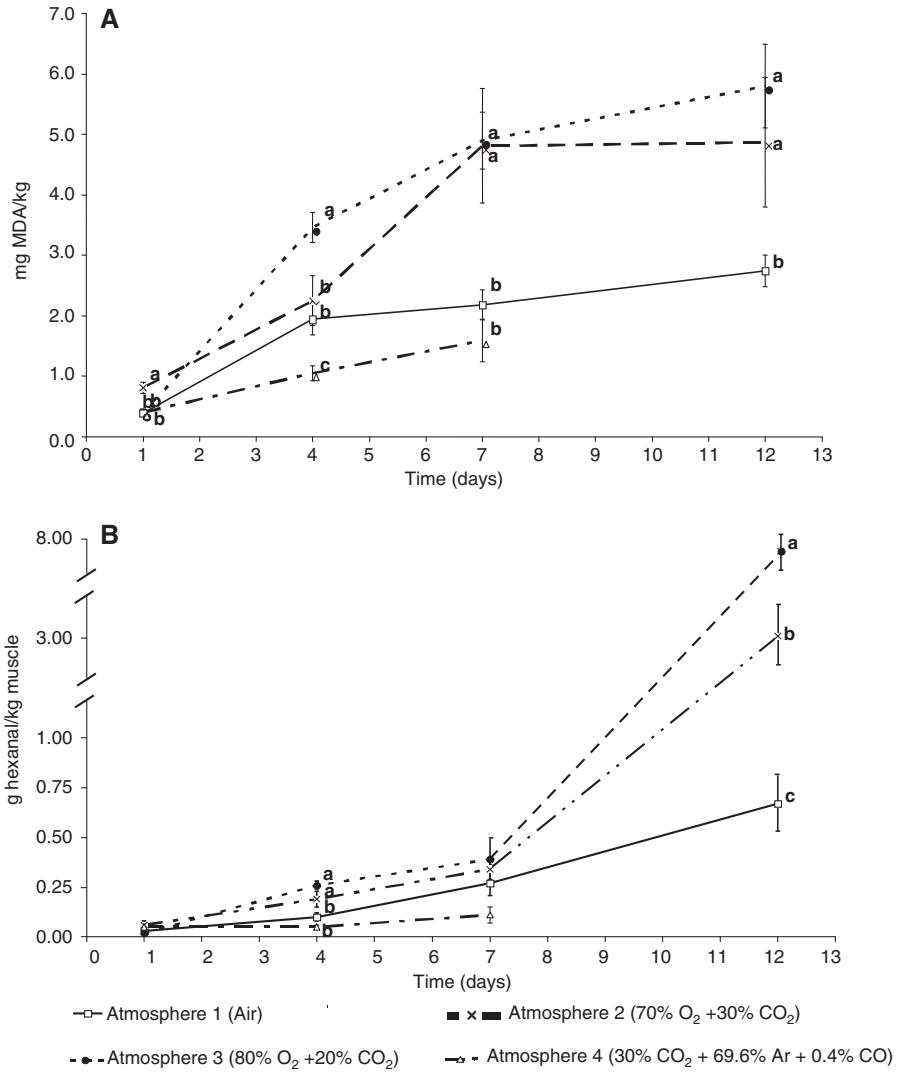

Figure 1. Evolution of malondialdehyde content (mg MDA/kg muscle) (Figure 1A) and hexanal content (g hexanal/kg muscle) (Figure 1B) during chill storage (3±1 °C) of raw lamb meat packaged in different modified atmospheres.

In this sense, the lower oxidative stability of unsaturated fatty acids is widely known and has been stated by several authors (Gray, Goma and Buckley 1996; Elmore *et al*. 1999; Gandemer 2002). Lipid oxidation is one of major chemical reactions that may limit shelf life of the meat and meat products packed in MA (Renerre and Labadie 1993; Mohamed *et al*. 2008). Lipid oxidation promotes rancidity, considered unpleasant or defective by consumers (Jeremiah 2001) as well as being related to discoloration of raw meat (O'Grady *et al*. 2001; Mohamed *et al*. 2008; Gutiérrez

et al. 2011). Rancidity is mainly ascribed to the presence of hexanal, from linoleic acid oxidation. In the present study, hexanal content showed an upward trend during storage (Figure 1b). A significant sharp increase in hexanal content took place after 7 days of storage, which was consistent with previous observations carried out in raw pork meat (Fernando, Berg and Grün 2003). Regarding the effect of different packaging atmospheres on the concentration of hexanal content during storage of raw lamb meat, the highest concentrations of hexanal were found in atmospheres 2 and 3 (70% O_2 + 30% CO_2 and 80% O_2 + 20% CO_2, respectively). These differences were statistically significant after 4 and 12 days of packaging ($P<0.001$ and $P<0.01$, respectively). The results found for hexanal in the present study agree with those previously mentioned for TBARS values in this report, the meat packed in atmospheres with high percentages of oxygen showing the lowest oxidative stability. In this sense, a significant and positive Pearson's correlation coefficient was found between hexanal content and TBARS ($R=0.56$; $P<0.001$). On the other hand, meat samples from atmosphere 4 (69.6% Ar, 30% CO_2 and 0.4% CO) had the lowest values for hexanal content throughout the storage period (7 days), which can be ascribed to the absence of oxygen in the package. Higher oxidation stability is the main advantage of using trace amounts of CO in MAP, as well as its capacity for maintaining a stable and bright red colour in meat, which has been reported in lamb in a parallel study by Gutierrez *et al*. (2011). A significant Pearson's correlation coefficient was not found between hexanal content and fatty acids of the PL fraction ($P>0.05$).

In a previous paper by Gutiérrez *et al*. (2011), rancid odour of lamb meat as assessed by a trained panel, remained practically steady from day 1 to day 7 of refrigeration but dramatically increased after 12 days, in accordance with the increase in hexanal content observed in the present work. Regarding the effect of gas mixtures, that study showed a more intense rancid odour in lamb meat packed with high oxygen contents (atmospheres 2 and 3) in parallel with hexanal contents determined in the present paper.

Conclusions

Results revealed that in general, there is no prominent effect of either time of storage under refrigeration or packaging atmosphere on the composition of fatty acids in NL and PL fractions. Oxidative stability of lamb meat packed with high oxygen concentration ranging from 70% (70% O_2 + 30% CO_2) to 80% (80% O_2 + 20% CO_2) was the lowest. The gas mixture consisting of 69.4% argon, 30% carbon dioxide, and 0.4% carbon monoxide is recommended, based on a higher oxidative stability of meat during conservation in refrigeration.

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