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Effects of grape pomace in growing lamb diets compared with vitamin E and grape seed extract on meat shelf life

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Abstract

The effect of dietary treatment (CTRL, control; VIT-E, 500 mg kg^{-1} vitamin E; GSE, 50 mg grape seed extract kg^{-1}; GP-5, 5% dried red grape pomace kg^{-1}) on shelf life of lamb meat was studied. After slaughter (27 kg LBW), *m. longissimus thoracis et lumborum* was sliced, packaged under modified atmosphere (80:20% / O_2:CO_2) and stored in retail conditions for 14 days. At each sampling day (0, 4, 7, 11, 14), microbiological, physico-chemical and sensory characteristics were analysed. Meat from VIT-E presented lower microbial counts than CTRL, GSE and GP-5, without differences between polyphenol treatments (GSE and GP-5) and CTRL. Vitamin E reduced meat discoloration and lipid oxidation (TBARS values) from day 7 with respect to the other treatments. Although not significant, an improvement in TBARS values of about 20% was observed for GSE and GP-5, compared with CTRL, from day 7 of storage. VIT-E dietary treatment was more effective in preventing sensory spoilage than the other treatments.

Keywords: Grape pomace; lamb; meat shelf life

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1. Introduction

Due to changes in shopping and consumption habits, producers try to extend the shelf life of meat by storage practices such as modified atmosphere packaging. Shelf life is conditioned by oxidative processes, which are brought about by temperature, oxygen exposure, light and microbial growth. It has been reported that optimum colour stability in red meat is obtained by using gas mixtures containing high concentrations of oxygen together with low proportions of carbon dioxide, which exhibits antimicrobial activity and therefore restricts the growth of aerobic spoilage bacteria (Jeremiah, 2001). A gas composition of 20 to 30% and 70 to 80% (O_2:CO_2) is generally used for meat packaging. Meat quality is increasingly important in animal production in order to meet consumer demands, with colour and flavour being among the most relevant attributes. The gas O_2 is responsible for the desirable bright red colour (oxymyoglobin) at the time of purchase. However, the presence of high oxygen concentrations in packages may enhance lipid oxidation in meat (Fernandes et al., 2014). Besides microbial development, one of the main reasons for the deterioration of meat products during processing, storage and retail display is lipid and myoglobin oxidation, which generates products that are undesirable from a sensory point of view, making the meat unfit for consumption. Therefore, control of this process is essential to preserve the quality and shelf life of the product (Falowo, Fayemi, & Muchenje, 2014). This objective has been approached in several studies by the exogenous addition of antioxidants, or by adopting feeding systems that can improve the antioxidant status of muscle. Indeed, synthetic additives have been widely used in animal nutrition and the food industry in order to preserve meat, but they have been questioned because of their toxicity,

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pathogenicity and carcinogenic effects on humans and animals (Hayes, Allen, Brunton, O'Grady, & Kerry, 2010). The increasingly demanding consumer preference for natural products and health benefits has intensified the search for alternative methods to retard lipid oxidation in foods, such as the use of natural antioxidants, which could be a suitable alternative in animal feedstuffs, thus avoiding any further manipulation of the meat.

The use of agro-industrial by-products, such as winemaking residues, as feed in ruminant diets could be adopted as a strategy to reduce feeding costs and also to cope with the need to recycle waste material which is costly to dispose of. Grape pomace is the main residue generated by the wine industry, consisting of grape seeds, skin and pulp. Grape pomace as feed could be interesting because of the presence of a wide range of polyphenols, mainly composed of flavonoids, including anthocyanins, flavonols and flavanols such as condensed tannins, which are characterised by their high antioxidant and antimicrobial properties (Guéndez, Kallithraka, Makris, & Kefalas, 2005). In this regard, winemaking by-products have been associated with effective antioxidant activity in beef (Ahn, Grün, & Fernando, 2002), lamb meat (Jerónimo et al., 2012) and sheep plasma (Gladine, Rock, Morand, Bauchart, & Durand, 2007), so they could be an alternative to synthetic antioxidant additives.

The aim of the present study was to investigate the shelf life of lamb meat during storage in retail sale conditions after it had been packaged under modified atmosphere and grape pomace from red wine had been included in the diet of the lambs. Vitamin E was included in another group as a positive control because it is one of the antioxidants most frequently used in animal nutrition, and grape seed extract (GSE) is a

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commercially available natural extract from grape seed, rich in polyphenols.

2. Material and methods

2.1. Animals and experimental design

Forty-eight weaned male Merino lambs (initial age 8–9 weeks) housed in individual pens were assigned randomly on the basis of live body weight (LBW, 14.3 ± 2.05 kg) to four homogeneous dietary treatments: control (CTRL, 50 mg of vitamin E per kg of concentrate), vitamin E (VIT-E, 500 mg of vitamin E per kg of concentrate), grape seed extract (GSE, 50 mg of grape seed extract per kg of concentrate, "GRAPE-AOX", Cargill Animal Nutrition Spain) and grape pomace (GP-5, 5% of dry grape pomace from red wine production per kg of concentrate). All concentrates (Table 1) were formulated so as to be isonitrogenous and isoenergetic in terms of net energy (UFV, feed unit for maintenance and meat production), except GP-5 concentrate, which displayed the same energy: protein ratio as the other groups.

After 10 days of adaptation to the experimental diets, the lambs were allowed *ad libitum* access to experimental concentrates, barley straw and fresh drinking water. Feed was provided once daily at 9:00 h. Lambs were weighed weekly until they reached a slaughter weight of 27 kg LBW. Average daily gain was 276 ± 47.1 g and no differences attributable to any experimental treatment were observed ($P > 0.05$). At the conclusion of the trial, lambs were slaughtered by stunning and exsanguination from the jugular vein; they were eviscerated and skinned. After slaughter, the carcasses, weighing 12.9 kg on average, were chill-stored at 4 °C for 24 h. All handling practices followed the

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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. The pH values were determined on *longissimus* muscle 24 h post mortem. Ultimate pH was within the normal range of fattening lambs (5.6 ± 0.02). It reflected good slaughter management of animals and a regular trend of the post mortem glycolysis in muscle.

After cooling the carcasses, the *m. longissimus thoracis et lumborum* (LTL) was removed from both sides of the carcasses to carry out the various analyses. In a previous study, incorporation of vitamin E, grape seed extract and grape pomace into lamb diet had minimal effects on the muscle fatty acid profile (Guerra-Rivas et al., 2013).

2.2. Shelf life analysis

2.2.1. Sample preparation

After slicing, LTL chops (about 3 cm thick) from each carcass were placed in trays and were randomly assigned to different storage periods (0, 4, 7, 11 and 14 days). Then the trays were flushed with the selected gas mixture (80:20% / O_2:CO_2), closed by heat-sealing with a packer (TECNOVAC mod: Linvac 400) with a high barrier film (with an oxygen transmission rate of 1.8 cm^3/m^2/24 h/bar at 20 °C and 65% RH, supplied by Fibosa Packaging S.L., Tordera, Spain). The trays were placed randomly on a cabinet illuminated with white fluorescent light (620 lux) at 4 ± 1 °C, simulating retail display conditions for storage. The trays were rotated daily to minimize light intensity differences and possible temperature variations. On each sampling day, the corresponding trays were removed for subsequent analysis. Half of the

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trays to be analysed at each sampling point in each treatment were used for carrying out microbial analyses and the other half were used for the other measurements.

2.2.2. Microbiological analysis

For microbiological assays, after opening the pack 10 g was taken aseptically from each tray and homogenised with 90 ml of tryptone water (Scharlau, Spain) for 2 min in a sterile plastic bag in a PK 400 Masticator (IUL, S.A., Barcelona, Spain). Serial decimal dilutions were made in sterile tryptone 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 of the samples that were performed were: total viable counts (TVC) determined on 3 M Petrifilm Aerobic Count Plate (Bioser, Barcelona, Spain) incubated at 30 °C for 72 h; enterobacteria on 3 M Petrifilm Enterobacteriaceae Count Plate (Bioser, Barcelona, Spain) incubated at 42 °C for 24 h; *Pseudomonas* spp. on Pseudomonas Agar (Oxoid, Spain) supplemented with Cetrimide, Fucidine and Cephaloridine (CFC, Oxoid, Spain) incubated at 30 °C for 48 h; lactic acid bacteria (LAB) on MRS Agar (Scharlau, Spain) incubated at 30 °C for 72 h, and *Brochothrix thermosphacta* on STAA Agar (Oxoid, Spain) supplemented with STAA selective supplement (Oxoid, Spain) incubated at 25 °C for 48 h. Presumptive colonies were differentiated from pseudomonads by performing an oxidase test using Oxidase Test Sterile Swabs (Scharlau, Spain). The detection limit of the above techniques was 1 log cfu g^{-1} except for pseudomonads, for which the limit was 2 log cfu g^{-1}.

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2.2.3. Instrumental colour measurements

Surface instrumental colour of LTL muscle slices was measured during storage after opening the packages, using a reflectance spectrophotometer (Konica Minolta CM-2600d; Osaka, Japan). The illuminant used was D65 (colour temperature of 6504 K) and the standard observer position was 10°. Colour results were expressed as CIE *L*a*b** values: *L** (lightness), *a** (redness) and *b** (yellowness). The measurements were performed on each slice of muscle at three different locations. For each parameter, at each time, the value was calculated as the average value of three determinations per replica. The hue angle (*H**), which defines colour (0° is red; 90° is yellow), was calculated as arctangent (*b*/a**), and the chroma (*C**), a measure of colour intensity (0 is dull; 60 is vivid), was computed as $\sqrt{(a^{*2} + b^{*2})}$.

The oxidation state of myoglobin was also measured indirectly in LTL muscle by spectrophotometry during storage for each time. Haem pigment percentages were estimated according to Krzywicki (1979) from 400 to 740 nm. Metmyoglobin (MMb) was calculated as: MMb = (1,395 – a_1), where: $a_1 = [(D_R^{572} - D_R^{730})/(D_R^{525} - D_R^{730})]$; D_R = (–log R).

2.2.4. Lipid oxidation analysis

The extent of lipid oxidation in LTL muscle during storage was assessed by measuring thiobarbituric acid-reactive substances (TBARS). TBARS were determined in meat samples according to the method described by Maraschiello, Sarraga, and García-Regueiro (1999). TBA

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values were expressed as micrograms of malonaldehyde per gram of meat.

2.2.5. Sensory evaluation

Sensory analysis was carried out in raw meat. For each sampling day, samples were evaluated for appearance, display of muscle oxidation, presence of off-odours and overall rating of the sample by a panel of six people selected and trained in accordance with the International Standard method for selection, training and monitoring of assessors (UNE-EN ISO 8586:2014). The taste panel performed the trial under controlled conditions in booths, at 22 °C.

The appearance was assessed in unopened trays, using a structured scale with numerical scores from 1 (excellent, fresh meat) to 5 (extremely undesirable). Likewise, in intact trays, display of muscle oxidation measured as the percentage of discoloured or brownish meat, 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 had been removed, the panellists were asked to score odour by sniffing, using a 5-point scale (1, no off-odours; 2, slight off-odours; 3, small off-odours but not spoiled; 4, clearly recognizable off-odours and 5, extremely strong off-odour). Overall rating was also evaluated using a 5-point scale (1 = excellent; 2 = good; 3 = acceptable; 4 = fair; 5 = unacceptable).

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2.3. Statistical analysis

Data were analysed by repeated-measures analyses using the MIXED procedure of the SAS 9.2. package, according to the model $Yi_{jk} = \mu + T_i + W_k + T_iW_k + B_j + E_{ijk}$; where Y_{ijk} is the response variable, μ the overall mean, T_i the treatment (T) effect (CTRL, VIT-E, GSE and GP-5), W_k the storage period effect (D), T_iW_k the interaction (T x D), B_j the block effect, and E_{ijk} the residual effect. The statistical significance of differences was defined as P values <0.05 and trends as P values <0.10. Pearson's correlation coefficients between the microbial, sensory attributes and physical–chemical analysis were calculated to evaluate their contribution to the meat lamb quality.

3. Results and discussion

3.1. Microbial results

Table 2 summarizes the results of the microbial analysis of LTL muscle slices packaged under a gas mixture (80:20% / O_2:CO_2) during refrigerated storage, from lambs belonging to the various experimental dietary treatments.

The initial bacterial load is important for determining the shelf life of meat. A high number of microorganisms in meat before storage shortens the shelf life, since the microorganism limit will be achieved more rapidly (Berruga, Vergara, & Gallego, 2005). In our work, no differences between treatments were observed initially in the lamb meat before storage. As has been widely reported, atmospheres containing 20% CO_2 control the growth of bacteria, but modified atmosphere packaging with a high concentration of oxygen allows the growth of aerobic microorganisms in refrigerated storage conditions, associated with meat

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spoilage (Buys, Nortjé, Jooste, & Von Holy, 2000). Therefore, as expected, all the microbial populations increased significantly during refrigerated storage ($P < 0.05$). However, the evolution of the microbial groups studied varied according to the treatment. When counts were above 7 log cfu g^{-1} the product was considered unsuitable for consumption, according to the limit for total microbial count for cuts of meat established by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), since higher microbial loads lead to sensory loss due to off-flavours, off-odours and slime. This level has previously been suggested as being indicative of bacterial spoilage. Counts of TVC remained stable for all the dietary treatments from the beginning to the seventh day of storage, when they began to increase significantly ($P < 0.05$). However, while the counts in meat from the CTRL, GSE and GP-5 groups continued increasing until the end of storage, VIT-E values remained constant from day 7 onwards. As a result, counts of this microbial group presented significantly lower ($P < 0.05$) average values in meat from VIT-E treatment than average values from the CTRL, GSE and GP-5 groups from 11 days of storage. Furthermore, the VIT-E treatment did not reach counts above the limit of 7 log cfu g^{-1}, while the other treatments showed counts exceeding this limit at 14 days of storage. Enterobacteria counts, generally considered a good hygiene indicator, began to increase significantly ($P < 0.05$) after 11 days of storage. From this sampling point, meat from the VIT-E treatment presented significantly lower ($P < 0.05$) average values than the other treatments. The final values of the VIT-E treatment in both microbial groups (TVC and enterobacteria) were much lower than those reported by Berruga et al. (2005), who obtained counts similar to those observed in our study for the CTRL, GSE and GP-5 treatments. With regard to lactic acid bacteria, which behave as facultative anaerobes and

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are able to grow under relatively high concentrations of CO_2, no statistical differences were detected between treatments from 0 to 11 days of storage. At day 14, VIT-E showed lower ($P < 0.05$) values than the other treatments. The behaviour of *Pseudomonas* spp. was similar in all groups, beginning to increase from day 7 to the end of storage, when the highest values were reached. However, from day 11 the VIT-E treatment showed lower values than the others ($P < 0.05$).

Our results seem to indicate that vitamin E was effective ($P < 0.05$) in preventing microbial development. In contrast, Lauzurica et al. (2005), using vitamin E in lamb diets, reported that dietary vitamin E supplementation did not affect microbial growth in meat packaged under modified atmosphere. Similarly, although most studies on the effect of dietary vitamin E observe an improvement in pigment and lipid stability, no important effects of vitamin E on microbial growth during storage have been reported (Ripoll, Joy, & Muñoz, 2011). Since we have not found evidence in the literature of the presence of specific antibacterial compounds resulting from vitamin E dietary treatment, we can only hypothesize that these antibacterial effects might be related to its antioxidant capacity.

It is interesting to note that no statistical differences ($P > 0.05$) in microbial results were observed between the polyphenol treatments (GSE and GP-5) and the CTRL group. These results are in agreement with Morán et al. (2012), who did not find that inclusion of rosemary polyphenols in lamb diets had an effect on microbial spoilage. In contrast to our results, Rota, Herrera, Martínez, Sotomayor, and Jordán (2008) provided evidence for the efficacy of polyphenols as antimicrobial agents, capable of altering bacterial cell membranes and microbial enzymatic metabolism with high antibiotic activity. In this

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regard, several studies have shown that dietary supplementation with rosemary (Ortuño, Serrano, Jordán, & Bañón, 2014) or quercetin flavonoid (Andrés et al., 2013) reduced microbial populations responsible for meat spoilage during storage owing to the accumulation of these compounds in the muscles during the life of animals (Raccach, 1984). In this regard, Reddy et al. (2013) reported that the addition of GSE to mutton slices significantly reduced total psychrophilic and coliform counts in meat during refrigerated storage; therefore, grape by-products could exert a protective effect against microbiological spoilage. In any case, most of these studies lack crucial information for assessing dose effects, particularly concerning the degree of degradation of active polyphenol compounds in the feed given to lambs, which could explain our results. Given that the cut-off point for microbiological shelf life was set at a bacterial count of 7 log cfu g^{-1}, the microbiological shelf life of meat from the VIT-E group would be longer than that of meat from the polyphenol treatments (GSE and GP-5) and CTRL.

3.2. Colour coordinates and metmyoglobin percentage.

Results of the colour measurements and metmyoglobin haem pigment percentage are shown in Figure 1. In general, storage time significantly affected the evolution of meat colour parameters. Modified atmosphere with a high proportion of oxygen enhances meat colour owing to the formation of oxymyoglobin, which maintains the desirable bright red colour of meat and which appears in approximately the upper 5 mm of the meat cut. Thus high O_2 levels prolong the colour of the meat before metmyoglobin becomes visible on the surface, which is responsible for meat browning (Fernandes et al., 2014).

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The behaviour of the colour coordinates was fairly similar for all treatments during the first days of storage. In this regard, lightness (L^*), yellowness (b^*) and hue angle (H^*) remained nearly stable during the first 7 days, while the redness parameter (a^*) increased during the first 4 days of storage and then decreased. Although the intensity of the red colour (chroma, C^*) remained stable throughout the trial, it reached higher numerical values between days 0 and 4 of storage, following a similar tendency to a^*. These results agree with similar studies regarding the evolution of colour in lamb meat during retail storage under modified atmosphere packaging (Bodas et al., 2012; Fernandes et al., 2014) as time of storage progresses.

From day 7 onwards, lightness (L^*) generally increased with time of storage. With regard to the dietary treatment effects, lower L^* average values were obtained in the VIT-E treatment throughout the complete storage period when compared with CTRL, GSE and GP-5 (41.86 vs. 44.23, 44.29 and 43.76, respectively), but the difference was only statistically different ($P < 0.05$) from 11 days of storage. Lamb colour stabilization through dietary vitamin E has also been reported in other studies with lambs (Karami, Alimon, Sazili, Goh, & Ivan, 2011; Ripoll et al., 2011). Vitamin E is able to improve the overall muscle antioxidant status by lowering the formation of some oxidation markers and consequently extending meat colour stability (Descalzo et al., 2007). The stabilization and improvement of meat colour by vitamin E supplementation is not completely understood, but it has been speculated that it is principally due to its ability to prevent the oxidation of myoglobin and/or oxymyoglobin to metmyoglobin and thus meat discoloration (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Some authors have observed that dietary inclusion of phenolic

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compounds produces lighter meat because they are iron-chelating agents promoting a lower blood haemoglobin concentration and probably lower myoglobin concentration before slaughter (Samman et al., 2001). Our results do not support that suggestion and are in agreement with various authors (Bodas et al., 2012; Andrés et al., 2013) who did not find statistically significant differences in L^* in lamb meat supplemented with polyphenol-rich substances (naringin and quercetin, respectively).

Redness (a^* value) is one of the most important colour parameters for evaluating meat oxidation, since consumers prefer fresh, red-coloured meat. In the present trial, lambs fed VIT-E showed higher overall a^* values ($P < 0.05$) than lambs fed CTRL, GSE and GP-5 (9.55 vs. 8.03, 7.77 and 7.27, respectively), but the difference was only significant at 11 and 14 days of storage, similarly to what was found by Lauzurica et al. (2005) when they included vitamin E in lamb diets. Our results contrast with Karami et al. (2011) and Atay, Gokdal, Eren, Cetiner, and Yikilmaz (2009), who did not report differences in a^* index when lambs received vitamin E supplementation compared with a control group. The GSE and GP-5 supplementation did not affect the a^* value, which is in agreement with several authors (Karami et al., 2011; Andrés et al., 2013), who did not find statistically significant differences in a^* in lamb and goat meat supplemented with different natural sources of polyphenols compared with a control group. However, other studies (Luciano et al., 2009; Ortuño et al., 2014) have reported lower decreases in redness in meat from lambs fed a phenol-rich source compared with meat from lambs fed a control diet, probably as a result of decreased myoglobin oxidation by the phenolic presence. No effect of dietary treatment ($P > 0.05$) on yellowness (b^*) was observed, which is in line with what was reported by Atay et al. (2009), who did not find differences in b^* when they

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included vitamin E in lamb diets compared with a control group. In contrast, Karami et al. (2011) reported higher *b** values in vitamin E fed goats compared with a control group. Like Bodas et al. (2012), we did not observe *b** differences resulting from the addition of dietary phenolic compounds. However, the present results contrast with other studies (Luciano et al., 2009; Andrés et al., 2013), which reported lower *b** values during storage in meat of lambs fed with different polyphenol sources compared with a control group.

There were no significant differences in chroma ($P > 0.05$) among the groups, which is in agreement with Andrés et al. (2013) in meat of lambs fed with polyphenols. However, these results contrast with other authors (Ortuño et al., 2014) who recorded higher *C** values in meat from animals supplemented with polyphenols in their diets, related to lower meat discoloration. Hue angle (*H**) provides a more realistic view of meat browning than individual colour coordinates (Luciano et al., 2009). In this regard, Ripoll, Joy, Muñoz, and Albertí (2008) stated that human evaluators are not able to appreciate individual *L**, *a**, *b** coordinates, but they are able to understand real colour (hue); thus an increase in *H** values over time is considered a good descriptor of meat browning as it correlates well with the visual appraisal of meat discoloration and with the accumulation of MMb on the meat surface (Luciano et al., 2011). Our data showed that the administration of vitamin E reduced ($P < 0.05$) the rate of increase in *H** values over time compared with the other diets from day 11; therefore, VIT-E seems to delay meat discoloration. Our results are in agreement with Ripoll et al. (2011), who found lower *H** values in lambs fed with vitamin E compared with a control group. However, the present results contrast with Karami et al. (2011), who found higher *H** in meat from goats supplemented with vitamin E. Our

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results are also in accordance with previous works that showed that colour of meat was not affected by dietary phenol sources (O'Grady, Carpenter, Lynch, O'Brien, & Kerry, 2008). The lack of differences ($P > 0.05$) in H^* index between the GSE and GP-5 groups compared with CTRL could be explained by the greater variability values. This response contrasts with results reported by other authors (Luciano et al., 2011; Jerónimo et al., 2012; Morán et al., 2012) employing polyphenol-rich plants, who found lower H^* values in meat from lambs fed a diet supplemented with these phenolic substances, owing to the protective effect of these substances against meat discoloration, preventing MMb formation.

The results of the colorimetric parameters, particularly H^* values, are consistent with the percentages of MMb observed in the VIT-E treatment, since lower percentages of MMb were observed ($P < 0.05$) in this group after 11 days of storage, when all the treatments except VIT-E reached values above the limit of 40% that has been reported to be a level that causes rejection. Similar results were observed by Lauzurica et al. (2005), who reported a delay in MMb formation when they included vitamin E in lamb diets.

With regard to the polyphenol treatments, it should be noted that, despite showing percentages above the limit of acceptance, GSE presented lower values than GP-5 at 14 days of storage, while CTRL values were intermediate. In this regard, Luciano et al. (2011) reported that consumption of tannins could improve the colour stability of lamb meat, since meat from lambs fed with a polyphenol source presented lower increases in MMb percentage compared with meat from animals fed with a control diet.

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3.3. Lipid oxidation

Initial TBARS concentrations were not significantly different between the four experimental treatments, ranging from 0.03 to 0.09 μg MDA g^{-1} muscle. As expected, meat lipid oxidation increased strongly ($P < 0.05$) with storage time (Figure 2), reaching high MDA values, probably as a result of the oxidizing conditions during storage (high O_2:CO_2 atmosphere and intense lighting). The limiting threshold for oxidized meat acceptability varies according to the animal and the study. In lambs, a TBA value of 4.4 mg MDA kg^{-1} meat was taken to mark the initiation of lipid oxidation/rancidity by Soldatou, Nerantzaki, Kontominas, and Savvaidis (2009). Dietary administration of antioxidants could be an interesting strategy because, for instance, supplementing animal diets with vitamin E has been extensively shown to effectively enhance the resistance of meat to oxidative deterioration. Vitamin E is the primary lipid-soluble antioxidant in biological systems and breaks the chain of lipid oxidation in cell membranes (Buckley, Morrissey, & Gray, 1995). In the present study, vitamin E supplementation in the diet was effective in preventing muscle MDA formation during storage in modified atmosphere packaging, since significantly lower ($P < 0.05$) TBARS values were found from day 7 onward with respect to the other treatments. These findings are consistent with results found by other authors in sheep dietary studies on α-tocopheryl acetate (Lauzurica et al., 2005; Ripoll et al., 2011; Muíño et al., 2014), who reported that dietary vitamin E inhibited MDA formation powerfully in lamb meat, especially during longer retail display periods.

On the other hand, a number of secondary compounds in plants, such as phenolic compounds and essential oils, possess antioxidant properties,

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and therefore their use as natural antioxidants in animal feeding could be promoted. In this regard, several studies have indicated that polyphenols in diets of small ruminants, such as rosemary or its derivatives (Morán et al., 2012; Ortuño et al., 2014), or other phenol-rich substances (Karami et al., 2011; Andrés et al., 2014) clearly delayed lipid oxidation of meat in retail display conditions, reducing MDA formation. In the current study, as seen in Figure 2, lower numerical values of TBARS, although not significant ($P > 0.05$), were found in the GSE and GP-5 groups from day 7 of storage compared with CTRL, achieving an MDA percentage improvement of 18.3% and 35.3% at day 7, 2.8% and 16.6% at day 11, and 23.3% and 11.4% at day 14, for the GSE and GP-5 treatments, respectively, with respect to CTRL, which could be attributed to the phenolic content of the GSE and GP-5 diets. The lack of significant differences in our study could be due to the doses employed, the time of feeding and the meat oxidative susceptibility. Indeed, Jerónimo et al. (2012) indicated that the inclusion of different polyphenol sources in lamb diet, including grape seed extract, reduced meat lipid oxidation during storage. In that study the diets were supplemented with vegetable oil, making the meat more prone to oxidation.

It is interesting to note that, in spite of the lack of significant differences in oxidation stability between VIT-E and wine by-products (GSE and GP-5) at the end of storage, the meat from antioxidant treatments (VIT-E, GSE and GP-5) did not reach the rancidity threshold established (4.4 µg MDA g^{-1} meat), but this level was exceeded by the CTRL treatment. This finding could be an indication of the antioxidant effects of wine by-product additives.

This effect of natural antioxidants, in particular phenolic substances, has been attributed to their ability to attenuate oxidative damage of a

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tissue indirectly by enhancing the natural defences of the cell and/or directly by scavenging the free radical species or through activation of antioxidant enzymes, combating disorders generated by phytochemical reactive oxygen species (ROS) (Du et al., 2010). Although several studies (Moñino, Martínez, Sotomayor, Lafuente, & Jordán, 2008; Luciano et al., 2011) have shown that their dietary inclusion favoured the antioxidant stability of meat, their mechanisms of action remain to be established. Direct antioxidant activity of dietary polyphenols would imply their absorption through the gastrointestinal tract and their transfer to tissues (Luciano et al., 2009). In the case of polymeric and high molecular weight substances, such as condensed tannins, their absorption could be limited and it is unlikely that oligomers larger than trimers could be absorbed in the small intestine in their native form (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Hydrolysis of polymers into compounds with a low degree of polymerization or monomers would make their absorption possible. However, the effect of dietary polyphenols on meat oxidative stability may be indirect, through interaction between phenols and other antioxidant compounds or pro-oxidant compounds present in meat.

3.4. Sensory evaluation.

Figure 3 shows the results of sensory evaluation of the LTL muscle slices packaged under a gas mixture (80:20% / O_2:CO_2) during refrigerated storage. As expected, no differences were found between treatments ($P > 0.05$) in the recently cut meat (day 0), all of the samples having a score of 1. In general, sensory quality decreased gradually during the whole period of storage and reached the highest scores at the end of the experimental period. However, the changes in deterioration

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scores varied between dietary treatments. It should be noted that for all attributes the mean score of 3 was considered as the borderline of consumer acceptability, following the guidelines of Land and Shepherd (1988).

At the day 7 sampling point, GSE showed lower general appearance scores than CTRL and GP-5, VIT-E scores being intermediate and not different. However, this potential antioxidant effect of GSE disappeared at the end of storage, when only lower scores of VIT-E were detected ($P < 0.05$). From day 11 onwards the perception of discoloration was lower in VIT-E than in the other groups, which had already reached scores above 3 at this sampling point. These results are consistent with the relative metmyoglobin percentage observed, since at that sampling point the values reached for all groups except VIT-E were higher than 40%, the limit established by several works as acceptable.

Similarly, the presence of off-odour increased gradually and significantly during storage in all groups. However, after 11 days of display VIT-E showed significantly lower values ($P < 0.05$) than those of CTRL and GSE, with GP-5 having intermediate values. It is interesting to note that at that point only VIT-E and GP-5 showed scores below 3, which was established as the threshold for rejection. Although VIT-E had the lowest values at the end of storage, all treatments presented values above this limit.

The overall rating of the samples reflects the changes in the other sensory attributes studied. Thus, according to the panellists, all the samples except meat slices from the VIT-E group reached values above 3 at 11 days of storage (3.93, 3.80 and 3.27 for the CRTL, GSE and GP-5 groups, respectively, and 2.60 for the VIT-E treatment).

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VIT-E dietary treatment was more effective ($P < 0.05$) in preventing sensory spoilage in the last days of storage than the other experimental treatments, coinciding with its favourable effect on preventing meat formation of free radicals (Arnold, Scheller, Arp, Williams, & Schaefer, 1992) and improving colour stability (Faustman & Casens, 1990). This agrees with the findings of Muíño et al. (2014), who observed that a trained panel judged the colour, flavour and overall appearance of dietary vitamin E supplemented lamb meat more favourably than non-supplemented meat.

In spite of the slight differences observed at day 7 in general appearance and at day 11 in off-odour and overall rating, where values of GSE and GP-5 were intermediate between CTRL and VIT-E, the inclusion of these polyphenol supplements in the diet did not achieve an extension of the shelf life of lamb meat in comparison with CTRL. As a result, only lamb meat from the VIT-E dietary treatment showed acceptable sensory quality after 11 days of storage in retail conditions. These results are in accordance with other studies (Jerónimo et al., 2012; Muíño et al., 2014), which did not detect effects of inclusion of polyphenols from different sources in lamb diets on sensory meat properties. On the other hand, some studies have observed better values in meat and fat colour when polyphenols are included in lamb diets (Ortuño et al., 2014). Moreover, some authors have also indicated that polyphenol addition in lamb diets reduces off-flavours and rancid-odour perceptions of meat on display, owing to their amelioration of oxidative stability. In this regard, some studies found more favourable lamb meat odour values (Priolo et al., 2009; Ortuño et al., 2014) when lamb diets were supplemented with polyphenols. Given that some positive results in

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sensory parameters were observed, more research on the use of such wine by-products in sheep feeding might be recommendable.

3.5 Interrelation among parameters defining shelf life of lamb meat

The effect of ewe´s diet on shelf life of lamb meat has been highlighted through the different parameters analyzed. The different factors involved in the shelf life of meat, such as microbial counts, colour, lipid oxidation and sensory quality were strongly interrelated. The relationship between microbial counts and TBARs has been demonstrated through the positive correlation between the counts of different microbial groups and lipid oxidation measured as TBARs. For instance, the Pearson coefficients between TBARs and TVC and Enterobacteria counts were r = 0.70, r = 0.69 respectively ($P < 0.001$), which is consistent to different studies in meat packaged under high oxygen modified atmospheres, which reported that results of microbial counts are related to lipid oxidation. The correlation between TBARs and sensory parameters, in particular with the presence of off-odours (r = 0.76; $P < 0.001$), confirms the effect of lipid oxidation on the development of metabolites that give rise off-odours. In case of colour, the sensory parameters were highly correlated ($P < 0.001$) with metamyoglobin content, being the coefficients r = 0.85 and r = 0.87 for appearance and off-colour respectively. The correlation between these sensory parameters and L^*, a^* and H^* colorimetric parameters were also positive and significant ($P < 0.001$), presented correlation coefficients ranged between r = 0.62 and r = 0.72. From these data, it seems that the use of metamyoglobin content rather than colorimetric parameters could be useful to be related to sensory quality.

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4. Conclusions

The results obtained in the present study suggest that whole dried red wine grape pomace can be included in lamb diets at 5% of concentrate without adverse effects on the shelf life of meat during storage in retail sale conditions. Although grape seed extract and grape pomace were not as effective as vitamin E in preventing meat deterioration, lambs fed wine by-products showed numerically lower TBARS meat values from day 7 of storage under retail display conditions compared with lambs not supplemented with polyphenols. Consequently, the use of grape pomace as feed in lamb diets could be adopted as a strategy to reduce feeding cost and also to cope with the need to recycle waste material which is costly to dispose of without adverse effects on shelf life of meat during storage in retail sale conditions. More research is necessary to test different doses and ways of supplementing winery by-products in lamb diets.

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Table 1. Ingredients and chemical composition of experimental concentrates (g kg^{-1} DM).

	Treatment			
	CTRL	VIT-E	GSE	GP-5
Ingredients				
Barley	740	740	740	701
Soya	200	200	200	189
Molasses	30.0	30.0	30.0	30.0
Mineral premix	30.0	30.0	30.0	30.0
Grape seed extract (mg kg^{-1}) [a]	-	-	50.0	-
Grape pomace[b]	-	-	-	50.0
Vitamin E (mg kg^{-1})	50.0	500	50.0	50.0
Analysed composition				
Dry matter (DM)	982	985	980	876
Ash	68.2	73.8	80.9	69.3
Neutral detergent fibre	154	157	153	172
Acid detergent fibre	56.6	61.1	61.8	81.1
Crude protein	189	189	187	187
Ether extract	43.9	43.8	44.6	46.6
UFV [c] (kcal kg^{-1} DM)	1.15	1.15	1.15	1.10
PDI [d]:UFV	9.80	9.80	9.80	9.80

[a] Grape seed extract composition: extractable polyphenols 329.5 g kg^{-1} DM, condensed tannins 412.5 g kg^{-1} DM, anthocyanins 4.4 g kg^{-1} DM.

[b] Grape pomace composition: DM 955 g kg^{-1}, ash 89,3 g kg^{-1}, NDF 375.9 g kg^{-1} DM, ADF 317.2 g kg^{-1} DM, CP 118.7 g kg^{-1} DM, EE 73.3 g kg^{-1} DM, extractable polyphenols 44.1 g kg^{-1} DM, condensed tannins 44.5 g kg^{-1} DM, anthocyanins 3.47 g kg^{-1} DM, total phenolics acids 78.6 g kg^{-1} DM, *trans* resveratrol 0.63 g kg^{-1} DM, total flavonols 2.15 g kg^{-1} DM, total flavanols 9.32 g kg^{-1} DM. Fatty acid composition (% of total fatty acid methyl esters): C12:0 (<0.01), C14:0 (0.10), C15:0 (<0.01), C16:0 (6.20), C16:1 (0.10), C18:0 (4.90), C18:1 (21.90), C18:2 (14.80), C18:3 (51.30), C20:0 (0.20); C22:0 (0.10).

[c] UFV = feed unit for maintenance and meat production (FEDNA Tables, 2010).

[d] PDI = protein truly digestible in the small intestine (FEDNA Tables,

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2010).

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Table 2. Effect of experimental lamb diets and storage time on microbial counts (log cfu g^{-1}) (mean values) on *m. longissimus thoracis et lumborum* from lambs during refrigerated storage at 2 ºC.

Microorganisms	Days	Treatment				SED[1]	*P*-level[2]		
		CTRL	VIT-E	GSE	GP-5		T	D	T x D
Total viable counts	0	$^{A}2.48$	$^{A}2.68$	$^{A}2.12$	$^{A}2.44$	0.328	0.006	<0.001	0.043
	4	$^{A}2.26$	$^{A}2.25$	$^{AB}3.00$	$^{AB}2.28$				
	7	$^{B}3.89$	$^{AB}3.51$	$^{B}3.92$	$^{B}3.48$				
	11	$^{C}5.69^{a}$	$^{B}4.18^{b}$	$^{C}6.24^{a}$	$^{C}6.14^{a}$				
	14	$^{C}6.08^{a}$	$^{B}4.43^{b}$	$^{D}8.03^{c}$	$^{D}7.89^{c}$				
Enterobacteria	0	$^{A}1.00$	1.00	$^{A}1.00$	$^{A}1.35$	0.352	0.006	<0.001	0.568
	4	$^{A}1.00$	1.00	$^{AB}2.16$	$^{A}1.00$				
	7	$^{AB}2.12$	1.00	$^{AB}2.32$	$^{AB}1.75$				
	11	$^{B}3.40^{ab}$	1.85^{a}	$^{BC}3.50^{b}$	$^{B}3.24^{ab}$				
	14	$^{B}3.70^{a}$	1.57^{b}	$^{C}4.51^{a}$	$^{B}3.08^{ab}$				
Lactic acid bacteria	0	$^{AB}2.20$	1.97	$^{A}1.92$	$^{A}2.15$	0.535	0.052	0.001	0.242
	4	$^{A}1.60$	1,56	$^{A}2.05$	$^{A}1.60$				
	7	$^{AB}3.62$	2.27	$^{A}2.39$	$^{AB}3.41$				
	11	$^{AB}3.64$	2.36	$^{AB}4.52$	$^{A}2.52$				
	14	$^{B}4.30^{a}$	1.60^{b}	$^{B}6.71^{a}$	$^{B}5.29^{a}$				
Pseudomonads	0	$^{A}2.00$	$^{A}2.00$	$^{A}2.00$	$^{A}2.00$	0.349	<0.001	<0.001	0.026
	4	$^{A}2.00$	$^{A}2.00$	$^{A}2.00$	$^{A}2.00$				
	7	$^{B}3.65$	$^{AB}2.73$	$^{B}3.80$	$^{B}3.66$				
	11	$^{BC}5.01^{a}$	$^{AB}2.67^{b}$	$^{C}5.79^{a}$	$^{C}5.71^{a}$				
	14	$^{C}5.91^{a}$	$^{B}3.85^{b}$	$^{D}7.90^{c}$	$^{D}7.95^{c}$				
Brochothrix thermosphacta	0	1.00	1.00	$^{A}1.00$	1,00	0.161	0.524	0.192	0.673
	4	1.00	1.00	$^{A}1.00$	1.00				
	7	1.00	1.00	$^{A}1.00$	1.00				

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	11	1.00[a]	1.42[ab]	[B]2.06[b]	1.00[a]
	14	1.00	1.00	[A]1.00	1.00

[1] SED: standard error of the difference.

[2] Probability of significant effects due to experimental dietary treatment (T), sampling day (D) and interaction effects (T x D).

[a, b, c] Averages with different letter in the same row are significantly different ($P < 0.05$).

[A, B, C, D] Averages for each parameter with different letter in the same column are significantly different ($P < 0.05$).

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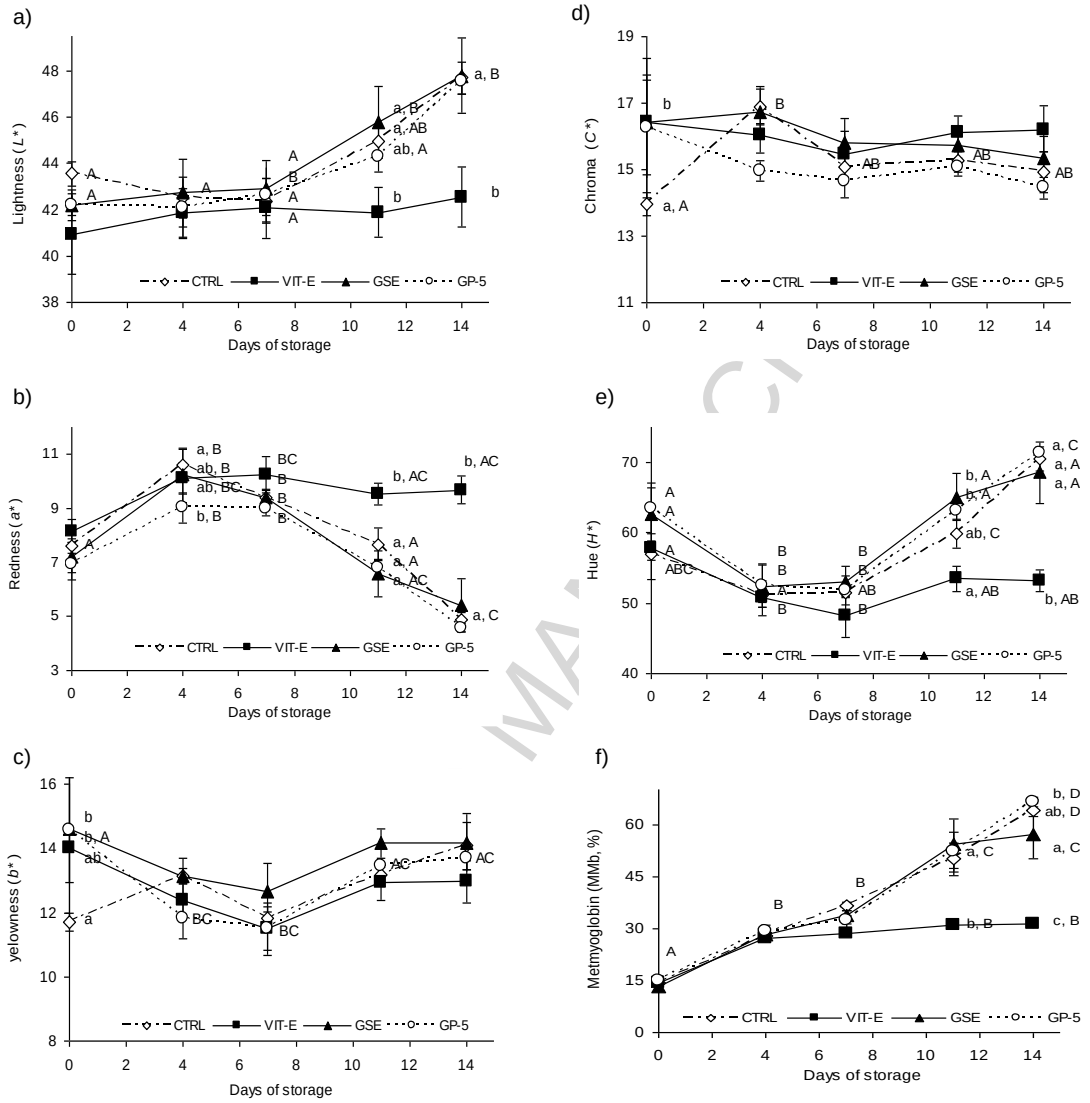

Figure 1. Effect of experimental lamb diets and storage time on evolution of colour parameters: lightness (a), redness (b), yellowness (c), chroma (d), hue (e) and metmyoglobin haem pigment (f) on *m. longissimus thoracis et lumborum* stored at 2 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error.

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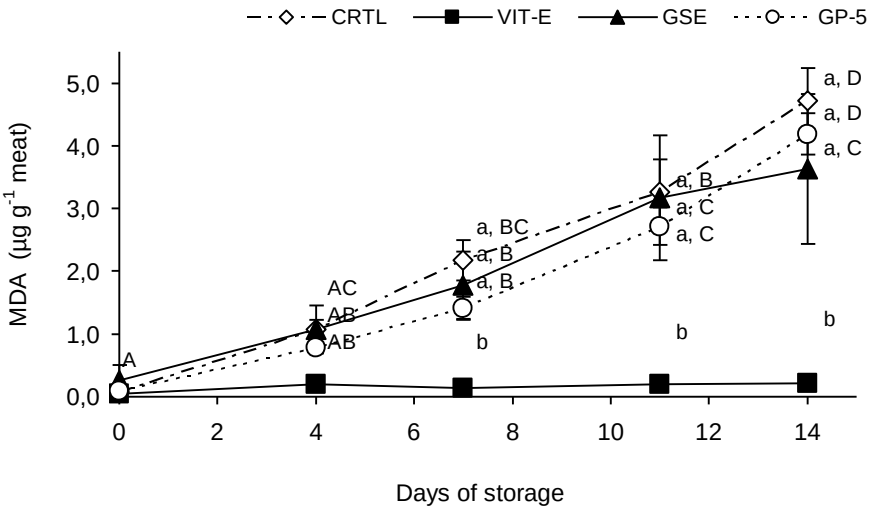

Figure 2. Effect of experimental lamb diets and storage time on evolution of TBARS (µg g^{-1} meat) on *m. longissimus thoracis et lumborum* stored at 2 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error.

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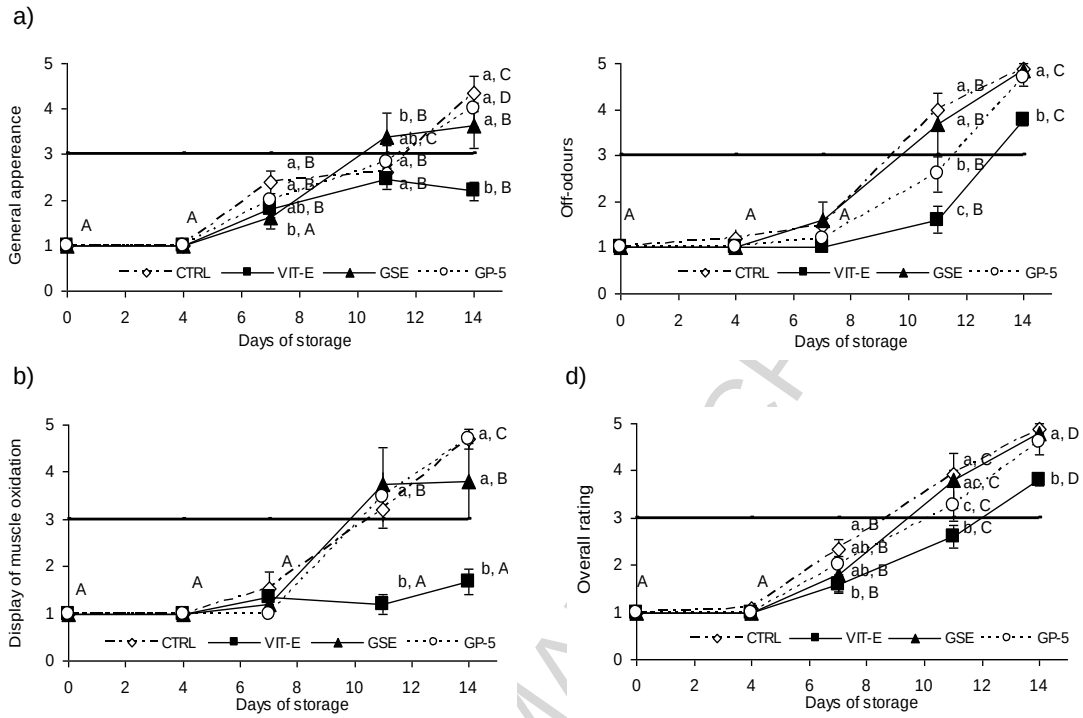

Figure 3. Effect of experimental lamb diets and storage time on general appearance (a), display of muscle oxidation (b), off-odours (c) and overall rating (d) evaluated by a panel of trained members on *m. longissimus thoracis et lumborum* stored at 2 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error.

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Highlights

- The effect of grape pomace on lamb meat shelf life was studied.
- Meat from vitamin E lambs showed lower microbial counts than lambs fed grape
- pomace.
- Vitamin E was more effective in preventing sensory spoilage than grape
- pomace.
- The use of grape pomace decreased lipid oxidation by about 20%.

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