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The effect of grape seed extract or *Cistus ladanifer* L. on muscle volatile compounds of lambs fed dehydrated lucerne supplemented with oil

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ABSTRACT

Thirty-six Merino Branco lambs were assigned to six dietary treatments: control diet (C) consisting of 90% dehydrated lucerne and 10% wheat bran; control diet with 6% of oil blend (CO); control with 2.5% of grape seed extract (GS); control with 2.5% of grape seed extract and 6% of oil blend (GSO); control with 25% of *Cistus ladanifer* (an aromatic bush widespread in Portugal; CL); control with 25% of *C. ladanifer* and 6% of oil blend (CLO). The muscle *longissimus dorsi* was then subjected to the analysis of volatile compounds (SPME–GC/MS). The CLO diet increased the concentration of heptanal, 3-hydroxy-2-butanone and 2-ethyl-phenol in muscle compared to the CL diet. When lambs received the CL diet, their meat contained lower amounts of 4-heptenal compared to the other treatments. The meat of the GS and GSO lambs contained similar amounts of volatile compounds deriving from lipid oxidation (such as heptanal, 2-nonenal, 4-heptenal and 3-hydroxy-2-butanone). These results indicate that in the presence of grape seed extract, oil supplementation did not enhance the production of lipid-derived volatile compounds. Verbenone and 2,2,6-trimethyl-cyclohexanone were detected only in the meat of the *Cistus*-fed lambs, suggesting that these compounds could be markers of feeding *Cistus*-containing diets. Meat volatile compounds profile allowed to discriminate between the lambs receiving *C. ladanifer* and those not receiving this bush in the diet.

1. Introduction

A large number of studies have recently aimed at ameliorating meat quality by manipulating animal diets. Most of these researches have focused on improving meat fatty acid composition, because it is strongly related to human health (Enser et al., 1998). Among meat fatty acids, *cis*-9, *trans*-11 C18:2 (conjugated linoleic acid or CLA) and $n-3$ polyunsaturated fatty acids (PUFA) have been shown to have healthy properties (Ip, Chin, Scimeca, & Pariza, 1991; Parodi, 2003). One of the dietary strategies that mostly enhances the accumulation of CLA and $n-3$ PUFA in ruminant products is feeding green grass (Aurousseau, Bauchart, Calichon, Micol, & Priolo, 2004; French et al., 2000; Santos-Silva, Bessa, & Santos-Silva, 2002). However, pasture is not always available in many areas and animals are often fed indoors with concentrates. It has been shown that supplementing oils to lambs fed high concentrate diets failed to enhance CLA concentration in meat due to shifts in rumen biohydrogenation pathways (Bessa, Portugal, Mendes, & Santos-Silva, 2005). On the contrary, including vegetable oils into pelleted forage (e.g. dehydrated lucerne) is effective in enhancing meat CLA content (Bessa, Lourenço, Portugal, & Santos-Silva, 2008; Bessa et al., 2005). Moreover, when the oil blend includes linseed oil also meat $n-3$ PUFA are increased (Bessa et al., 2007).

However, if on one hand high PUFA concentration is favourable for human health, on the other hand it is often responsible for the reduction of meat oxidative stability (which implies a shorter product shelf life) and for the appearance of rancid flavour (Nute et al., 2007). In order to contrast the oxidation of PUFA in meat, some strategies, such as vitamin E supplementation, have been found to be effective (Monahan et al., 1992). It has been recently reported that the inclusion of quebracho tannins into concentrate increased lamb meat colour stability (Luciano et al., 2009). Tannins are plants secondary compounds and they belong to the wide family of phenols. Phenolic compounds have antioxidant properties and modulate gene expression (Chen et al., 2002; Diebolt, Bucher, &

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Adriantsitohaina 2001) and protein expression (Vasta et al., 2009). The effects of tannins on ruminant physiology and products quality is diverse depending on their vegetal source (Muller-Harvey, 2006; Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). It has been shown that feeding tannin-containing feed strongly affects meat colour (Priolo, Waghorn, Lanza, Biondi, & Pennisi, 2000), flavour (Priolo et al., 2009; Schreurs et al., 2007a) and fatty acid composition (Vasta et al., 2007a; Vasta et al., 2009). However, to date there are no studies concerning the effect of feeding a tannin containing diet on lamb meat volatile compounds.

The aim of the present study was to investigate the volatile compounds profile in the muscle *longissimus dorsi* of lambs receiving a basal diet rich in dehydrated lucerne supplemented with grape seed extract or *Cistus ladanifer* L. (both of them being tannins sources), with or without oil. Also, considering that *C. ladanifer* is a bush typical of some areas of Portugal, we have envisaged the possibility of discriminating the meat from animal fed *C. ladanifer* from the meat of lambs not receiving this bush in their diets.

2. Materials and methods

2.1. Animals and management

Thirty-six Merino Branco ram lambs were weaned at the age of 60 days and housed in an experimental farm of the Animal Production Unit of Instituto Nacional dos Recursos Biológicos (UPA-INRB). Lambs average initial weight was 24.8 ± 1.55 kg (mean ± s.d.). Animals were randomly assigned to 12 groups of three lambs each. The animals were assigned to one of six dietary treatments each one of them consisting of two groups of three lambs. The six experimental diets were as follows: control diet (C) consisting in 90% dehydrated lucerne and 10% wheat bran; control diet plus 6% oil blend (CO); control plus 2.5% grape seed extract (GS); control plus 2.5% grape seed extract and 6% oil blend (GSO); control plus 25% *C. ladanifer* (CL); control plus 25% *C. ladanifer* and 6% oil blend (CLO). All the diets were pelletted. Grape seed extract (AHD international LLC, Atlanta, GA, USA) contained (on DM basis) 95% condensed tannins (CT). Leaves and young stems of *C. ladanifer* shrubs (a local widespread aromatic bush) were harvested in Portugal (39°30′36″N/8°19′00″W) in March 2008, dried at room temperature, cut into small particles and milled to 3 mm. The oil blend was composed of sunflower oil and linseed oil (1:2 vol/vol). After an adaptation period of 7 days to the experimental conditions, lambs were fed the experimental treatments for 6 weeks. Water was always available for the animals.

At the end of the experimental period, the lambs were slaughtered in the UPA-INRB experimental abattoir. The carcasses were kept at 10 °C for 24 h and then were chilled at 2 °C. On the third day after slaughter, the muscle *longissimus dorsi* was excised, wrapped in aluminium foil, vacuum packed and stored at −80 °C. Volatile compound analyses were performed at the Sezione di Scienze delle Produzioni Animali, University of Catania, where the samples were transported on dry ice.

2.2. Sample preparation and volatile compounds analysis

The muscle *longissimus dorsi*, still frozen, of each lamb ($n = 36$) was trimmed of external visible fat and finely sliced (slices thickness: 1 mm maximum) by a scalpel cut. Six grams of raw sliced meat were placed in a 20 ml glass vial and capped with a PTFE septum. For the extraction of headspace volatile compounds the solid phase microextraction (SPME) technique was used. The vial containing the sample was placed in a water bath set at 60 °C (±2 °C) for 10 min; a 75 μm PVB/PDMS fibre (Supelco, Bellefonte, PA) was then exposed to the headspace over the sample at 60 °C (±2 °C) for 30 min. Once finished the adsorption time, the fibre was removed from the vial and immediately inserted into the GC (TRACE 2000, Thermo-Finnigan, San Jose, CA) injector set at 250 °C and the desorption time was 4 min. For the GC analysis, the injector operated in splitless mode at a temperature of 250 °C and was provided with a 0.75 mm inlet liner (Supelco, Bellefonte, PA). Helium was used as carrier gas with a flow rate of 1.0 ml/min. Volatile compounds were separated using a Supelco SPB 5 column (60 m × 0.32 mm × 1 μm). The GC oven temperature was ramped as follows: 40 °C held for 5 min; then up to 230 °C with a rate of 3 °C/min and held at 230 °C for 5 min, with a total acquisition programme of 73 min. The GC/MS interface was heated at 280 °C. Volatile compounds mass spectra were generated by a MS equipped with an ion trap (Polaris Q, Thermo-Finnigan, San Jose, CA); the acquisition was performed in electron impact (EI) mode (70 eV) by 10 microscans/s, scanning the mass range 33–230 *m/z*. Compounds identification was performed by comparison with mass spectra of the NIST 7 Mass Spectral Library (2000), by comparison with linear retention indices (LRI) (Kondjoyan & Berdagué, 1996) and by data reported in the literature. The LRI were calculated by previous injection of standards of *n*-alkanes from 5 to 17 carbon atoms. The peak area of the volatile compounds was integrated from specific ions for each molecule to avoid overlapping between the compounds. Meat samples underwent a singular analysis. In order to avoid memory effects due to the SPME fibre and to the GC column, in each day of analysis four samples from different treatments were analysed. Also the sequence of samples from different dietary treatments was changed over the nine days of analysis.

2.3. Feed analyses

Feed were analysed for DM, crude protein, CP (ISO 5983, 1997), ether extract (ISO 6492, 1999); neutral detergent fibre (NDF) was assayed according Van Soest, Robertson, and Lewis (1991) with sodium sulphite, without alpha amylase and expressed with residual ash method. Extraction and analysis of phenolic compounds were carried out as described by Khazaal, Markantonatos, Nastis, and Orskov (1993). Total phenols (TP) were determined by Folin–Ciocalteu's reagents, according to Julkunen-Tiito (1985) and the concentration was measured as tannic acid equivalent using tannic acid (100 773, Merck KGaA, Darmstadt, Germany) as standard. Total extractable CT were measured using butanol-HCl method (Porter, Hrstich, & Chang, 1986). The levels of condensed tannins were quantified in GS and GSO diets using grape seed purified CT as standard. The concentration of CT in the CL and CLO diets was quantified using *C. ladanifer* purified CT as standard. For C, CO diets the levels of CT were quantified twice using both grape seed and *C. ladanifer* purified CT, thus two different CT values for these diets are presented in Table 1.

2.4. Statistical analysis

Data were analysed by ANOVA as a completely randomized design, including in the model treatment effects and experimental error. Individual animals were considered as experimental units. When ANOVA was significant ($P < 0.05$) means were separated by pairwise comparison. The Anderson–Darling test was used for testing if data followed a normal distribution. Where not normally distributed ($P < 0.05$), a $\log_{10}$ transformation of the data was performed before analysis of variance; $\log_{10}$ of peak area mean values are presented in the table. Principal component analysis was performed on the data from the 36 samples. The data set consisted of those volatile compounds affected ($P < 0.05$) by the dietary treatment.

Table 1
Chemical composition of the diets.

	Diets					
	C	CO	GS	GSO	CL	CLO
Chemical composition (g/kg DM)						
Dry matter[a]	927	929	927	930	904	907
Crude protein	149	143	138	132	150	128
Neutral detergent fibre	535	516	532	519	399	416
Ether extract	18.6	68.7	20.7	67.8	34.1	87.9
Total fatty acids	14.2	66.0	14.5	66.7	24.4	78.4
Total phenols	9.1	7.5	17.6	16.2	17.5	16.5
Grape seed CT[b]	0.95	0.70	14.9	13.3	–	–
Cistus ladanifer CT[c]	8.48	8.17	–	–	21.0	20.7
Fatty acid composition[d]						
C16:0	16.0	7.8	17.6	8.08	13.1	7.7
C18:0	1.6	2.8	2.0	2.8	2.1	2.8
C18:1 *cis*-9	16.0	19.1	15.7	18.8	17.6	18.6
C18:2 *n*−6	29.6	31.0	33.1	31.3	36.8	32.8
C18:3 *n*−3	11.0	29.9	14.0	29.9	8.5	29.4

[a] Expressed as g/kg of feed.
[b] Condensed tannins (CT) quantified using purified grape seed CT as standard.
[c] Condensed tannins quantified using purified *Cistus ladanifer* CT as standard.
[d] Expressed as g/100 g of total extracted fatty acids.

3. Results

The chemical composition of each of the diets is shown in Table 1. Oil inclusion target was 60 g/kg DM, but the total fatty acid enrichment was 52 g/kg DM for both CO and GSO diets and 54 g/kg DM for CLO diet. Oil inclusion in the diets resulted in an increase of linolenic (C18:3 *n*−3) acid. The CL diet contained the highest levels of C18:2 *n*−6 as compared to the other diets. Also, among the oil-free diets, the CL treatment contained the highest level of total fatty acids (24.4 g/kg DM). The dietary inclusion of grape seed or of *Cistus* increased the total phenols concentration by 8.7 g/kg DM, compared to the C and the CO treatments. Condensed tannins values were lower than total phenolics in grape seed diets and higher than total phenolics in *Cistus*-diets. The enrichment in grape seed CT, computed by the difference of grape seed diets and control diets (i.e. GS–C and GSO–CO), was 13.9 g/kg DM and 12.6 g/kg DM for GS and GSO diets, respectively. The enrichment in *Cistus* CT, computed by the difference of *Cistus*-diets and control diets (i.e. CL–C and CLO–CO) were 12.5 g/kg DM for both GS and GSO diets. This shows that the enrichment of condensed tannins into the diets was achieved to a similar extent by the addition of grape seed extract or of *Cistus*.

Table 2 reports the volatile compounds, grouped according to their chemical families, detected in lamb *longissimus dorsi* muscle. A total of 106 volatile compounds were detected and among them 47 were identified using both the mass spectra and the linear retention index while six compounds were tentatively identified on the basis of their mass spectra. None of the organic acids identified was affected ($P > 0.05$) by the dietary treatments. However, nonanoic acid was not detected in the meat of the lambs of the CL and CLO groups while was present in the samples from the other four treatments. Among the seventeen identified aldehydes, pentanal, 4-heptenal, heptanal, 2-heptenal, 2-nonenal, 2,4-heptadien-1-al and 2-ethyl benzaldehyde were affected by the diets ($P < 0.05$). Pentanal was found at lower ($P < 0.05$) level in the meat of the lambs fed the CL treatment compared to the meat from animals fed the CO diet (4.41 vs. 5.00 $\log_{10}$ peak area, respectively). The meat of the CL lambs contained lower amounts ($P < 0.05$) of 4-heptenal compared to the muscle of the lambs from the other treatments. Also, this volatile compound was found at higher level ($P < 0.05$) in the meat of the lambs receiving the CO diet as compared to the meat of the lambs receiving the C diet (5.44 vs. 4.75 $\log_{10}$ peak area). When lambs were fed the *C. ladanifer* plus oil their meat contained higher ($P < 0.05$) levels of heptanal as compared to the meat of the lambs fed the *Cistus*-diet without oil supplementation. The accumulation of 2-heptenal was in tendency higher ($P = 0.063$) in the meat of the CO lambs as compared to the meat of the lambs from the C group (4.64 vs. 2.74 $\log_{10}$ peak area, respectively). 2,4-Heptadien-1-al was found at greater amounts ($P < 0.05$) in the meat of the lambs receiving oil supplementation as compared to the meat of the animals from the C and the CL treatments. 2-Ethyl-benzaldehyde was higher ($P < 0.05$) in the meat of the CO lambs as compared to the CL animals.

Among the ketones, 2-butanone was detected at higher ($P < 0.05$) levels in the meat of the lambs receiving the CL diet as compared to the meat from the animals fed the GSO treatment. Feeding the CL diet resulted in a lower ($P < 0.05$) accumulation of 3-hydroxy-2-butanone as compared to the GS, GSO and CLO diets. The 2,2,6-trimethyl-cyclohexanone was detected exclusively in the meat from the lambs of the CL and CLO groups ($P < 0.0005$).

Seven alcohols were identified in meat samples. Among them, only 2-ethyl-phenol (tentatively identified) was affected by the treatments ($P = 0.007$), being at lower amount in the meat of the CL lambs as compared to the other groups (1.75 vs. 3.70 $\log_{10}$ peak area, on average).

Most of the 10 identified hydrocarbons contained methyl-groups. 2,3-Dimethyl-hexane and the cyclopropyl benzene were not detected in the meat of the lambs of the C, CO, GS and GSO treatments and were present at higher levels in the muscle of the CLO lambs than the CL animals ($P < 0.05$). 3-Ethyl-2-methyl-1,3-heptadiene was detected in none of the samples from the C and the GSO groups while was present in the meat of some of the animals of the other four groups. Among the sulphur-containing compounds, the accumulation of dimethyl sulfone was strongly affected by the treatment ($P < 0.0005$). This volatile compound was lower ($P < 0.05$) in the meat of the lambs fed the *Cistus*-diets as compared to the meat from the animals fed the other treatments (on average, 4.21 vs. 4.79 $\log_{10}$ peak area). The other sulphur-containing compounds identified were not affected by the feeding system.

None of the furans was affected by the six different treatments ($P > 0.05$). Among the terpenes, only verbenone was affected by the diets ($P < 0.0005$). This terpene was found exclusively in the meat of the *Cistus*-fed lambs.

The principal component (PC) 1 accounted for the 53.1% of the variability. The PC 1 enabled to discriminate between the lambs of the CL and the CLO groups, while was of scarce value for discriminating the lambs from the other four treatments (Fig. 1). The PC 2 allowed the discrimination of the samples from the lambs fed the CL and CLO diets from the meat of the lambs of the other four dietary treatments. In regards to the PC 2, all the animals form the CL and CLO treatments scored negative values, while all the lambs – with the exception of two animals – fed the C, CO, GS and GSO treatments were positive. The PC 2 accounted for the 25.2% of the variability of the data set.

4. Discussion

Most of the volatile compounds present in meat are responsible for its flavour (Mottram, 1998). Some of these are transferred directly from feed to tissues (e.g. the terpenes) while other compounds arise from ruminal microorganisms metabolism (e.g. skatole) and others are originated by the animal metabolism (Vasta & Priolo, 2006). This is the case of some aldehydes, alcohols, ketones and hydrocarbons, which arise from PUFA oxidation (Mottram, 1998). Other meat volatile compounds are originated during cooking via heat-induced reactions, such as the Maillard or the Strecker reactions (Mottram, 1998).

Table 2
Volatile profile of muscle *longissimus dorsi* from lambs fed different diets[A].

Compounds [*m/z* (relative intensity)]	Specific ion[B]	LRI[C]	Method of identification[H]	Treatment[D]						SE[E]	*P*[F]
				C	CO	GS	GSO	CL	CLO		
Organic acids											
Acetic acid											
45 (100), 43 (98), 60 (92)	60	571	MS	4.42	4.26	4.33	4.45	4.61	4.33	0.175	NS
Hexanoic acid[I]	60	990	MS, LRI	4.47	4.61	4.66	4.77	4.38	4.58	0.218	NS
Benzoic acid[d]	105	1182	MS, lri	4.30	4.33	4.34	4.41	4.32	4.03	0.189	NS
Octanoic acid[I]	60	1186	MS, LRI	3.99	4.14	4.17	4.14	4.21	3.91	0.222	NS
Nonanoic acid	60	1285	MS, LRI	2.13	1.39	2.69	1.98	nd[G]	nd	0.732	NS
Decanoic acid	55	1379	MS, LRI	0.70	1.27	0.68	0.68	1.94	2.66	0.768	NS
Aldehydes											
3-Methyl-butanal	58	651	MS, LRI	3.68	3.92	3.66	3.83	3.13	3.92	0.289	NS
Pentanal	58	695	MS, LRI	4.54[a,b]	5.00[b]	4.65[a,b]	4.75[a,b]	4.41[a]	4.80	0.125	*
Hexanal	56	799	MS, LRI	5.82	6.17	5.91	5.93	5.77	6.01	0.110	NS
2-Hexenal	55	853	MS, LRI	3.28	3.78	3.35	4.06	3.15	3.48	0.223	NS
4-Heptenal	67	925	MS, LRI	4.75[b]	5.44[c]	4.76[b]	5.30[b,c]	4.16[a]	4.92[b,c]	0.130	***
Heptanal	55	927	MS, LRI	5.90[a,b]	6.12[a,b]	5.94[a,b]	5.95[a,b]	5.64[a]	6.19[b]	0.118	*
2-Heptenal	55	981	MS, LRI	2.74	4.64	4.08	4.45	3.29	4.42	0.458	*
Benzaldehyde[I]	106	994	MS, LRI	5.85	5.91	5.92	5.80	5.97	5.97	0.040	NS
Octanal[I]	81	1031	MS, LRI	5.26	5.38	5.29	5.24	5.17	5.24	0.046	NS
2,4-Heptadien-1-al	81	1040	MS, LRI	4.09[a]	4.70[b,c]	4.13[a,b]	4.76[c]	3.98[a]	4.54[c]	0.138	***
Phenyl acetaldehyde[I]	91	1077	MS, LRI	4.20	4.45	4.39	4.35	4.21	4.39	0.126	NS
Nonanal[I]	67	1135	MS, LRI	6.02	6.01	6.00	6.00	5.92	5.94	0.040	NS
2-Nonenal	55	1191	MS, LRI	4.71[a,b]	5.07[b]	4.72[a,b]	4.86[a,b]	4.43[a]	5.03[b]	0.110	**
2-Ethyl-belzaldehyde[e]	105	1204	MS, lri	4.31[a,b]	4.64[b]	4.42[a,b]	4.59[a,b]	4.26	4.54[a,b]	0.097	*
2,4-Dimethyl-benzaldehyde											
133 (100), 134 (64), 91 (50), 105 (46)	133	1222	MS	2.84	2.82	2.81	3.56	3.49	2.11	0.845	NS
2-Decenal[I]	70	1295	MS, LRI	3.98	4.13	3.99	4.08	3.54	3.98	0.159	NS
2,4-Decadienal[e,f]	81	1350	MS, lri	2.84	2.36	3.51	3.01	2.77	3.64	0.881	NS
Ketones and hydroxyketones											
2-Butanone[i,g]	57	579	MS, lri	4.09[a,b]	4.26[a,b]	4.21[a,b]	4.02[a]	4.36[b]	4.28[a,b]	0.120	*
3-Hydroxy-2-butanone	45	708	MS, LRI	3.80[a,b]	4.53[a,b]	5.14[b]	5.10[b]	1.59[a]	5.57[b]	0.794	**
6-Methyl-2-heptanone[f]	58	955	MS, lri	3.36	4.27	3.99	4.07	3.88	3.99	0.293	NS
2-3-Octanedione	99	1009	MS, LRI	5.17	5.51	5.24	5.35	5.13	5.25	0.108	NS
2,2,6-trimethyl cyclohexanone	67	1044	MS, LRI	nd[a]	nd[a]	nd[a]	nd[a]	4.54[b]	4.55[b]	0.063	***
Alcohols											
1-Penten-3-ol	67	675	MS, LRI	3.11	4.02	3.78	3.88	2.87	3.77	0.372	NS
3-Methyl-3-buten-1-ol	67	731	MS, LRI	2.50	3.24	3.48	3.67	3.64	3.94	0.438	NS
1 Pentanol	55	765	MS, LRI	4.94	4.75	4.77	4.78	4.66	4.99	0.252	NS
2,3-Butanediol	57	782	MS, LRI	0.70	1.40	0.77	1.47	0.70	2.27	0.842	NS
2-Ethyl phenol											
107 (100), 122 (38), 77(24)	107	944	MS	3.14[b]	4.01[b]	3.73[b]	3.78[b]	1.75[a]	3.86[b]	0.425	**
2-Ethyl-1-hexanol[I]	70	1056	MS, LRI	5.34	5.28	5.27	5.30	5.19	5.13	0.029	NS
2-Octen-1-ol	67	1070	MS, LRI	3.68	4.27	4.30	4.30	4.22	4.32	0.336	NS
Hydrocarbons											
Benzene	78	665	MS, LRI	4.59	4.63	4.47	4.58	3.97	4.58	0.350	NS
3-Methyl-2-heptane[h]	55	750	MS, lri	0.82	nd	nd	0.55	0.59	1.08	0.546	NS
2,3-dimethyl-hexane	67	755	MS, LRI	nd[a]	nd[a]	nd[a]	nd[a]	1.92[b]	3.64[c]	0.358	***
Toluene[I]	91	781	MS, LRI	5.17	5.22	5.19	5.08	5.19	5.12	0.036	NS
1,2-Dimethyl benzene (o-xylene)	106	899	MS, LRI	4.88	5.00	4.81	4.79	4.66	4.72	0.134	NS
Cyclopropyl benzene											
117 (100), 118 (69), 91(42), 65 (11)	117	981	MS	nd[a]	nd[a]	nd[a]	nd[a]	0.77[a]	2.89[b]	0.487	***
1,2,3-Trimethyl benzene[i]	91	997	MS, lri	1.43	0.78	nd	0.64	0.61	1.31	0.696	NS
1,3,5-Trimethyl benzene	105	1001	MS, LRI	3.53	5.21	5.02	4.99	4.88	4.96	0.470	NS
3-Ethyl-2-methyl 1,3 heptadiene											
81 (100), 138 (97), 67 (98), 109 (93)	81	1066	MS	nd	0.59	1.41	nd	2.06	2.74	0.676	*
4-Ethyl-1,2-dimethyl benzene	119	1089	MS, LRI	2.87	4.36	4.30	4.12	4.23	4.46	0.403	NS
Sulphur-containing compounds											
Dimethyl sulfide[I,h]	62	532	MS, lri	5.15	4.94	5.05	5.02	5.28	5.17	0.053	NS
Carbon disulfide	76	542	MS, LRI	4.42	4.26	4.33	4.45	4.61	4.33	0.153	NS
Dimethyl sulfone 79 (100), 94 (43), 45 (13)	94	939	MS	4.87[b]	4.73[b]	4.80[b]	4.77[b]	4.18[a]	4.24[a]	0.118	***
Furans											
2-Ethyl furan[d,g]	81	710	MS, lri	3.66	4.73	4.36	3.84	2.21	3.85	0.678	NS
2-Penthyl furan	81	1022	MS, LRI	4.47	5.14	5.14	5.55	5.40	5.52	0.435	NS
Terpenoids											
α-Pinene	93	961	MS, LRI	nd	1.28	0.69	0.69	nd	2.09	0.644	NS
Menthol[g,j]	81	1181	MS, lri	2.22	2.47	2.60	0.76	0.62	0.62	0.777	NS
Verbenone	107	1293	MS, LRI	nd[a]	nd[a]	nd[a]	nd[a]	3.24[b]	3.96[b]	0.267	***

[a,b,c] Means with different letters within the same row are statistically different ($p < 0.05$).
[A] Values ($\log_{10}$ specific ion peak area units) are means of six lambs per dietary treatment.
[B] MS mass fragment of which area was integrated.
[C] Linear retention index.

Table 2 (*continued*)

[D] The treatments were: dehydrated lucerne without (C) or with (CO) vegetal oil supplementation; dehydrated lucerne plus grape seed extract without (GS) or with (GSO) vegetal oil supplementation; dehydrated lucerne plus *Cistus ladanifer* without (CL) or with (CLO) vegetal oil supplementation.
[E] Standard error.
[F] NS, not significant different; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.
[G] nd, not detected.
[H] MS: mass spectrum identified using Mainlib/NIST/Wiley 7 Mass Spectral Database; LRI: in agreement with Kondjoyan and Berdagué (1996); lri: in agreement with linear retention index, obtained with DB5 columns, reported in literature as listed below.
[I] Compounds which were not logarithmically transformed for statistical analysis because were normally distributed according to Anderson–Darling test.
[d] Machiels and Istasse (2003).
[e] Elmore, Campo, Enser, and Mottram (2002).
[f] Elmore et al. (2000).
[g] Vasta et al. (2007b).
[h] Insausti et al. (2005).
[i] Spadone, Takeoka, and Liardon (1990).
[j] Cornu et al. (2005).

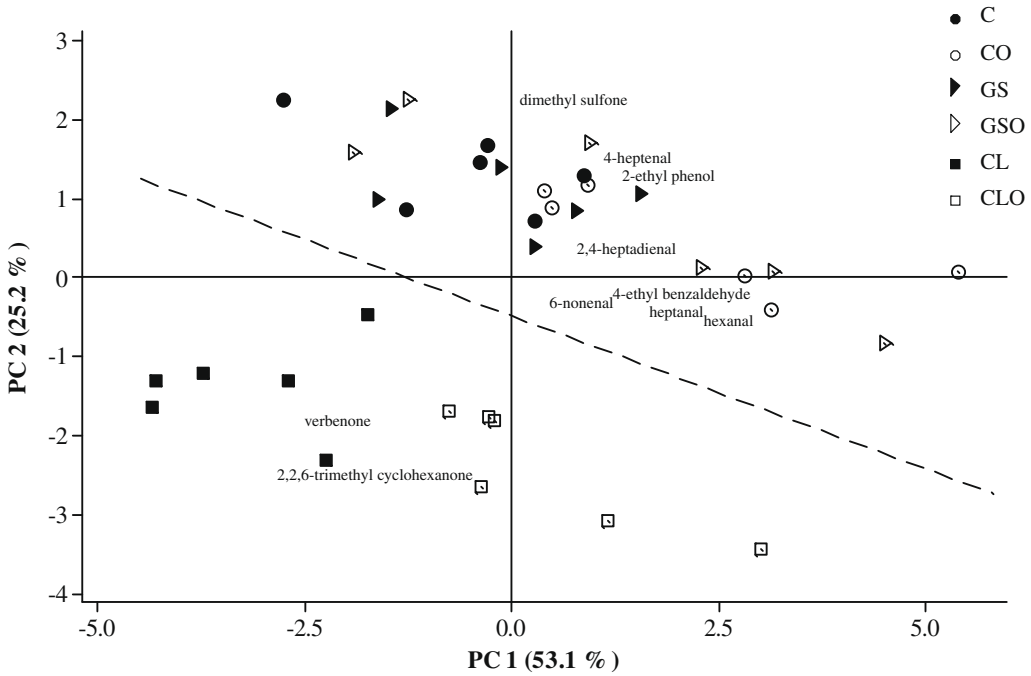

Fig. 1. Discrimination of lambs according to the feeding treatment, based on muscle volatile compounds. Principal component analysis of the volatile compounds affected ($P < 0.05$) by the feeding treatment.

So far, most of the studies concerning volatile compounds in meat animals have focused on heated adipose tissue (Priolo et al., 2004; Sivadier, Ratel, & Engel, 2009) and on roasted beef or sheep meat (Elmore et al., 2005; Insausti, Goñi, Petri, Gorraiz, & Beriain, 2005). This implies that most of the volatile compounds reported in muscle are originated by heat-induced reactions. In the present study we have chosen to perform the analyses of compounds from the headspace of *longissimus dorsi* muscle incubated at 60 °C. In agreement with Vasta, Ratel, and Engel (2007b), this procedure allows to extract and analyse those compounds which can be considered as original information displayed by muscle rather than by cooked meat.

A number of studies have investigated the effect of feeding vegetal or fish oils-enriched diets on the appearance of volatile compounds in cooked meat (Elmore, Mottram, Enser, & Wood, 1999; Elmore et al., 2005), showing that meat PUFA undergo oxidative reactions, producing a large number of odour-active compounds, comprising off-flavours. This result has been confirmed also *in vitro* (Campo et al., 2003).

In the present study, for the first time, we have supplemented the sunflower and linseed oil together with grape seed extract or with *C. ladanifer,* which contain high amounts of polyphenols and in particular of tannins (Table 1). It has been recently reported that the supplementation of grape seed or quebracho extract into lamb diets is effective in obtaining a milder caudal fat odour by reducing ruminal biosynthesis and the accumulation of strong odour-active compounds, such as skatole (Priolo et al., 2009; Schreurs et al., 2007a). When we have planned the present study we have hypothesized that supplementing a source of polyphenols could affect meat volatile compounds.

The CL treatment resulted in lower amounts of 4-heptenal in muscle compared to all the other treatments (Table 2). This compound arises from oxidation of linolenic acid. Elmore, Mottram, Enser, and Wood (2000), found that 4-heptenal detected in cooked lamb was highly correlated with the fish or linseed oil supplementation. As compared to control group (C), oil supplementation resulted in an increase of 4-heptenal, while *Cistus* supplementation resulted in a reduction of this compound ($P < 0.05$). If the first result had been previously described (Elmore et al., 2000), the protection against oxidation of tanniniferous supplement had not been described yet. Another compound originated by the oxidative degradation of C18:3 $n-3$ is 2,4-heptadien-1-al. This compound was increased by oil supplementation (Table 2) as expected. Compared to the CL diet, the CLO treatment resulted in higher amounts

of 2-ethyl-phenol and of heptanal, 2-nonenal and of 3-hydroxy-2-butanone, which derive from lipid degradation. Also, these volatile compounds did not differ between the C, CO, GS and GSO treatments. These results suggest that the vegetable oil enhanced the appearance of these volatile compounds only when it was associated to the *C. ladanifer*, rather than when it was given together with lucerne, with or without the grape seed extract. The fact that the appearance of these volatile compounds was similar in the lambs from the GS and GSO treatments suggests that the presence of grape seed extract in the GSO diet has prevented the formation of these lipid-deriving compounds. We suppose that this result may be due to the presence of polyphenols from the grape seed extract. The different response to oil supplementation between diets containing the *C. ladanifer* or the grape seed extract could depend on the different type of polyphenols present in these treatments. It is well known that polyphenols, and in particular the tannins, from different plants differ for their chemical structure and reactivity in animal digestive tracts (Muller-Harvey, 2006). However, also other dietary factors could have been involved in the appearance of meat volatile compounds. Oil supplementation can exert different effects on carcass traits and meat quality depending on the type of basal diet given to animals. In a recent study, we have found that meat fatty acid composition of lambs given dehydrated lucerne plus oils was different as compared to the fatty acid profile of lambs receiving the oil supplementation together with a concentrate diet (Bessa et al., 2005). Probably, some secondary compounds or some enzymes present in *C. ladanifer* could have played a role on the digestion of the PUFA ingested by the animals.

In the present study we have detected several volatile compounds (e.g. C_7–C_{10} saturated and monounsaturated aldehydes, 3-hydroxy-2-butanone, 1-penten-3-ol, 2-octen-1-ol) which according to other authors (Elmore et al., 2000; 2005) are highly associated to fat-enriched diets, but in our study these compounds were not affected by treatments. This could be explained considering that the works from Elmore et al. (2000; 2005) were conducted on meat from animals fed fat-enriched diets (which is similar to our study) cooked at 140 °C (Elmore et al., 2000) or grilled (Elmore et al., 2005), which is not our case. The cooking conditions have probably triggered the oxidation of PUFA, thus originating the volatile compounds deriving from lipid degradation. In our case, the volatile compounds extraction was conducted with much milder conditions (60 °C) because we aimed to assess the effect of the six treatments on muscle metabolism itself. This could also explain why, compared to most of the studies conducted on cooked meat, in the present work we detected only a few etherocyclic compounds (such as furans) which arise from Maillard's and Strecker's reactions. For the same reason, we did not observe remarkable differences in the volatile compounds profile of the meat of the lambs receiving lucerne alone (control group) or with oil supplementation.

The C_6–C_{10} aliphatic acids were detected also in lamb adipose tissue by Southerland and Ames (1996). 2,3-Octanedione was not affected by the treatments. This volatile compound has been widely indicated as tracer of animal feeding system (pasture vs. concentrate) (Priolo et al., 2004; Sivadier et al., 2009; Vasta & Priolo 2006; Young, Berdagué, Viallon, Rousset-Akrim, & Theriez, 1997) when detected in sheep adipose tissue. However, regardless of animals feeding system, Vasta et al. (2007b) have reported that the concentration of 2,3-octanedione in lamb muscle increased by the increase of the temperature used for the extraction of volatile compounds, thus suggesting that also this volatile compound could derive from a heat-induced reaction.

The sulphur-containing compounds that we have detected could have originated from the degradation of the amino acids cysteine and methionine (Mottram, 1998). Only the dimethyl sulfone was affected by the feeding system, being lower in the meat of the animals from the CL and CLO treatments. A hypothesis for this result is that those microorganisms responsible for its production in the rumen were impaired by *Cistus* tannins. A similar result has been recently described for skatole by Schreurs et al. (2007b) and by Priolo et al. (2009). Why grape seed tannins had not this effect is not clear. However, it is well known that tannins (and their effects upon ruminal microorganisms) strongly differ from plant to plant (Muller-Harvey, 2006). This hypothesis is supported by the results of the principal component analysis (Fig. 1) which clearly shows that the muscle of the lambs receiving *C. ladanifer* displays a volatile profile highly distinguishable from that displayed from the meat of the other feeding treatments and this was observed regardless oil supplementation in the diet. In particular, the 2,2,6-trimethyl-cyclohexanone, 2,3-dimethyl-hexane and verbenone were detected only in the meat of the CL and CLO groups. Similarly to most of the terpenoids detected in meat or milk, verbenone could have been directly transferred from *C. ladanifer* to lamb meat. Ramalho, de Freitas, Macedo, and Silva (1999) have detected the 2,2,6-trimethyl-cyclohexanone in *C. ladanifer* leaves and therefore also this compound could derive directly from the diet.

5. Conclusions

In the present study we have investigated the effect of the inclusion of grape seed extract and *C. ladanifer* in dehydrated lucerne based diets with or without oils on the appearance of volatile compounds in lamb muscle. Muscle volatile compounds allowed the discrimination between the lambs receiving *C. ladanifer* from those not receiving this bush. Feeding *C. ladanifer* led to the presence of 2,2,6-trimethyl-cyclohexanone and of verbenone which derive from the direct transfer from feed to tissue. The concentration of heptanal, 2-nonenal, 4-heptenal and 3-hydroxy-2-butanone, which arise from lipid oxidation, was similar in the meat of the lambs fed the GS and GSO diets. These results suggest that in the presence of the grape seed extract, oil supplementation did not enhance the production of these volatile compounds. 2,4-Heptadien-1-al was higher in the meat of the lambs receiving the oil supplementation as compared to the lambs not supplemented. Further research is needed to assess if phenolic compounds can be transferred from feed to muscle and, if so, if they can play a direct role on muscle metabolism.

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