Effect of dietary grape seed extract and Cistus ladanifer L. in combination with vegetable oil supplementation on lamb meat quality

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A R T I C L E   I N F O

Article history:
Received 29 June 2010
Received in revised form 28 June 2012
Accepted 25 July 2012

Keywords:
Lamb meat
Grape seed extract
Cistus ladanifer L.
Oil supplementation
Colour stability
Lipid oxidation

A B S T R A C T

Thirty-six Merino Branco lambs were assigned to six dietary treatments: control diet (C) consisting of 90% dehydrated lucerne and 10% wheat bran; C with 6% of oil blend (CO); C with 2.5% of grape seed extract (GS); GS with 6% of oil blend (GSO); C with 25% of C. ladanifer (CL), and CL with 6% of oil blend (CLO). Meat lipid and colour stability was then evaluated during 7 days of storage. The effect of inclusion of grape seed extract and C. ladanifer in diets on meat sensory properties was also evaluated. Meat antioxidant potential, determined after oxidation induction by a ferrous/hydrogen peroxide system, decreased with oil supplementation (P < 0.001), but inclusion of grape seed extract and C. ladanifer in diets protected the meat against lipid oxidation (P = 0.036). Meat colour was not affected by diets. Inclusion of grape seed extract and C. ladanifer in diets did not change the sensory properties of meat.

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1. Introduction

Ruminant meat is characterized by high contents of saturated fatty acids (SFAs) and low levels of polyunsaturated fatty acids (PUFAs), which has been linked with an high cardiovascular diseases risk in humans (Givens, 2005). The supplementation of ruminant diets with lipids sources rich in PUFA is an effective means to improve the nutritional value of meat fat, decreasing the SFA and promoting the enrichment in PUFA, including the health enhancing fatty acids (FA), such as conjugated linoleic acid (CLA) and non−3 PUFAs (Sinclair, 2007). However, the high content of PUFA in meat is associated with its increased susceptibility to oxidation (Morrissrey, Sheehy, Galvin, Kerry, & Buckley, 1998) and, thus meat quality deterioration.

The synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used in the animal nutrition and food industries in order to improve the oxidative stability of foods. However, for satisfying the consumers on concern over food safety and toxicity of synthetic antioxidant, the interest in natural antioxidants in substitution of the synthetic ones has increased in recent years.

Proanthocyanidins, also known as condensed tannins (CTs), are oligomers and polymers of flavanoid units linked by carbon–carbon bonds (Hagerman, 1998), arising from the secondary metabolism of plants. The effective antioxidant activity of CT sources, such as grape seed extract, has been reported when added to minced meat, including beef (Ahn, Grün, & Fernando, 2002), pork (Carpenter, O’Grady, O’Callaghan, O’Brien, & Kerry, 2007) and turkey (Lau & King, 2003). Feeding studies conducted with poultry also showed that dietary supplementation with grape seed and green tea extracts (Smet et al., 2008), grape pomace concentrate (Brenes et al., 2008) and high-tannin sorghum (Du, Cherian, Stitt, & Ahn, 2002) limits lipid oxidation in meat. The inclusion of grape seed and peel extract directly in rumen of sheep was shown to be effective in reducing the susceptibility to lipid oxidation in plasma (Gladine, Rock, Morand, Bauchart, & Durand, 2007). Although the effect of dietary CT sources on lipid oxidation in ruminant meat has been little explored, Luciano, Monahan, Vasta, Biondi et al. (2009) have reported that the inclusion of quebracho tannins in concentrate fed to lambs did not affect lipid oxidation in semimembranosus muscle, although reduced the meat discoloration.

Cistus ladanifer L. is a very abundant shrub in marginal fields of Mediterranean countries, with high contents of CT (Dentinho, Navas, & Potes, 2005). The antioxidant activity of C. ladanifer phenolic extract in vitro was reported recently (Andrade, Gil, Breitenfeld, Domingues, & Duarte, 2005).
samples (1.5 cm thickness) were used to evaluate the lipid and colour stability during storage of lamb meat. We also investigated if these sources of CT affected the sensorial properties of cooked meat.

2. Materials and methods

2.1. Animal and management

Details on diets, animal handling procedures and growth performances have been reported elsewhere (Jerónimo et al., 2010; Vasta et al., 2010). Briefly, 36 Merino Branco ram lambs weighing 24.8 ± 1.55 kg (mean ± SD) were randomly distributed to 12 pens which were allocated to one experimental diet according to a completely randomized experimental design with a 3×2 factorial arrangement. This 3×2 factorial arrangement resulted in 6 diets: C. basal diet composed of 900 g dehydrated lucerne/kg DM and 100 g wheat bran/kg DM; CO, basal diet with 6% DM of oil blend; GS, basal diet with 2.5% DM of grape seed extract; GSO, basal diet with 2.5% DM of grape seed extract and 6% DM of oil blend; CL, basal diet with 25% DM of C. ladanifer and CLO, basal diet with 25% DM of C. ladanifer and 6% DM of oil blend. Grape seed (Vitis vinifera L.) extract powder contained 95% proanthocyanidines in DM (AHD international LLC, Atlanta, GA, USA). Leaves and soft stems of C. ladanifer shrubs were harvested in Portugal (39°30′ N) in March 2008, dried at room temperature, cut in small particles and milled to a final particle size of 3 mm. The oil blend was composed by a mixture of sunflower and linseed oils in a proportion of 1:2 (v/v). Diets were prepared in an industrial unit, where grape seed extract and C. ladanifer were mixed with milled dehydrated lucerne. These mixtures were then pelleted and oil was sprayed on the pellets.

The total phenols content was 9.1 and 7.5 g/kg DM, respectively, in C and CO diets, but ranged from 17.6 g/kg DM in diets with CT sources without oil (GS and CL) to 16.4 g/kg DM in diets with CT sources and oil (GSO and CLO) (Vasta et al., 2010). The enrichment in grape seed CT, computed by the difference of CT concentration in grape seed diets and control diets (i.e. GS minus C and GSO minus CO) was 13.9 g/kg DM and 12.6 g/kg DM for GS and GSO diets, respectively. The enrichment in C. ladanifer CT, computed by the difference of CT concentration in C. ladanifer diets and control diets (i.e. CL minus C and CLO minus CO) were 12.5 g/kg for both CL and CLO diets (Jerónimo et al., 2010; Vasta et al., 2010).

After 6 weeks of trial, the lambs were slaughtered in the experimental abattoir. The average slaughter weight was 36.2 kg ± 1.60 (mean ± S.E.M.), which was not affected by dietary treatments. Carcasses were kept at 10 °C for 24 h, and then chilled at 2 °C until the third day after slaughter.

2.2. Sample collection

Seventy-four hours after slaughter four samples of longissimus dorsi muscle of carcass left halves were collected. One sub-sample of muscle, after removing the epimysium, was minced, vacuum packed, freeze-dried and stored at −80 °C until lipid analysis. Three sub-samples (1.5 cm thickness) were used to evaluate the lipid and colour stability during 0, 3 and 7 days of storage at 2 °C in an illuminated cooler. At 0 day of storage the colour parameters were determined after 1 h of blooming and samples were vacuum packed. The other samples were individually placed on polystyrene trays, over-wrapped with oxygen permeable polyvinyl chloride film and displayed for 3 and 7 days. At the end of storage time, meat samples were allowed to bloom for 1 h, before the determination of the colour parameters. After that, the samples were vacuum packed and stored at −80 °C until analysis. Seventy-four hours after slaughter loins and ribs of carcass right halves were collected and frozen at −20 °C, until being used for sensorial analysis.

2.3. Lipid analysis

Intramuscular lipid extraction and FA methyl esters preparation are fully described by Jerónimo et al. (2010a). Briefly, intramuscular lipids were extracted using dichloromethane and methanol (2:1 v/v) and FA were transesterified with sodium methoxide in methanol followed by hydrochloric acid in methanol (1:1 v/v). Quantification of muscle lipid fatty acid methyl esters (FAME) was done using nonadecanoic acid (19:0) as internal standard. Fatty acid methyl esters were analysed using a HP6890A chromatograph (Hewlett-Packard, Avondale, PA, USA), equipped with a flame-ionization detector (GC-FID) and fused silica capillary column (CP-Sil 88; 100 m×0.25 mm i.d.×0.20 μm of film thickness; Chrompack, Varian Inc., Walnut Creek, CA, USA). Gas chromatography conditions and FA identification were the same as described in Jerónimo et al. (2010).

2.4. Measurement of meat lipid oxidation after induction of oxidation in vitro

For the evaluation the meat lipid stability (antioxidant potential) the general procedure of Mercier, Gatellier, and Renere (2004) was followed, which involves the induction of oxidation in meat homogenate by ferrous iron and hydrogen peroxide, followed by the measurement of oxidized lipids. Meat homogenates were prepared by homogenising 1 g tissue in 10 ml of sodium phosphate 100 mM (pH 7.0) using an Ultra-Turrax T25 homogenizer (IKA Werke GmbH & Co. KG, Staufen, Germany) for 1 min at 20000 rpm. These homogenates were incubated with 100 μl of mixture of ferrous sulphate (0.5 mM) and hydrogen peroxide (1 mM) at 37 °C water bath for 30 min. After incubation time, 2 mg of butylated hydroxytoluene (BHT) was added to homogenate to stop the oxidation. Aliquots of 2 ml of homogenate were immediately frozen at −80 °C until lipid oxidation measurement by thiobarbituric acid reactive substances (TBARS) as described by Lynch and Frei (1993). Briefly, homogenate samples (0.5 ml) were incubated with 0.25 ml of 1% (w/v) 2-thiobarbituric acid in 50 mM of NaOH and 0.25 ml of 2.8% (w/v) trichloroacetic acid in boiling water bath for 10 min. The pink chromogen was extracted with 2 ml of n-butanol and its absorbance measured at 535 nm in a UV/VIS Spectrophotometer (Ultrospec III, Pharmacia LKB Biochrom Ltd., Cambridge, England). The 1,1,3,3 tetraethoxypropane standard curve was used for calculating the TBARS concentration and the results were expressed as mg of malonaldehyde (MDA)/kg of meat.

2.5. Meat colour measurement

Minolta CR-300 chromometer (Konica Minolta, Lisboa, Portugal) was used to measure meat colour coordinates employing the CIE L* a* b* system, where L* is lightness, a* redness and b* yellowness. Measurements were made using the C illuminant and 2° standard observers. Hue angle (H°) was calculated as tan−1(b*/a*)×(180/π) and colour saturation (chroma, C*) as (a*²+b*²)²/4. Overall colour variation between each day of storage and the day 0 of measurements was calculated as ΔE0–n = ((ΔL*0–n)² + (Δa*0–n)² + (Δb*0–n)²)². Where ΔL*0–n, Δa*0–n and Δb*0–n are the sum of the differences in L*, a* and b* from day 0.
2.6. Sensory analysis

For sensory analysis a panel of 30 naive assessors, ranging from 28 and 60 years old was used. Triangular tests were conducted according to ISO 4120 (1983), to evaluate if consumers could detect the inclusion of grape seed extract and C. ladanifer in lamb diets with or without oil blend and if so, what their preference was. Therefore, the comparisons were performed on meat samples of lambs fed diets with oil (i.e. CO vs. GSO and CO vs. CLO) and without oil (i.e. C vs. GS and C vs. CL). Twenty-four hours before the sessions, 1.5 cm thick chops were removed from right joints (loins and ribs) and thawed at 0 °C. Chops were wrapped in aluminium foil, and cooked in an industrial grill for 6 min and served warm to the consumer panel. Each assessor was offered simultaneously three chops, two corresponding to the same diet and one to the other. Sessions were conducted in two days and in each day assessors tasted 6 chops. They were asked to identify the sample that was different from the other two (question 1) and to indicate the chop that tasted better (question 2). When analysing the results, the answers to question 2 were considered only when the panelists answered correctly to question 1.

2.7. Statistical analysis

This trial was conducted using a 3 × 2 factorial design, where the 2 factors were the condensed tannin sources (CT, with 3 levels: control, grape seed extract and C. ladanifer) and the vegetable oil blend supplementation (0, with 2 levels: 0% and 6%). The interaction between CT sources and 0 was also evaluated (CT × O). The individual animals were considered as experimental units. The Shapiro–Wilk test was used in order to evaluate whether data followed a normal distribution. When not normally distributed (P<0.05) data was Box–Cox transformed before further analysis. Data of intramuscular FA composition were analysed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), considering the oil and CT sources and their interaction as fixed effects and the pen as random effect. The covariance of measurements from lambs within each pen was considered in the model. The colour and lipid oxidation were studied by repeated measure analysis of variance with the MIXED procedure of SAS with day of sampling as the repeated measurement. Least squares means and standard error of means (S.E.M.) are presented in tables. For Box–Cox transformed variables the S.E.M. is presented in tables, although means are back-transformed. For sensory analysis results, a significance table for triangle tests was used to analyse the answers to question 1, and a significance table for pair tests to analyse the answers to question 2 (Roessler, Pangborn, Sidel, & Stone, 1978). The level of statistical significance was set at P<0.05 for main effects and at P<0.10 for interactions.

3. Results and discussion

Intramuscular FA content and composition are presented in Table 1. Intramuscular FA content was higher (P=0.017) in lambs fed CLO diet (30.7 mg/g of fresh muscle) than meat from lambs fed other diets (20.6 mg/g of fresh muscle). As expected, supplementation of lamb diets with oils rich in 18:2n−6 and 18:3n−3 decreased SFA and increased PUFA, 18:1 trans-11 and CLA contents in meat (Bessa, Portugal, Mendes, & Santos-Silva, 2005; Bessa et al., 2007; Bolte, Hess, Means, Moss, & Rule, 2002; Jerónimo, Alves, Alfaia et al., 2010). An interaction between CT sources and oil was observed for several individual PUFA (18:2n−6, CLA, 20:4n−6 and 22:6n−3) and for total PUFA and n−6 PUFA partial sums, in which meat from lambs fed CLO diet had lower PUFA content (17.1% of total FA) than meat from other oil supplemented lambs (19.8% of total FA). This is likely explained by higher intramuscular FA content in lambs fed CLO diet than in lambs fed other diets. It is known that an increase in intramuscular FA is due to greater accumulation of triacylglycerols, which incorporate mostly SFA and monounsaturated FA (MUFA), while membrane polar lipids, which incorporate mostly the PUFA remain fairly constant (Raes, De Smet, & Demeyer, 2004; Wood et al., 2008). Thus, the lower content of PUFA in meat from lambs fed the CLO diet than meat from lambs fed other diets with oil resulted from the “dilution effect” caused by the increase in intramuscular FA (and therefore of SFA and MUFA) at the expense of the PUFA. Moreover, meat from lambs fed CLO diet showed a higher 18:1 trans-11 and CLA than that from other oil supplemented lambs. The interaction of C. ladanifer with oil supplementation and its implications on ruminal biohydrogenation and tissue lipid metabolism was thoroughly discussed in a preceding paper, where detailed FA composition of both intramuscular polar and neutral lipid fractions were presented (Jerónimo, Alves, Dentinho et al., 2010).

3.1. Meat lipid oxidation

As intended, meat from oil supplemented lambs had higher PUFA concentration than meat from unsupplemented lambs and, thus, can be expected to be more prone to lipid oxidation (Morrissey et al., 1998). It is well established that lipid oxidation decreases meat quality and acceptability by the consumers because it leads to colour deterioration and development of off-odours and off-flavours (Morrissey et al., 1998). Therefore, the susceptibility of PUFA to rapid oxidation might limit the nutritional strategies which aim at increasing PUFA concentration in meat. In present trial the lipid oxidation was measured after chemical oxidation by ferrous iron and hydrogen peroxide, allowing to determine the meat resistance against lipid oxidation in pro-oxidative conditions (Mercier et al., 2004).

As expected, lipid oxidation increased (P<0.001) with storage time and dietary lipid supplementation (Table 2), reflecting both the reduction of meat capacity for resist against lipid oxidation during storage and the tendency of PUFA to oxidize. An interaction between oil supplementation and storage time (P=0.056) was observed for lipid oxidation, in which at day 3 of storage the meat from oil supplemented lambs showed similar TBARS values to that from lambs fed diets with no oil stored after 7 days (Fig. 1). The inclusion of a CT sources in lamb’s diets reduced the meat lipid oxidation (−15%, P=0.036) as compared to that control diet (Table 2); improving thus the meat resistance against induced lipid oxidation. This protective effect only occurred after 3 days of storage (interaction CT source×time, P=0.078, Fig. 2). At day 3 of storage, the meat from lambs fed grape seed diets showed lower TBARS values than that from lambs fed control diets (−18%). Feeding C. ladanifer diets resulted in intermediate TBARS values between grape seed and control diets. Nevertheless, at day 7 of storage the grape seed extract and C. ladanifer were equally effective in protection against lipid oxidation, increasing the meat resistant against lipid oxidation in 16% comparatively to control diet. The antioxidant effect of dietary CT have been shown in poultry (Brenes et al., 2008; Du et al., 2002; Smet et al., 2008) and rodents (Gladine, Morand, Rock, Bauchart, & Durand, 2007; Gladine, Morand, Rock, Gruffat et al., 2007). However, information on potential antioxidant effect of dietary CT in ruminants seems to be restricted to a few studies. Gladine, Rock, Morand et al. (2007) reported that inclusion of 10% DM of grape seed and peel extract directly into the rumen of sheep improved the antioxidant status and reduced the susceptibility to lipid oxidation of plasma measured by an oxidation induced assay. Luciano, Monahan, Vasta, Biondi et al. (2009) reported that inclusion of 8.5% DM of quebracho in the diet did not improve the lipid oxidative stability in lamb meat, measured as TBAR without oxidative induction in meat. However, recently Luciano et al. (2011) showed that this incorporation of quebracho in lamb diets improves the overall antioxidant status of meat, increasing its reducing ability (ferric reducing antioxidant power assay) and radical scavenging ability (trolox equivalent antioxidant capacity assay); which is in accordance with higher resistance of meat from lambs fed CT sources against lipid oxidation observed in our work. Differences observed between methods using...
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or not oxidative induction might be explained by the fact that although dietary CT increase the meat antioxidant capacity, this might be evident only when oxidative pressure exceeds the antioxidant capacity of control meats which might not be attained in normal meat storage conditions.

Despite the several studies that show that dietary CT have beneficial effects on oxidative stability of meat, their mechanisms of action remain to be established. The direct antioxidant activity of dietary CT would imply their absorption through the gastrointestinal tract and their transfer in tissues (Luciano, Monahan, Vasta, Biondi et al., 2009). However, the polymeric nature and high molecular weight of CT should limit their transit through the rumen. Nevertheless, the effect of dietary CT on meat oxidative stability may be indirect, through the interaction between CT and other antioxidants compounds or with pro-oxidants compounds present in meat. Gladine, Morand, Rock, Gruffat et al. (2007) reported that rosemary and grape extract inclusion in rat diets increased significantly the vitamin E content in liver. Previous works showed that plant extracts, such as Gymnema montanum, grape and marigold extracts, increased the activity of antioxidant enzymes in kidney and liver of rats (Ananthan et al., 2004; Gladine, Morand, Rock, Gruffat et al., 2007) that rosemary and grape extract inclusion in rat diets increased significantly the vitamin E content in liver. Previous works showed that plant extracts, such as Gymnema montanum, grape and marigold extracts, increased the activity of antioxidant enzymes in kidney and liver of rats (Ananthan et al., 2004; Gladine, Morand, Rock, Gruffat et al., 2007). However, in the present trial, neither vitamin E content nor enzymes with antioxidant activity were determined.

In the present experiment we used the extract of grape seed that is composed mainly by proanthocyanidins (95%) and leaves and soft stems of C. ladanifer that contained secondary compounds, including several flavonoids other than proanthocyanidins and terpenoids (Gomes, Mata, & Rodrigues, 2005; Sosa, Alías, Escudero, & Chaves, 2005). Several flavonoid and terpenoid compounds have been shown to have antioxidant properties (Harborne & Williams, 2000; D'Penez et al., 2000). Nevertheless, the effect of dietary CT on meat oxidative stability may be indirect, through the interaction between CT and other antioxidants compounds or with pro-oxidants compounds present in meat. Gladine, Morand, Rock, Gruffat et al. (2007) reported that rosemary and grape extract inclusion in rat diets increased significantly the vitamin E content in liver. Previous works showed that plant extracts, such as Gymnema montanum, grape and marigold extracts, increased the activity of antioxidant enzymes in kidney and liver of rats (Ananthan et al., 2004; Gladine, Morand, Rock, Gruffat et al., 2007). However, in the present trial, neither vitamin E content nor enzymes with antioxidant activity were determined.

Table 1

<table>
<thead>
<tr>
<th>Control</th>
<th>S.E.M.</th>
<th>P values&lt;sup&gt;6&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Diet GSE</td>
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<tr>
<td>Diet C. ladanifer</td>
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<tr>
<td>Diet CT</td>
<td></td>
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<tr>
<td>Diet CT + GSE</td>
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</table>

S.E.M., standard error of mean; SFA, sum of saturated fatty acids; PUFA, sum of polyunsaturated fatty acids.

<sup>a</sup> Diet C, basal diet composed of 900 g dehydrated lucerne/kg DM and 100 g wheat bran/kg DM.
<sup>b</sup> Diet C, basal diet with 6% DM of oil blend (sunflower and linseed oils, 1:2 v/v).
<sup>c</sup> Diet GSE, basal diet with 2.5% DM of grape seed extract.
<sup>d</sup> Diet GSE, basal diet with 25% DM of rosemary and 6% DM of oil blend (sunflower and linseed oils, 1:2 v/v).
<sup>e</sup> Diet CT, basal diet with 25% DM of Cistus ladanifer.
<sup>f</sup> Diet CT, basal diet with 25% DM of Cistus ladanifer and 6% DM of oil blend (sunflower and linseed oils, 1:2 v/v).
<sup>g</sup> CT, condensed tannins source in diet; O, oil supplementation.
<sup>h</sup> Variables submitted to Box–Cox transformation; means presented are back-transformed values but S.E.M. is expressed in transformed scale; λ for 18:1 cis-9, 18:2 trans-6 and n-6 PUFA = 0.01.
<sup>i</sup> Include 18:2 cis-9, trans-11, 18:2 trans-8, cis-10 and 18:2 trans-7, cis-9 isomers.
<sup>j</sup> n-6 PUFA<sup>||</sup> = (18:2n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6).
<sup>k</sup> n-6 LC-PUFA<sup>||</sup> = (20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6).
<sup>l</sup> n-3 PUFA<sup>||</sup> = (20:3n-3 + 20:4n-3 + 20:5n-3 + 20:6n-3 + 22:5n-3 + 22:6n-3).
<sup>m</sup> n-3 LC-PUFA<sup>||</sup> = (20:3n-3 + 20:4n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3).

S. Jezzarelli (1995) demonstrated that rumen microorganisms do not hydrolyze CT. Conversely, studies in rats and humans indicated that CT are not inert within the gut, but undergo structural modifications operated by the intestinal microflora (Aiba & Fry, 2001; Déprez et al., 2000). Nevertheless, the effect of dietary CT on meat oxidative stability may be indirect, through the interaction between CT and other antioxidants compounds or with pro-oxidants compounds present in meat. Gladine, Morand, Rock, Gruffat et al. (2007) reported that rosemary and grape extract inclusion in rat diets increased significantly the vitamin E content in liver. Previous works showed that plant extracts, such as Gymnema montanum, grape and marigold extracts, increased the activity of antioxidant enzymes in kidney and liver of rats (Ananthan et al., 2004; Gladine, Morand, Rock, Gruffat et al., 2007). However, in the present trial, neither vitamin E content nor enzymes with antioxidant activity were determined.

In the present experiment we used the extract of grape seed that is composed mainly by proanthocyanidins (95%) and leaves and soft stems of C. ladanifer that contained secondary compounds, including several flavonoids other than proanthocyanidins and terpenoids (Gomes, Mata, & Rodrigues, 2005; Sosa, Alías, Escudero, & Chaves, 2005). Several flavonoid and terpenoid compounds have been shown to have antioxidant properties (Harborne & Williams, 2000; Matkowski, 2008). Thus, the flavonoid and terpenoid compounds present in C. ladanifer also may be responsible for the higher antioxidant capacity in the meat of the lambs fed C. ladanifer. Further studies should be conducted to identify the compounds that contribute to C. ladanifer antioxidant activity.
Table 2
Effect of storage time, oil supplementation and inclusion of condensed tannin sources on lipid oxidation (mg MDA/kg muscle) and surface colour parameters in raw longissimus dorsi muscle from Merino Branco lambs.

| Lipid oxidation | 0 day | 3 days | 7 days | S.E.M. | P values
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
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<td>31.0b</td>
<td>0.437</td>
<td>&lt;0.001</td>
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<tr>
<td>Cistus ladanifer</td>
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<td>13.2b</td>
<td>34.4b</td>
<td>0.06b</td>
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<td>14.5b</td>
<td>15.7b</td>
<td>16.1b</td>
<td>0.437</td>
<td>&lt;0.001</td>
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Effect of oiled supplementation (O)

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<td>0</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>7</td>
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<td></td>
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Effect of dietary condensed tannin sources (CT)

<table>
<thead>
<tr>
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<th>Cistus ladanifer</th>
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<tr>
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<td>4.45</td>
<td>11.1c</td>
<td>11.5b</td>
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<tr>
<td>3</td>
<td>9.17</td>
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<td>14.9b</td>
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<td>7</td>
<td>9.17</td>
<td>14.5bc</td>
<td>14.9b</td>
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<tr>
<td>S.E.M.</td>
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<td>0.052</td>
<td>0.470</td>
</tr>
<tr>
<td>P values</td>
<td>&lt;0.001</td>
<td>0.052</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Effect of interactions on TBARS (mg MDA/kg fresh muscle)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Grape seed extract</th>
<th>Cistus ladanifer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.6c</td>
<td>4.3c</td>
<td>4.8b</td>
</tr>
<tr>
<td>3</td>
<td>3.6c</td>
<td>4.3c</td>
<td>4.8b</td>
</tr>
<tr>
<td>7</td>
<td>3.6c</td>
<td>4.3c</td>
<td>4.8b</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0.272</td>
<td>0.086</td>
<td>0.417</td>
</tr>
<tr>
<td>P values</td>
<td>&lt;0.001</td>
<td>0.070</td>
<td>0.070</td>
</tr>
</tbody>
</table>

3.2. Meat colour

Dietary oil supplementation and inclusion of a CT sources in diets did not affect the meat colour coordinates (L*, a* and b*) but, as expected these parameters were affected by storage time (Table 2). Lightness (L*) values increased during firsts 3 days of storage, but between days 3 and 7 of storage remained unchanged (P<0.001). The yellowness (b*) values increased over the 7 days of storage, resulting from the increase in a* and the increase of b*. We found an interaction between inclusion of a CT sources in diets and time of storage (P<0.001), which at day 0 (1 h of blooming) the H* value was lower in meat of lambs fed grape seed extract diets than meat of lambs fed control diets, while meat of lambs fed C. ladanifer diets diet showed intermediate values. The ΔE, which measures the overall variation of meat colour during storage (Mancini & Hunt, 2005), was 5.43 at days 3 and 5.92 at day 7 of storage, indicating that at day 3 of storage the meat showed a more vivid colour (greater C* values) than meat stored 0 and 7 days. Hue angle (H*) allows more realistic perspective on meat browning than single colour coordinates (Luciano, Monahan, Vasta, Pennisi et al., 2009). Independently from dietary treatment, H* values increased (ranged from red to yellow) over the 7 days of storage, resulting from the decrease in a* and the increase of b*. We found an interaction between inclusion of a CT sources in diets and time of storage (P=0.040), which at 0 day (1 h of blooming) the H* value was lower in meat of lambs fed grape seed extract diets than meat of lambs fed control diets, while meat of lambs fed C. ladanifer diets diet showed intermediate values (Fig. 3), suggesting that dietary CT, especially grape seed extract has interfered with blooming development. However, after 3 and 7 days of storage this effect of the dietary CT sources on H* values was not observed, not differing between dietary treatments. This response is contrasting with results reported by Luciano, Monahan, Vasta, Biondi et al. (2009), who found lower H* values in minced meat from lambs fed diet supplemented with quebracho tannins after 7 and 11 days of storage as compared to meat from lambs fed diet without tannin supplementation.

It is widely accepted that lipid and myoglobin oxidation in meat diet are associated and, generally, both processes increase concurrently (Luciano, Monahan, Vasta, Pennisi et al., 2009). Previous works showed that colour and lipid stability of meat was not affected by PUFA enrichment in meat (Daly, Moloney, & Monahan, 2007; Ponnapalam, Graham, Sinclair, Egan, & Leury, 2001; Vatasever et al., 2000) or by dietary CT sources (O’Gardy, Carpenter, Lynch, O’Brien, & Kerry, 2008). In contrast, Luciano, Monahan, Vasta, Biondi et al. (2009) showed that the inclusion of quebracho tannins in sheep diets improved meat colour stability during refrigerated storage, but did not affect the lipid stability. In the present study, the absence of effect of the dietary treatments on meat colour stability may suggest that in normal conditions (without induction of the oxidation) and in period of storage used in experiment (7 days) the
oxidative pressure was not enough to cause the negative effects of oil supplementation and positive effects of inclusion of CT sources in diets on meat oxidative stability.

3.3. Sensory analysis

In a companion paper (Vasta et al., 2010), it was showed that C. ladanifer inclusion in lamb diets strongly affected meat volatile compounds profile, while grape seed extract inclusion had only minor effect on that profile. However, the consumer panel did not detect the effect of the grape seed extract and C. ladanifer inclusion in diets on meat sensory properties (Table 3). This result is in agreement with Priolo, Lanza, Biondi, Pappalardo, and Young (1998), who reported that trained panelists were unable to distinguish between meats from lambs fed diet with carob pulp (rich in CT) from meat of lambs fed control diet. In contrast with these results, Priolo et al. (2009) and Schreurs et al. (2007) reported that lamb meat flavour and odour is affected by CT, when supplementing the diets with 10% of quebracho or supplied 33 g/d of grape seed extract as liquid supplement, respectively. In these works, the dietary CT reduced the typical sheep meat odour and flavour. Our results showed that, at the levels of inclusion used, grape seed extract and C. ladanifer may be successfully used as supplements in lamb's diets without compromising the characteristic lamb meat sensory properties.

4. Conclusion

Dietary oil supplementation resulted in a beneficial effect on the FA composition of lamb meat but reduced the meat antioxidant potential. The increase of the lipid oxidation with oil supplementation was not reflected in meat colour stability during storage. Dietary grape seed extract and C. ladanifer did not improve the meat colour stability but in pro-oxidative conditions reduced the lipid oxidation of meat from lambs fed both diets with or with no oil. The sensory properties of lamb meat were not affected by the inclusion of grape seed extract and C. ladanifer in diets. Thus, our results suggest that use of these CT sources might be a good approach to reduce the meat deterioration induced by lipid oxidation in ruminant meat enriched in PUFA. However, further studies are needed to prove the effectiveness of this approach in normal conditions. Finally, further studies should be undertaken in order to elucidate the underlying mechanisms responsible for the oxidative stability of meat by grape seed extract and C. ladanifer.

Acknowledgements

Financial support through grant POCI/CVT/61202/2004 and individual fellowships to E. Jerónimo (SFRH/BD/23675/2005) and S.P. Alves (SFRH/BD/37793/2007), both from Fundação para a Ciência e a Tecnologia (FCT), are acknowledged.

References


Fig. 3. Effect of inclusion the grape seed extract and Cistus ladanifer in diets on Hue angle (H°) of longissimus dorsi muscle during storage time. Values are means, with SD represented by vertical bars. Values with different superscripts are signiﬁcantly different (*P<0.10).

Table 3 Results of sensory analysis by a consumer panel.

<table>
<thead>
<tr>
<th></th>
<th>Number of panellists</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>C vs. GS</td>
<td>30</td>
<td>11 (ns)</td>
</tr>
<tr>
<td>CD vs. GSO</td>
<td>30</td>
<td>13 (ns)</td>
</tr>
<tr>
<td>C vs. CL</td>
<td>30</td>
<td>13 (ns)</td>
</tr>
<tr>
<td>CD vs. CL</td>
<td>30</td>
<td>14 (ns)</td>
</tr>
</tbody>
</table>

D, number of panellists that were able to distinguish the meats. ns, not significant effect (*P<0.05).

Diet C, basal diet composed of 900 g dehydrated lucerne/kg DM and 100 g wheat bran/kg DM.

Diet CD, basal diet with 6% DM of oil blend (sunflower and linseed oils, 1:2 v/v).

Diet GSO, basal diet with 2.5% DM of grape seed extract and 6% DM of oil blend (sunflower and linseed oils, 1:2 v/v).

Diet GSO, basal diet with 2.5% DM of grape seed extract.

Diet GSO, basal diet with 2.5% DM of grape seed extract and 6% DM of oil blend (sunflower and linseed oils, 1:2 v/v).

Diet CL, basal diet with 25% DM of Cistus ladanifer.

Diet C, basal diet with 25% DM of Cistus ladanifer and 6% DM of oil blend (sunflower and linseed oils, 1:2 v/v).