Suckling lamb meat quality from ewes fed with different sources of fat, during storage under display conditions

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Abstract

We studied the effect of calcium soaps offered to ewes during lactation (CO, 3% Ca soap of palm oil; OLI, 3% Ca soap of olive oil; FO, 3% Ca soap of fish oil) on the microbial, physical-chemical and sensory properties of suckling lamb meat packaged with modified atmosphere packaging (80%O_2:20%CO_2) throughout storage; six ewes were tested per treatment. Microbial counts (*Enterobacteriae*, *Pseudomonas spp.*, psychrotrophic and lactic acid bacteria), lipid oxidation (TBARS), colour coordinates (L*, a*, b*), metmyoglobin content and sensory quality were analysed on days 0, 5, 8 and 13 of storage. Throughout storage, the evolution of most parameters throughout depended on the lambs' mothers' diet. From day 5 onward, microbial counts, as well as pigments and lipid oxidation, presented the highest values with FO. CO and OLI were rejected for poor sensory quality after 13 days, whereas FO reached the limit of rejection after 5 days. Therefore, the positive effect of increasing PUFA in sucking lamb meat via FO is limited due to a shorter shelf life.

Keywords: Suckling lamb meat quality; Meat shelf life; Calcium soap oil

1 Introduction

Nowadays, with regard to purchasing meat, consumers are increasingly using extrinsic cues to perceive quality, which are associated with nutrition and health (Grunert, 2006; Da Fonseca and Salay, 2008). In the Mediterranean, and more specifically in the Castilla and León regions of Spain, suckling lamb meat is a high quality product obtained from lambs fed only maternal milk and slaughtered before 35 days of age. However, the fatty acid composition of lamb meat does not adequately meet nutritional recommendations because of its high content of some saturated fatty acids (SFA). In order to add value to sucking lamb meat, an increase of some specific fatty acids would be desirable, such as n-3 polyunsaturated fatty acids (PUFA), oleic and CLA (conjugated linoleic acid) which are thought to be beneficial for human health.

Suckling lambs are cared for by their dams and fed exclusively maternal milk, so any feeding strategy to enhance the meat quality should be through the ewes' diet. Wood et al. (2003) have reviewed the effects of incorporating different sources of unsaturated fatty acids in the diets of domesticated animals; they concluded that, even in ruminants, there is a relationship between dietary fatty acids in animal feed and the technological and nutritional quality of meat. Specifically in lambs, Manso et al. (2009) reported that the inclusion of sunflower oil improved fatty acid composition without affecting animal performance. On the other hand, there is evidence that vegetable oil (Vieira et al., 2012) and fish oil (Díaz et al., 2011; Najafi et al., 2012) can be used as dietary supplements for lactating ewes, to modify and enhance the fatty acid composition of their meat and the fat obtained from their suckling lambs (Manso et al., 2009).

However, the transfer efficiency of unsaturated fatty acids in ruminant diets to milk and meat fat can be improved when oils are fed in a rumen-protected form, such as calcium (Ca) soaps; this can prevent biohydrogenation, even if only partially (Kitessa et al., 2003; Manso et al., 2006). Gallardo et al. (2014) compared the effects of dietary calcium soap oil (palm, olive and fish oil) supplementation and observed that suckling lamb meat from ewes fed diets supplemented with calcium soap fish oil had the highest concentrations of polyunsaturated fatty acids. On the other hand, some authors have observed that changes in fatty acid compositions lead to differences in scores for suckling lamb meat odour (Manso et al., 2009; Díaz et al., 2011; Vieira et al., 2012). In contrast, Najafi et al. (2012) observed that the use of fish oil during the fattening phase of goat kid production improved the percentage of PUFA without changing the sensory properties of the meat.

Due to changes in shopping and consumption habits, producers try to extend the shelf life of meat using storage practices such as gas mixture atmosphere packaging. At the time of purchase, among the major meat quality parameters, appearance is considered a key attribute due to its direct impact on consumers' impression of product freshness. It has been reported that optimum meat colour stability is obtained by using gas mixtures containing high concentrations of oxygen together with low proportions of carbon dioxide, which restricts the growth of aerobic spoilage bacteria (Jeremiah, 2001). However, the presence of high oxygen concentrations may enhance lipid and protein oxidation in meat (Smiddy et al., 2002). The main, non-microbial causes of meat quality deterioration during processing are protein and lipid oxidations. Meat discolouration, due to the oxidation of myoglobin (Mb) to metmyoglobin

(MMb) while in storage or on retail display, leads to significant product discarding (McKenna et al., 2005). Oxidation has also been described as an important factor responsible for quality loss through the formation of rancid odours and deterioration in flavour (Faustman and Cassens, 1991; Liu et al., 1995; Falowo et al., 2014). In small ruminants, meats containing high unsaturated fatty acid contents are more prone to oxidation than those with more saturated fatty acids (Manso et al, 2012; Najafi et al., 2012; Vieira et al., 2012). Some papers have dealt with the link between fatty acid modifications and product shelf life (Ansorena and Astiasarán, 2004; Valencia et al., 2006; Rubio et al., 2007). In meat, myoglobin and lipid oxidation are closely related (Renerre and Labadie, 1993) and are also affected by fatty acid composition. The degree of this oxidation depends on the balance between anti- and pro-oxidant substances. In this sense, certain vegetable oils often used in feeding diets may contain anti-oxidant compounds (Artajo et al., 2007; Nieto et al., 2010; Vieira et al., 2012).

Several works have studied the effects of ruminant diets on fatty acid composition and fat oxidation. However, specific studies related to the effects of protected fat in ewes' diets on the shelf life of their suckling lambs under storage conditions simulating retail conditions are limited. The aim of the present work was to evaluate the influence of feeding different calcium soap oil-supplemented diets to lactating ewes on the evolution of physical-chemical, microbial and sensory properties of suckling lamb meat packaged in modified atmospheres and stored under commercial display conditions.

2 Materials and methods

2.1 Animals

Animal characteristics are widely described in a previous work (Gallardo et al., 2014). Briefly, 18 pregnant Churra ewes (mean live body weight, LBW: 60.88 ± 1.366 kg) were selected before lambing and fed the same diet throughout the experimental period, but with no fat added (forage/concentrate ratio of 50:50). The ewes, aged 3–5 years with parities of 4–6, had been artificially inseminated. Two days after lambing, each ewe was assigned to one of three experimental diets based on their milk production, age, initial BW, and parity randomisation. Newborn lambs (six per treatment), covered by the protected geographical indication 'Lechazo de Castilla y León', were continuously housed with their respective mothers and nourished exclusively via suckling for the whole experimental period (from birth until they reached approximately 11.5 kg LBW). The three experimental diets consisted of an isoenergetic and isonitrogenous total mixed ration (39.38% dehydrated lucerne, 13.77% soybean meal, 11.83% corn grain, 10.38% oat grain, 7.89% barley grain, 7.86% beet pulp, 4.95% molasses and 1% vitamin mineral premix). They were supplemented with different calcium soaps of fatty acids: animals fed with 3% of Ca soap of palm oil (Magnapac®; Norel Animal Nutrition, Madrid, Spain) were considered as the control group (CO), whereas experimental diets were with 3% of Ca soap of olive oil (OLI; Olifat®; Anupal S.L., Zaragoza, Spain) and 3% of Ca soap of fish oil (FO; Strata-g Lactation®; Virtus Nutrition LLC, Corcoran, USA). All handling practises followed the recommendations of the European Council Directive 2010/63/EU for the protection of animals used for scientific purposes, and all of the animals were able to see and hear other lambs.

Treatment implications on intramuscular lipid composition have been thoroughly discussed in Gallardo et al. (2014). The authors reported the absence of differences in intramuscular fat content. Moreover, SFA percentages in intramuscular fat were unaffected by the ewes´ diet. Intramuscular fat had the highest monounsaturated fatty acid (MUFA) content in the CO and OLI treatments and the lowest MUFA in the FO lambs. Conversely, FO treatment reached the highest concentrations for all the n-3 FAs: 0.97 (18:3 n-3), 2.72 (20:5 n-3), 2.21 (22:5 n-3) and 1.53% (22:6 n-3). In addition, not only did FO intramuscular fat have the most cis-9, trans-11 18:2 (1.66%) and trans-11 18:1 (3.75%) fatty acids, but it also had the lowest n-6/n-3 ratio (1.80).

2.2 Samples and packaging

After carcasses cooled, *M. longissimus thoracis et lumborum* (LTL) were removed from both sides of the carcasses. Muscles were sliced into pieces (nine steaks of about 20 g were cut from each muscle), placed in trays that were flushed with the selected gas mixture ($80O_2$:$20CO_2$) and closed by heat-sealing with a packer (TECNOTRIP mod. TSB-100) and a high barrier film (55 µm thick, O_2 permeability 5 cc/m^2/24 h/bar at 23 °C and 50% RH and steam permeability of 19 g/m^2/24 h at 23 °C and 90% RH). The meat/headspace ratio in each package was 1/3. Because of microbial analysis is destructive, the pool of trays devoted to shelf life study were prepared in duplicate, one for microbial analyses and the other for sensory and physicochemical analysis. Finally, the trays were placed randomly in a refrigerated open-front display cabinet illuminated with white fluorescent light (620 lux) at 4 ± 1 °C. The cabinet was set to 12-h light-dark cycles to simulate the conditions of the point of sale. Trays were rotated daily to minimise light intensity differences. The packs were opened for subsequent analysis after 0, 5, 8 and 13 days of storage.

2.3 Microbial analyses

On each sampling day, microbiological testing was conducted. After pack opening, 10 g meat were aseptically taken and homogenised with 90 ml of peptone water for 2 min in a sterile plastic bag in a PK 400 Masticator (IUL, S.A., Barcelona, Spain), to obtain a mother solution. Serial decimal dilutions were made in sterile peptone water and, in duplicate, 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread onto total count and selective agar plates. The microbiological analyses were: psychrotrophic bacteria on Plate Count Agar (Scharlau, Spain) incubated at 7 °C for 10 days, enterobacteria on 3 M Petrifilm *Enterobacteriaceae* Count Plate (Bioser, Barcelona, Spain) incubated at 42 °C for 24 h, *Pseudomonas* spp. on Pseudomonads Agar (Oxoid, Spain) supplemented with Cetrimide Fucidine and Cephaloridine (CFC, Oxoid, Spain) incubated at 30 °C for 48 h and lactic acid bacteria (LAB) on MRS Agar (Scharlau, Spain) incubated at 30 °C for 48 h.

2.4 Instrumental colour measurement

Retail colour display was measured when the modified atmosphere packages were opened. Muscle colorimetric parameters [L* (lightness), a* (redness), b* (yellowness) (CIE, 1976)] were measured using a Minolta CM-2600d reflectance spectophotometer (Minolta Co., Osaka, Japan). Measures were taken in the CIE L*a*b* space under D65 illumination with a 10° observer visual angle, 11 mm aperture for illumination and 8 mm for measurement, as well as a SCI mode condition, previously calibrated against a white plate supplied by the manufacturer. For colour determination, the spectophotometer directly touched the muscle cross-section surfaces. Three different locations were measured on each tray and the results were expressed as an average of the three measurements.

2.5 Myoglobin and lipid oxidation

To determine myoglobin oxidation, samples were scanned using a 400–760 nm wavelength using a Minolta CM-2600d reflectance spectophotometer (Minolta Co., Osaka, Japan). Metmyoglobin percentage was estimated spectrophotometrically by measuring the reflectance at 525, 572 and 730 nm, according to Krzywicki (1979). The metmyoglobin relative percentage was calculated as follows: % MMb=[1,395−(A 572 − A 730) / (A 525 − A 730) x 100].

After the instrumental colour measurement, each sample was minced and the extent of lipid oxidation in LTL muscle during storage was assessed by a thiobarbituric acid reactive substance (TBARS) assay. This method is based on the reaction between malondialdehyde and thiobarbituric acid and the production of a coloured pigment, as determined by Maraschiello et al. (1999), using an UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). TBARS values were expressed as micrograms of malonaldehyde per gram of meat.

2.6 Sensory analysis

On each sampling day, samples were evaluated for appearance, colour and odour by a panel of eight people selected and trained in accordance with the International Standard method for the selection, training and monitoring of assessors (ISO ~~8586,~~ 8586; 2012). The taste panel performed the trial under controlled conditions in booths at 22 °C. Appearance was assessed in unopened trays, using a structured scale with numerical scores from 1 (excellent, fresh meat) to 5 (undesirable). Likewise, in intact trays, the percentage of discoloured or brownish meat surface was scored using a 5-point scale (1, none; 2, 1–10%; 3, 11–20%; 4, 21–60% and 5, 61–100%). Once the film was removed, panellists were asked to score odour by sniffing, using a 5-point scale (1, no off-odour; 2, slight off-odour; 3, small off-odour but not spoiled; 4, clearly recognizable off-odour and 5, extremely off-odour). For all attributes, a mean value of 3 was considered the borderline of consumer acceptability. Sensory shelf life was defined as the time in days to reach mean values of 3 (Greer and Murray, 1988; Land and Shepherd, 1988).

2.7 Statistical analysis

Data were analysed using a general linear model (GLM) in SPSS 16.0. The model included the effects of diet (CO, OLI and FO) and storage period (0, 5, 8 and 13 days) and their interactions. When the interaction between diet and storage period was significant, planned comparisons among means were performed using the Duncan method (comparing diets for each storage period and storage period for each diet, $p < 0.05$). Pearson's correlation coefficients between the microbial, physical-chemical and sensory attributes were used to evaluate their contribution to the lamb meat quality.

3 Results

3.1 Microbial counts

Counts of psychrotrophic bacteria, *Enterobacteriaceae*, *Pseudomonas* spp. and LAB of lamb meat during display are shown in Table 1. As expected, different microbial counts between treatments were observed throughout storage. In spite of numerical values seems to indicate differences between experimental treatments at the most of sampling times, in particular for *Enterobacteriaceae* and LAB, no statistically significant differences were detected ($p > 0.05$), probably due the high standard deviations. The different evolution of microbial counts during storage for each treatment could be observed considering the sampling time in which the microbial counts began to increase significantly. Regarding psychrotrophic bacteria counts, the initial value (day 0) for fresh suckling lamb ranged between 3.4 and 3.7 cfu/g. The values obtained in the CO and OLI treatments on day 8 of storage were significantly higher than those obtained on day 0. However, in the FO treatment, significant increases in psychrotrophic counts were observed after 5 days of storage. *Enterobacteriaceae* counts increased from average values of 2.6 at day 0 to 5.0 log cfu/g at the end of storage. Nevertheless, significant increases throughout the storage period were only observed in the FO and OLI treatments ($p < 0.05$). LAB counts differed between treatments. CO and FO showed significant increases at 5 days, while in the OLI treatment, 8 days of storage time were necessary to detect a significant increase. *Pseudomonas spp.* counts increased from an average of 2.0 log cfu/g to an average of 6.2 log cfu/g at the end of storage. In OLI, increases in absolute values (from 2.0 log cfu/g to 5.7 log cfu/g) were not statistically significant ($p > 0.05$). However, in both the CO and FO treatments, the values observed at the end of storage were significantly higher than initial counts ($p < 0.05$).

Table 1 Effect of ewes' fed diets (CTR, OLI and FISH) on Phycrotrophic bacteria, *Enterobacteriaceae,* Lactic acid bacteria and *Pseudomonads* spp. counts (log cfu/g) of suckling lamb meat packaged in modified

atmosphere throughout the storage (0, 5, 8 and 13 days) under commercial display conditions.

alt-text: Table 1

Microorganisms	[a]Ewes' diet	Days of storage				[b]RSD	Days p-value
		0	5	8	13		
Physcrotrophic bacteria	CTR	3.6^{A}	5.5^{AB}	6.9^{BC}	8.3^{C}	0,98	*
	OLI	3.7^{A}	4.8^{AB}	6.1^{BC}	8.1^{C}	0,72	*
	FISH	3.4^{A}	5.8^{B}	7.3^{BC}	8.0^{C}	0,58	*
	RSD	*0.44*	*0.00*	*0.34*	*0.86*		
	Ewes' diet p-value	*ns*	*ns*	*ns*	*ns*		
Enterobacteriaceae	CTR	3.0	3.8	3.1	4.3	1,79	ns
	OLI	3.0^{A}	2.0^{A}	2.7^{A}	5.2^{B}	0,56	*
	FISH	1.8^{A}	3.9^{AB}	4.6^{AB}	5.6^{B}	1,59	*
	RSD	*0.82*	*1.14*	*0.69*	*2.22*		
	Ewes' diet p-value	*ns*	*ns*	*ns*	*ns*		
Lactic acid bacteria	CTR	2.6 ^{A}	5.7^{B}	5.8^{B}	6.8^{B}	1,13	*
	OLI	3.4^{A}	4.3^{A}	5.8^{B}	7.4^{B}	0,52	*
	FISH	2.9^{A}	5.5^{B}	7.1^{B}	7.4^{B}	0,70	**
	RSD	*0.39*	*0.61*	*1.16*	*1.32*		
	Ewes' diet p-value	*ns*	*ns*	*ns*	*ns*		
Pseudomonads spp.	CTR	2.0^{A}	3.3^{AB}	5.2^{BC}	6.5^{D}	0,93	*
	OLI	2.0	3.6	3.3	5.7	1,53	ns
	FISH	2.0^{A}	3.7^{AB}	4.1^{AB}	6.3^{B}	0,83	*
	RSD	*1.11*	*1.80*	*0.92*	*0.70*		
	Ewes' diet p-value		*ns*	*ns*	*ns*		

A, B, C, D Averages for each parameter with different capital letter mean significant differences ($P < 0.05$) between days of storage within treatment.

a CTR: control group; OLI: Ca soap of olive oil; FISH: Ca soap of fish oil.

b RSD: residual standard deviation.

3.2 Physical-chemical analyses

3.2.1 Colour parameters, myoglobin and lipid oxidation

Results of colour coordinates and metmyoglobin percentages during storage are shown in Fig. 1. Significant differences in colorimetric parameters were only observed in lightness at day 0, when OLI treatment had the lowest values. In relation to the evolution of colorimetric parameters throughout storage, L* values remained constant during storage in the CO and FO groups ($p > 0.05$), while the OLI group increased at day 5 ($p < 0.05$) before remaining constant ($p > 0.05$). a*, related to redness,

increased over the first 5 days and then decreased to the end of the storage time in all treatments ($p > 0.05$). In turn, b* increased on day 5 for all treatments ($p > 0.05$), then remained invariable until the end of storage ($p > 0.05$).

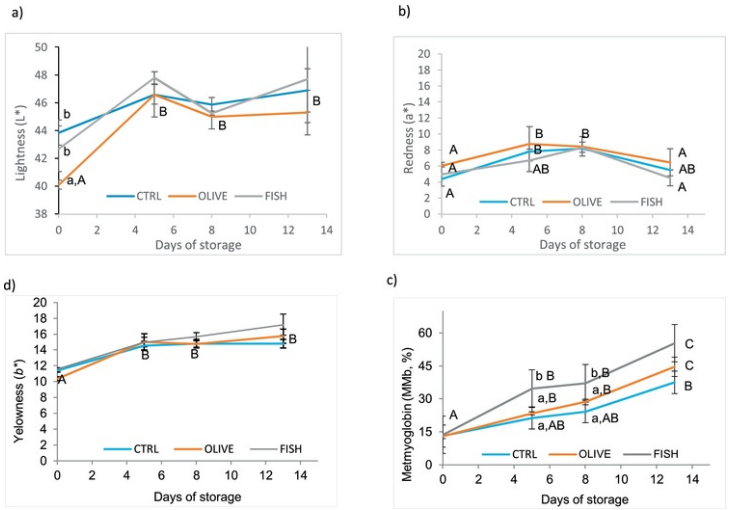

Fig. 1 Effect of experimental ewe's diet (CTR, OLI and FISH) and storage time on evolution of colour parameters: lightness (a), redness (b), yellowness (c) and metmyoglobin haem pigment (D) on *m. longissimus thoracis et lumborum* of suckling lambs stored at 2 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error.

alt-text: Fig. 1

With respect to myoglobin oxidation, the relative amount of metmyoglobin was significantly higher in the FO treatment at 5 and 8 days with respect to the other treatments ($p < 0.05$), but no significant differences were observed at the initial and final sampling points ($p > 0.05$). No differences were found between CO and OLI at any sampling point. In all treatments, the general tendency of metmyoglobin percentages was to increase during the first 5 days in storage ($p < 0.05$), remain almost constant until 8 days ($p > 0.05$) and then continue to increase until the end of the storage time ($p < 0.05$). However, although the initial mean values in lamb meat were similar in all treatments (13.3% on average), the extent to which values increased at each sampling point differed between treatments. In particular, after 8 days of storage, CO and OLI values were 24.3 and 28.6% metmyoglobin respectively, while FO reached values of 37.1%.

Lipid oxidation, measured by TBARS, is summarised in Fig. 2. When the TBARS of lamb meat were analysed for dietary treatment effects during storage, values followed a similar pattern as metmyoglobin percentages. Thus, at 5 and 8 days of storage, TBARS values for FO samples were significantly higher ($p < 0.05$) than those observed in samples from CO and OLI. However, no significant differences were found among treatments at the beginning or at the end of the storage period ($p > 0.05$). Regarding the evolution of TBARS throughout storage, significant differences with respect to the initial TBARS values were observed in FO after just 5 days, reaching 7.12 ($p < 0.05$), whereas the values for CO and OLI were 1.38 and 1.69, respectively ($p < 0.05$). TBARS values for CO and OLI differed significantly from the initial value after 8 days of storage, reaching values of 4.07 and 4.11. In both treatments, TBARS values continued to increase until the final sampling point ($p < 0.05$). However, in FO, after 8 days of storage, TBARS values were over 9.00 and remained constant until the end ($p > 0.05$).

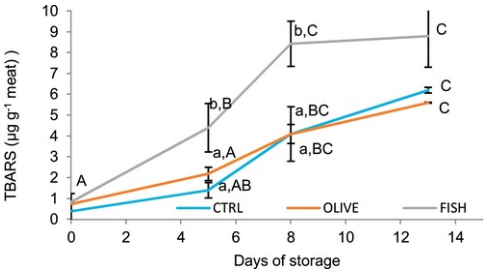

Fig. 2 Effect of experimental ewe's diet (CTR, OLI and FISH) and storage time on evolution of TBARS (µg g^{-1} meat) on *m. longissimus thoracis et lumborum* of suckling lambs stored at 2 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error.

alt-text: Fig. 2

3.2.2 Sensory analysis

Fig. 3 shows the effect of ewes' diet and storage time on the sensory attributes of the lamb meat. The sensory evaluation reflected a decrease of the typical appearance, colour and odour of lamb meat during storage; thus, scores were lowest in the recently packaged meat (day 0) and, as expected, no differences were found between treatments ($p > 0.05$) at this sampling point, with all samples having a score of 1. When the typical appearance of lamb meat was analysed for dietary treatment effects during storage, no differences ($p > 0.05$) were found between treatments at any sampling point. Scores for this parameter increased throughout storage, but in all treatments, they only reached scores above 3 (the established threshold for rejection) at the end of storage.

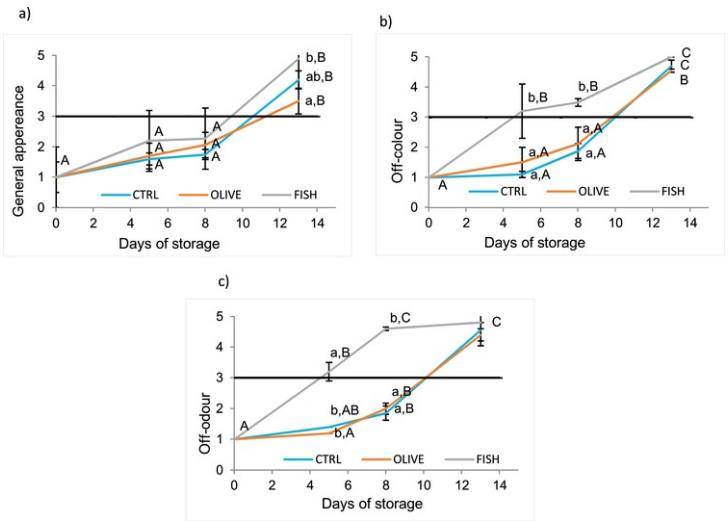

Fig. 3 Effect of experimental ewe's diet (CTR, OLI and FISH) and storage time on general appearance (a), off-colour (b) and off-odours (c) evaluated by a panel of trained members on *m. longissimus thoracis et lumborum* of suckling lambs stored at 2 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error.

Horizontal line represents threshold limit of acceptance.

alt-text: Fig. 3

With regards to colouration, evaluated as the percentage of discoloured meat surface in samples, differences between treatments were detected after 5 and 8 days of storage ($p < 0.05$). Suckling lamb meat from the FO group presented higher scores ($p < 0.05$) than those from the CO or OLI groups, with no differences between these last two treatments ($p > 0.05$). The threshold score was reached at day 5 in FO, while CO and OLI took 13 days to reach this score.

As well, the presence of off-odours, which includes all odour sensations different than those that are expected in suckling lamb meat, limited its shelf life. FO led to higher scores than the other groups after 5 ($p < 0.001$) and 8 days ($p < 0.5$), without significant differences at the end of storage ($p > 0.05$). As expected, off-odour scores increased gradually with days in storage in all groups ($p < 0.001$). However, in the FO group, significant differences with respect to initial values were found after just 5 days, when scores were already over the established threshold for rejection. In CO and OLI, 8 days of storage were required to detect significant differences ($p < 0.001$) with respect to the initial off-odour values.

4 Discussion

Counts of psychrotrophic bacteria were consistent with those observed in studies with MAP stored lamb and sheep meat (Soldatou et al., 2009; Nieto et al., 2010). In the FO treatment, significant increases between sampling points were observed earlier than in the other treatments (Table 1). CO and OLI treatments reached counts above 7 log cfu/g, considered the upper microbiological limit for acceptable quality meat as defined by the ICMFS (1986), at the end of storage, while FO showed counts exceeding this limit after 5 days of storage. These results could be due to the fatty acid profile of the meat, since FO showed a lower MUFA and a higher PUFA than CO and OLI. Similar results have been reported in shelf life studies of meat and meat products. Ouattara et al. (1997); Branen et al. (1980); Rubio et al. (2007) and Parfene et al. (2013) have indicated the antibacterial activity of fatty acids against meat spoilage organisms, such as psychrotrophic bacteria and LAB, with SFA having the greatest antibacterial activity. In general, *Enterobacteriaceae* counts increased during storage and the values at the end of storage were similar to those observed by Berruga et al. (2005), who reported that the presence of CO_2 limited the growth of *Enterobacteriaceae*.

As in psychrotrophic bacteria, LAB counts of samples from FO reached counts over the limit of 7 log cfu/g after 8 days of storage, while the values for both the CO and OLI treatments were 5.8 log cfu/g at 8 days. LAB behave as facultative anaerobes and are able to grow under high CO_2 concentrations; they constitute a substantial part of the natural microflora of MAP meats. In fact, LAB was the major microbiological flora in all treatments and was strongly correlated with other microbial groups ($r = 0.91$ with psychrotrophic bacteria and $r = 0.84$ with *Pseudomonas* spp.). LAB growth was faster in the CO than OLI, since a significant increase was only detected after 8 days storage in the OLI treatment, whereas samples from CO and FO showed significant increases at 5 days. Given that no differences were found between the CO and OLI groups in the SFA and MUFA content in intramuscular fat (Gallardo et al., 2014), other factors are responsible for the different evolution of LAB counts in the OLI and CO treatments. An antimicrobial effect of olive oil has been widely reported, since essential oils and crude extracts from olive plants have shown important antimicrobial activity in meat and meat products (Karabagias et al., 2011; Jayasena and Jo, 2013; Falowo et al., 2014). The initial counts of *Pseudomonas* spp. were similar to those obtained for Soldatou et al. (2009) in lamb meat samples stored in air, but our values were much lower than after 13 days. This difference in the final counts of *Pseudomonas* spp. between the present study and samples packaged in air are in accordance with the general agreement that Gram negative bacteria, such as *Pseudomonas* spp. and *Enterobacteriaceae,* are more sensitive to CO_2 than Gram positive bacteria. This type of result has been reported by Sheridan et al. (1997) for lamb and Kennedy et al. (2004) for beef steaks. From our microbial results, particularly from counts of psychrotrophic bacteria, the shelf life of meat from the CO and OLI treatments is 8 days, while suckling lamb meat with FO supplementation is 5 days.

There is no agreement among different studies in relation to changes in L*, a*, b* of lamb meat during storage under retail conditions. In our work, the patterns for lightness (L*), redness (a*) and yellowness (b*) were similar for all treatments. L* and b* gradually increased throughout the storage period, in agreement with results observed in other studies, which reported that increases in L* and b* in lamb meat are related to oxidation (Vergara and Gallego, 2001; Berruga et al., 2005; Linares et al., 2007). On the other hand, a*, related to redness, tended to increase during the first 8 days and then decreased to the end of storage, with lower redness values associated with browning. A similar pattern was described by Gómez and Lorenzo (2012) and Guerra-Rivas et al. (2016). However, Linares et al. (2007) and Karabaggias et al. (2011) indicated that a* values did not show a specific trend and remained more or less constant with storage time. In our study, significant differences in a* were only detected in CO and FO.

Metmyoglobin percentages were in agreement with the evolution of colorimetric parameters. Metmyoglobin percentages in lamb meat were similar in all treatments at day 0 (13.3% on average) and, also followed an analogous pattern throughout storage, increasing during the first 5 days and remaining almost constant until 8 days before continuing to increase until the end of storage. This decrease in redness from 8 days observed in our work is consistent with Warner et al. (2017), who reported a colour shelf life of 7–16 days of retail display for lamb meat packaged under high oxygen gas mixtures atmospheres; they demonstrated that, under these conditions, muscles show initiation of browning at 7 days, which is at the lower end of colour stability. However, in our study, the extent to which values increased at each sampling point differed between treatments. Metmyoglobin percentage values revealed that pigment oxidation occurred significantly faster in the FO treatment. After 8 days of storage, metmyoglobin percentage values of the CO and OLI were still at an acceptable level, while FO reached values of 37.1% metmyoglobin, near the limit of 40% that has been reported to cause consumer rejection (Greene et al., 1971); the OLI and CO groups needed 13 days of storage to reach a metmyoglobin percentage of about 40%. FO had a greater proportion of metmyoglobin than the other treatments, shortening the shelf life of the meat. These results show that feeding strategy affects both the rate and the extent of myoglobin oxidation.

The increase of lipid oxidation with storage time was expected. The higher values of TBARS for FO, with respect to CO and OLI, are consistent with its fatty acid composition. As intended, FO was rich in PUFA and had a higher PUFA concentration than CO and OLI (Gallardo et al., 2014). According to similar studies (Díaz et al., 2011; Manso et al., 2012), meat containing high PUFA contents, such as with pasture feeding, supplements finishing diets with certain free oils or oil seeds, so FO meat is expected to be more prone to lipid oxidation. After 5 days of storage, the TBARS values for CO and OLI were 1.4 and 2.2, respectively, while FO reached values of 7.1 mg MDA/kg. The limiting threshold for oxidised meat acceptability varies between studies. Considering the values reported in lambs, we have assumed the value of 4.4 mg MDA/kg meat, suggested by Soldatou et al. (2009), as a reference for rancidity detection in lamb meat. Thus, TBARS values in FO reached levels above rancidity detection at day 5, whereas in the OLI and CO groups, these values were not reached until day 13 of storage. Therefore, the susceptibility of PUFA to rapid oxidation might limit the nutritional advantage of feeding strategies whose aim is to increase the PUFA concentration in meat (Wood et al., 2003; Leticia et al., 2017). On the other hand, Lorenzo and Gómez (2012) found a relationship between microbial counts (Total viable counts, *Pseudomonas* spp. and psychrotrophic bacteria) and lipid oxidation in foal meat packaged under high oxygen modified atmospheres. Likewise, the microbiological results obtained in our study seem to indicate that lipid oxidation, dependent on fatty acid composition, was related to microbial growth, since a positive correlation between the counts of different microbial groups and TBARS was observed ($r = 0.73$, $r = 0.54$, $r = 0.74$ and $r = 0.70$ for psychrotrophic bacteria, LAB, *Enterobacteriaceae* and *Pseudomonas* spp., respectively).

The absence of statistically significant differences in TBARS values between the OLI and CO groups at any sampling times could be attributed to the absence of significant differences in the fatty acid profiles of intramuscular fat. In this sense, despite all existing knowledge, it is not yet possible to make an unequivocal conclusion regarding the effect of phenolic compounds on meat fatty acid composition (Vasta and Luciano, 2011). However, our finding contrasts with numerous studies that have demonstrated the antioxidant activity of olive oil polyphenols (Visioli and Galli, 2002; Servili et al., 2009; Bubonja-Sonje et al., 2011; Luciano et al., 2013; Falowo et al., 2014). As has been reported by Briante et al. (2002) and Dejong and Lanari (2009), several compounds of olives and olive oil, such as polyphenols (e.g. oleuropein, hydroxyl-tyrosol, rutin, quecitina) as well as cafeic, vanilllic and p-coumaric acids, are effective radical scavengers, subsequently inhibiting lipid oxidation. A similar observation was recently reported by Dua et al. (2015), who found that oleuropein was a successful natural preservative against lipid oxidation in mutton ribs under aerobic packaging conditions. The lack of antioxidant activity by OLI in our study could be due to the doses employed and the time of feeding. Subsequently, we hypothesise that a higher level of inclusion of calcium soap of

olive oil in the diet of ewes may be needed to have an effect on lipid oxidation.

High Pearson correlation coefficients were found between colour (appearance and off-colour), and colour coordinates and metmyoglobin percentage. This correlation was significant for L*, b* and metmyoglobin, being particularly high in the case of metmyoglobin content ($r = 0.87$ and $r = 0.92$ for appearance and off-colour, respectively). This is consistent with Ripoll et al. (2013), who reported that the use of metamyoglobin content rather than colorimetric parameters could be useful to for assessing sensory quality. Also, sensory results relative to off-colour are consistent with lipid oxidation. It is well known that lipid oxidation, in addition to rancid odours, off-flavour development and drip losses, decrease the shelf life of meat (Richards et al., 2002). The rate and extent of lipid oxidation are influenced by a number of factors, including iron content, the distribution of unsaturated fatty acids, pH and antioxidant levels (Gatellier et al., 2005; Ortuño et al., 2016). In this sense, several works (Chan et al., 1997; Dua et al., 2015; Ortuño et al., 2016) have reported an association between lipid oxidation and discoloration in meat, with it depending on the balance between anti- and pro-oxidant substances, including the concentration of PUFAs. At day 5, lamb meat from FO showed a metmyoglobin percentage close to the rejection threshold and meat surface discolouration scores above the limit for rejection by sensory analysis. The same pattern occurred in lamb meat from CO or OLI, but with sensory colour scores and metymyoglobin percentages only reaching the threshold for rejection after 13 days.

Odour deterioration occured much faster in FO than in the other treatments, with 56.2% and 65.5% higher scores at 5 days than those obtained in the CO and OLI treatments, respectively. Many studies have focussed on the role of fatty acids in meat odour and flavour formation and have stated that modifications of the fatty acid profile may exert changes in lipid-derived volatile compounds arising from lipid oxidation, although not all fatty acids contribute to this effect in the same amount (Dransfield, 2008; Wilches et al., 2011; Dua et al., 2015). As is generally accepted, increasing PUFA levels, particularly n-3 PUFA, affects odour because of their greater susceptibility to oxidation during storage, which leads to colour deterioration and the development of off-odours and off-flavours. In general, the dietary inclusion of vegetable oils, rich in PUFAs, results in a higher presence of off-odours and off-flavours in lamb meat compared to non-supplemented diets (Francisco et al., 2015). In particular, fish oil-supplemented diets, according to Nute et al. (2007), reduce lamb meat flavor, which was linked to a high proportion of C20:5 n-3 and C22:6 n-3. As has been previously published (Gallardo et al., 2014), higher percentages of these fatty acids were observed in suckling lamb meat from the FO group than in the OLI and CO groups. Díaz et al. (2011) reported that lamb odour decreased with the storage period, while higher abnormal odours, such as rancid odours, were found with longer storage time in lambs fed a fish oil-supplemented diet.

Off-odour scores were positively and strongly related to TBARS values ($r = 0.87$). According to the scores given by panellists, off-odours were clearly detected in FO samples at day 5; this perception coincided with a TBARS value (5.62) that clearly exceeds the limit for lipid oxidation/rancidity perception of 4.4 mg MDA/kg (Soldatou et al., 2009). In turn, at day 5, CO and OLI showed TBARS values of 1.39 and 2.19 mg MDA/kg, respectively, that agree with the scores given by the panellists (less than 1.5), meaning that they corresponded to samples without off-odours. After 8 days of storage, CO and OLI treatments reached TBARS values (4.1 in both treatments) close to 4.4 mg MDA/kg, which coincides with the sensory analysis results in which only a slight off-odour was detected (scores close to 2).

For all sensory parameters, CO and OLI samples only reached a score of 3, the established threshold for rejection, at the end of storage (13 days). Likewise, Ortuño et al. (2015) noted a storage time of 11 days to reach the threshold for rejection in lamb meat, which had been established as the loss of half of the initial freshness. In contrast, in our study, FO exceeded the threshold for rejection after just 5 days of storage, which implies an important decrease in shelf life with respect to the other treatments.

5 Conclusion

In summary, the supplementation of ewe diets with different calcium soap oils modified the shelf life of meat from lambs nourished exclusively by suckling. The meat of suckling lambs from ewes fed with calcium soap of fish oil showed a shorter shelf life than calcium soap with palm oil and olive oil, when packaged under modified atmospheres and stored under display conditions. Therefore, from a consumer's point of view, results indicate that the advantage of improving the nutritional value of suckling lamb meat by supplementation with polyunsaturated fatty acids in the ewes' diet could be minimised because of a shorter shelf life under display conditions.

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Highlights

- The effect of oil calcium soap in ewes diet on suckling lamb meat quality was studied.
- Shelf life of suckli: residual standard deviation lamb meat depended on their mothers' diet.
- Ewes’ fed with calcium soap ofte panel performed the tri fish oil provided lamb meat with shorter shelf life.
- Higher microbial counts and lipid oxidation were obtained with fish oil calcium soap.
- Olive and palm oil calcium soaps give meat with similar shelf life.

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