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Volatiles in raw and cooked meat from lambs fed olive cake and linseed

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This study was conducted to determine the effects of feeding olive cake and linseed to lambs on the volatile organic compounds (VOCs) in raw and cooked meat. Four groups of eight male Appenninica lambs each were fed: conventional cereal-based concentrates (diet C), concentrates containing 20% on a dry matter (DM) basis of rolled linseed (diet L), concentrates containing 35% DM of stoned olive cake (diet OC), or concentrates containing both rolled linseed (10% DM) and stoned olive cake (17% DM; diet OCL). The longissimus dorsi *muscle of each lamb was sampled at slaughter and was subjected to VOC profiling through the use of SPME-GC-MS. In the raw meat, the concentration of 3-methylpentanoic acid was higher in treatment C as compared with treatments L, OC and OCL ($P < 0.01$). Moreover the level of nonanoic acid was greater in treatments C and OC than in treatment L ($P < 0.05$). With respect to alcohols, in raw meat the amount of 2-phenoxyethanol in treatment OCL was lower than in treatments C ($P < 0.01$) and OC ($P < 0.05$), while in cooked meat the amount of 1-pentanol was higher in treatment C than in treatment OC ($P < 0.05$). Apart from these compounds, none of the lipid oxidation-derived volatiles was significantly affected by the dietary treatment. Therefore, the results suggest that the replacement of cereal concentrates with linseed and/or olive cake did not cause appreciable changes in the production of volatile organic compounds in lamb meat.*

Keywords: lamb, volatile organic compounds, polyunsaturated fatty acids, olive cake, linseed

Implications

Linseed supplementation in lamb diet increased the meat unsaturated fatty acids but reduced the resistance to oxidation, while linseed combined with olive cake improved meat oxidative stability due to the antioxidant compounds in olive cake. The results of the present investigation demonstrate that linseed and/or olive cake can be included into a concentrate-based diet for lambs, in partial replacement of conventional feedstuffs, with no appreciable effect on the appearance of volatile organic compounds in either raw or cooked meat.

Introduction

Sheep meat is one of the dietary sources of polyunsaturated fatty acids (PUFA) such as linoleic (C18:2 n-6) and α-linolenic (C18:3 n-3), and of monounsaturated fatty acids (MUFA) such as oleic acid (C18:1 n-9), which confer beneficial effects to human health (Enser *et al.*, 1996). On the other hand sheep meat also contains significant amounts of saturated fatty acids (SFA) with negative impact to human health. Taking into account the beneficial effects of the healthy fatty acids and the well-established influence of diets to meat fatty acid composition, dietary manipulation for livestock has been a common practice to obtain relatively healthier products. However, an increase in unsaturation in the intramuscular fat could lead to a decrease in meat oxidative stability, as the degree of unsaturation determines the susceptibility to oxidation (Luciano *et al.*, 2009). During the lipid oxidation process, free radicals are produced, which can propagate leading to oxidative damage to both the lipid and protein components in the meat. These damages are correlated with the deterioration of meat quality traits including colour, flavour and nutritional value (Santé-Lhoutellier *et al.*, 2008). Therefore, when animal feeding strategies are implemented to increase the levels of PUFA in the muscle, it is also important to provide adequate amounts of dietary antioxidants that could protect the meat from oxidation.

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Recently, we have conducted a study to increase the concentration of PUFA n-3 fatty acids in lamb meat by feeding diets containing linseed (Mele *et al.*, 2014). Considering that PUFA n-3 fatty acids are susceptible to oxidation, we have added olive cake into the diets and we found that the combination of linseed and olive cake effectively increased the content of n-3 PUFA in the muscle (Mele *et al.*, 2014) and preserved the meat from rapid oxidation (Luciano *et al.*, 2013). Volatile organic compounds (VOCs) are produced through lipid oxidation, or through Maillard reaction or Strecker degradation (Mottram, 1998), which are responsible for meat flavour development. The VOCs are also strongly correlated with animal diet (Vasta and Priolo, 2006); consequently they have been used as tracers of animal feeding (Priolo *et al.*, 2004). In this study we have evaluated the effect of dietary stoned olive cake alone or in combination with rolled linseed on the meat VOCs. We have hypothesized that the differences found previously in the meat fatty acid composition and oxidative stability could lead to differences in the meat volatiles. Therefore, using the solid-phase microextraction (SPME) and subsequent GC-MS analysis, we have determined the volatile compounds in the same meat samples used by Luciano *et al.* (2013) and Mele *et al.* (2014).

Material and methods

Animals and dietary treatments

The detailed animal management and diet composition was published by Luciano *et al.* (2013) and by Mele *et al.* (2014). Briefly, the feeding trial was conducted at the Experimental Station of the Department of Applied Biology at the University of Perugia, Italy and involved 32 Appenninica male lambs. Animals were weaned at the age of 40 ± 5 days and at the live weight of 17.8 ± 1.6 kg, and were randomly distributed into four groups (based on the dietary treatments) of eight lambs each. The adaptation period to the experimental diets lasted 20 days, followed by the 40-day experimental feeding with: barley- and oat-based concentrate with low lipid level and high non-structural carbohydrate content (diet C); concentrates containing 20% on a dry matter (DM) basis of linseed (diet L); concentrates containing 35% DM of olive cake (diet OC); or a mixture of linseed (10% DM) and olive cake (17% on DM; diet OCL). All ingredients were made into pellets (3 mm diameter) using a pelleting machine (CMS IEM-Colognola ai Colli Verona, Italy) operating at 35°C to 40°C. The olive cake was prepared by the mechanical extraction of virgin olive oil using an RCM Rapanelli three phases decanter mod. 44 eco (Rapanelli Inc., Perugia, Italy). The fresh olive cake remained in the process was stored at room temperature for 36 h, and dried through a fluid bed dryer, with an initial temperature of the drying air flow at 120°C. The maximum temperature attained by olive cake during the drying process was 45°C. The dried olive cake was stored at room temperature. The protein content and energy level of the diets were adjusted in order to obtain isonitrogenous and isoenergetic treatments. The concentrates were given to the animals, together with the grass hay (forage) following the 30 : 70 forage:concentrate ratio on the basis of the expected DM intake. The weight of each lamb was recorded at the beginning of the experiment and every 10 days thereafter until the day before slaughter. The amounts of feed offered and refused by animals under each group were recorded daily, and samples of these feeds were collected weekly and stored at − 30°C until analysis of CP and ether extract.

Slaughter procedure

At the end of the experimental period, the lambs were slaughtered and were weighed immediately to obtain the hot carcass weight and carcasses were kept at 4°C for 24 h. The *longissimus dorsi* (LM) muscle between the 2nd and the 13th ribs was removed, vacuum-packed and stored at − 80°C until analysis of the VOCs.

Sample preparation and volatile compounds extraction

The analysis of VOCs was done in both raw and cooked meat. The raw meat represented the original state of animal production while the cooked meat represented the cooking condition, in which the heat employed increases the production of VOCs.

Before slicing, the visible fat of the frozen LM ($n = 32$) was trimmed off with a scalpel to a thickness of not more than 1 mm. Six grams of meat was placed in a 20-ml capacity glass vial and then capped with a PTFE septum. The vial was equilibrated for 20 min in a water bath at 45°C (±2°C). Then the solid-phase microextraction (SPME) for VOC extraction was performed by exposing a 75 μm PVB/PDMS fibre (Supelco, Bellefonte, PA, USA) to the headspace of the sample for 30 min, at 45°C. The same sample preparation was done on cooked meat VOC extraction except that cooking in hot water bath at an internal temperature of 70°C (±2°C) for 30 min was done before equilibration and headspace extraction.

GC-MS analysis

Following the VOC extraction, the SPME fibre was withdrawn from the glass vial and was immediately inserted into the GC (TRACE 2000, Thermo-Finnigan, San Jose, CA, USA). The injector temperature was set at 250°C at 4 min desorption time. The GC injector, with an inlet liner of 0.75 mm (Supelco) was operated in a splitless mode at 250°C. The carrier gas used was helium, set at a flow rate of 1.0 ml/min. The extracted volatile compounds were separated through a Supelco SPB 5 column (60 m × 0.32 mm × 1 μm). The GC oven temperature was held at 40°C for 5 min and then ramped to 230°C at the rate of 3°C/min and maintained at this temperature for 5 min. The total acquisition time was 73 min. The temperature of the GC/MS interface was at 280°C. The mass spectra of the volatile compounds were obtained by using an MS equipped with an ion trap (Polaris Q; Thermo Finnigan, San Jose, CA). The electron impact acquisition mode employed was with 70 eV, and 10 microscans/s using a scanning range of 33 to 230 m/z. The identities of the headspace-extracted VOC were determined by comparing

their mass spectra with those in the NIST 7 Mass Spectral Library (2000) and by comparing the calculated linear retention indices (LRI) with those found in literature (Kondjoyan and Berdagué, 1996; NIST Mass Spec Data Center, 2011). The LRI calculation was done by using n-alkanes standards of 5 to 17 carbon-containing atoms. The peak area of the VOCs was integrated using the specific ions for each molecule.

Statistical analysis

The effects of the dietary treatment (Diet: C, L, OC and OCL) on the VOCs in both raw and cooked meat were analysed using one-way ANOVA of the Minitab software version 16. When ANOVA showed a significant effect of the diet ($P < 0.05$), the means were separated by pair-wise comparison using the Tukey's test. All the data were normalized before the analysis of variance, consequently they were tabulated as $\log_{10}$ of the peak area. Pearson correlations were also performed between the VOC measured in both raw and cooked meat and data published on the tocopherol content (Luciano *et al.*, 2013) and fatty acid composition (Mele *et al.*, 2014) of the same muscle used in the present experiment.

Results and discussion

As shown by Mele *et al.* (2014), the inclusion of linseed increased the levels of C18:3 n-3 in the L diet, the inclusion of olive cake increased the levels of C18:1 n-9 in the OC diet, while the C diet had the highest C18:2 n-6 content. Although the diets contained different quantities of SFA, MUFA and PUFA, similar growth rates among animals were observed since the diets were formulated to be isoenergetic and isonitrogenous (Luciano *et al.*, 2013; Mele *et al.*, 2014).

Fatty acid oxidation and Maillard reaction or Strecker degradation (Mottram, 1998) in meat result in the production of volatile compounds. In this study, the headspace VOCs were extracted from raw and cooked meat of lambs fed different diets. The temperature used for the VOC extraction was 45°C (±2°C), which is lower than 60°C used by Vasta *et al.* (2010), to reduce the interference of volatiles formed from heat-related reactions, especially for the raw meat samples. Moreover, the cooking temperature used was 70°C (±2°C) because at low internal temperatures (72°C) minimal degradation of meat components takes place, consequently the influence of the dietary treatments and the production of volatile compounds may be easily correlated (Almela *et al.*, 2010). Temperature is critical in VOC studies because changes in extraction temperature modifies the identities and total amount of volatiles derived from meat (Ahn *et al.* 1999). The extraction procedure used in this study was static which, when combined with low extraction temperature, could limit the amount and number of VOCs that can be detected in the samples.

There were 20 volatile compounds identified in the raw meat samples (Table 1) and 30 volatile compounds were detected in the cooked meat samples (Table 2). The VOCs were grouped according to their chemical families: five aldehydes, two ketones, five hydrocarbons, two alcohols, three fatty acids, one ester, and two sulfur-containing compounds in the raw meat, and 10 aldehydes, three ketones, nine hydrocarbons, three alcohols, one ester, two sulfur-containing, one furan, and one sulfur-and-nitrogen compound in the cooked meat. Most of the VOCs in raw meat have previously been reported in lamb meat (Vasta *et al.*, 2010), heifer muscle (Vasta *et al.*, 2011), and turkey thigh (Ahn *et al.*, 1999). The chemical families of VOCs that were found in the cooked meat were similar to those identified in cooked meat from lambs fed diets containing different fat sources (Elmore *et al.*, 2000). Since most of the VOCs detected in meat in the current study were derived from the oxidation of fatty acids, we correlated the VOC profile of meat with the published data on the fatty acid composition (Mele *et al.*, 2014) and tocopherol content (Luciano *et al.*, 2013) which were measured in the same muscle samples.

The dietary treatment did not affect the concentration of aldehydes in either raw or cooked meat. Aldehydes are mainly produced from the oxidation of lipids (Vasta *et al.*, 2011), and high amount of aldehydes can be associated with great PUFA oxidation in animal fats (Elmore *et al.*, 2005; Vasta *et al.*, 2012). Overall, the aldehydes concentration in the raw meat was lower than in the cooked meat, which is in agreement with the positive relation between volatile concentration and extraction temperature observed by Ahn *et al.* (1999). In raw meat, the total aldehyde concentration (the sum of the aldehydes detected) was not correlated with the PUFA concentration in the muscle but was positively correlated with some MUFA, such as C14:1 *trans*-9 (0.353, $P = 0.047$) and C16:1 *trans*-10 (0.422, $P = 0.016$). Conversely, in cooked meat, aldehydes concentration showed a positive correlation with the concentration of PUFA in the muscle, such as C18:3 *cis*-6 *cis*-9 *cis*-12 (0.385, $P = 0.030$), C22:5 *cis*-4 *cis*-7 *cis*-10 *cis*-13 *cis*-16 (0.463, $P = 0.008$), and in tendency with C22:4 *cis*-7 *cis*-10 *cis*-13 *cis*-16 (0.330, $P = 0.065$). This is consistent with the fact that, generally, cooking represents a stress for meat, which increases the susceptibility of PUFA to lipid peroxidation as compared with raw meat (Nieto *et al.*, 2011). Indeed, elevated cooking temperatures increase the rate of chemical reactions (Ahn *et al.*, 1999). Moreover, during cooking, endogenous antioxidant defences of muscle are inactivated and the iron-containing meat proteins such as myoglobin and haemoglobin are denatured, releasing free iron, a major oxidative catalyst in meat systems (Campo *et al.*, 2003), which then enhances the interaction between the oxidants and the unsaturated fatty acid substrates (Nieto *et al.*, 2011). Regarding the individual aldehydes, hexanal showed a tendency to be affected by the dietary treatment ($P = 0.097$), being lower in raw meat from lambs fed OCL diet when compared with the C diet. Hexanal and other aldehydes such as pentanal could be derived from the oxidation of linoleic acid (C18:2 n-6; Elmore *et al.*, 2005). However, Mele *et al.* (2014) found no effect of the dietary treatment on the concentration of linoleic acid in the intramuscular fat from the same animals used here, and no significant correlation was found between these aldehydes

Table 1 *Volatile organic compounds detected in the raw* longissimus dorsi *muscle from lambs fed with diets containing olive cake and linseed*[A]

Compound [m/z (relative intensity)]	Specific ion[B]	LRI	Diet[C]				s.e.m.	*P* value
			C	L	OC	OCL		
Aldehydes								
2-ethyl-4-pentenal	55	1029	3.408	3.334	3.102	2.945	0.152	0.712
4-ethylbenzaldehyde	105	1174	2.552	2.742	2.117	1.522	0.215	0.191
Hexanal	82	799	3.557	3.135	2.176	1.762	0.281	0.080
Nonanal	67	1104	3.223	2.785	3.035	3.214	0.242	0.918
Octanal	81	1004	2.081	1.265	0.420	1.698	0.299	0.238
Ketones								
2-butanone[c]	43	596	2.458	1.953	1.926	1.572	0.276	0.747
3-hydroxy-2-butanone[c]	45	707	2.118	2.196	3.273	3.127	0.289	0.362
Alcohols								
1-octen-3-ol	57	967	3.259	2.689	2.422	2.226	0.303	0.669
2-phenoxyethanol[g]	94	1228	2.887[a]	1.821[ab]	2.648[a]	0.756[b]	0.247	0.004
Acids								
Decanoic acid	55	1357	2.786	1.866	1.935	1.541	0.265	0.405
Nonanoic acid[e]	129	1259	2.840[a]	0.768[b]	2.823[a]	2.012[ab]	0.276	0.017
3-methylpentanoic acid	60	947	2.965[a]	0.446[b]	3.018[a]	0.413[b]	0.308	<0.001
Ester								
3-butenoic acid, ethyl ester[h]	55	764	0.436	0.967	2.212	2.111	0.315	0.120
Hydrocarbons								
5-(1- methylethylidene)-1,3-cyclopentadiene	91	849	3.083	2.608	2.718	3.080	0.253	0.883
Benzene	78	662	2.673	2.857	2.838	2.376	0.133	0.570
Trans-2,3-epoxybutane[f]	43	578	1.877	1.831	2.859	2.301	0.330	0.688
m-xylene[e]	91	873	2.767	3.307	3.030	2.801	0.183	0.726
p-xylene[d]	106	855	3.394	3.608	3.705	3.074	0.129	0.331
Sulfur-contaning								
Carbon disulfide	76	537	2.900	3.351	3.466	2.960	0.155	0.500
Dimethyl sulfide[c]	62	519	3.884	4.039	3.881	3.722	0.052	0.206

LRI = linear retention index.
[a,b]Means with different letters within the same row are statistically different ($P < 0.05$); Mass spectrum identified using Mainlib/NIST/Wiley 7 Mass Spectral Database; LRI in agreement with Kondjoyan and Berdagué (1996) and NIST Mass Spec Data Center (2011); LRI: in agreement with linear retention index, obtained with DB5 column; [c]Vasta *et al.* (2007); [d]Vasta *et al.* (2011); LRI: in agreement with linear retention index obtained with other column; [e]OV-101; [f]methyl silicon; [g]BPX-5; [h]SE-30.
[A]Values (log_{10} specific ion peak area units) are means of eight lambs per dietary treatment.
[B]MS mass fragment of which area was integrated.
[C]The dietary treatments were: a barley- and oat-based concentrate with a low level of lipids and a high content of non-structural carbohydrate (diet C); concentrate containing 20% on a dry matter basis (DM) of linseed (diet L); a concentrate containing 35% DM olive cake (diet OC); a diet containing a mixture of linseed (10% DM) and olive cake (17% on DM; diet OCL).

and linoleic acid. Octanal and nonanal were both present in the raw and cooked meat and originate from the oxidation of oleic acid (C18:1 n-9; Meynier *et al.*, 1998), which is consistent with the fact that, in the present study, the concentration of these aldehydes in cooked meat was positively correlated with that of oleic acid (0.345, $P = 0.053$). Despite the fact that the inclusion of olive cake in the diet increased the muscle concentration of oleic acid (Mele *et al.*, 2014), these aldehydes were not affected by the dietary treatment. The Strecker aldehydes, 2-methylbutanal and 3-methylbutanal were identified in the cooked meat and are generally associated with grilled lamb meat aroma profile (Elmore *et al.*, 2005). Benzaldehyde was also detected in cooked meat and it has been reported to be derived from linolenic acid (C18:3 n-3) degradation (Nuernberg *et al.*, 2005). Linolenic acid was found at higher concentrations in meat from lamb fed linseed-containing diets, L and OCL, in comparison with the other treatments (Mele *et al.*, 2014). However, in the current study, none of the volatile compounds that typically originate from linolenic acid degradation were found to be significantly affected by the diets. On one hand, it should be considered that individual PUFAs readily form free radicals, which is the initial step in the oxidative degradation of fatty acids, but once the free radicals are formed the subsequent steps in the reaction become more independent of the nature of the fatty acids (Elmore *et al.*, 1997 and 1999). This could partly explain why, in the present study, despite the differences in the fatty acid composition of the muscle, only slight correlations were found between these lipid oxidation-derived VOCs and the individual fatty acids. Furthermore, it should be stressed that, besides the susceptibility of PUFA to oxidation (Elmore *et al.*,1997; Luciano *et al.*, 2009), the oxidative stability of meat ultimately depends on the balance between the

Table 2 *Volatile organic compounds detected in the cooked* longissimus dorsi *muscle from lambs fed with diets containing olive cake and linseed*[A]

Compound [m/z (relative intensity)]	Specific ion[B]	LRI	Diet[C]				s.e.m.	*P* value
			C	L	OC	OCL		
Aldehydes								
2-ethyl-4-pentenal	55	1029	3.198	2.877	2.770	3.206	0.143	0.629
4-ethyl-benzaldehyde	105	1174	2.998	2.598	2.089	2.301	0.222	0.519
Hexanal	82	799	5.290	5.160	5.133	5.164	0.033	0.361
Nonanal	67	1104	4.670	4.520	4.486	4.477	0.037	0.217
Octanal	81	1004	3.736	1.630	3.009	3.521	0.337	0.109
Benzaldehyde[c]	105	949	4.760	4.228	4.696	4.131	0.208	0.640
2-methyl butanal[c]	58	662	2.924	2.546	3.346	2.536	0.220	0.536
3-methylbutanal[c]	58	651	3.561	3.644	3.597	3.606	0.024	0.711
Heptanal[c]	96	875	3.883	2.647	4.105	4.182	0.254	0.108
Pentanal	43	696	4.107	3.649	3.954	4.007	0.133	0.671
Ketones								
2-butanone[c]	43	596	4.044	4.053	4.054	4.037	0.026	0.996
3-hydroxy-2-butanone[c]	45	707	1.355	1.824	2.898	3.095	0.323	0.166
2,3-octanedione[c]	142	973	2.001	2.892	3.070	2.755	0.306	0.642
Alcohols								
1-octen-3-ol	57	967	3.693	3.699	3.941	3.592	0.220	0.957
2-phenoxyethanol[g]	94	1228	2.398	0.993	1.085	1.026	0.248	0.124
1-pentanol	55	763	3.899[a]	2.041[ab]	1.383[b]	2.380[ab]	0.340	0.052
Ester								
Ethyl ester-3-butenoic acid[h]	55	764	3.913	2.953	3.580	3.768	0.173	0.215
Hydrocarbons								
5-(1- methylethylidene)-1,3-cyclopentadiene	91	849	3.782	2.888	3.803	3.292	0.205	0.345
Trans-2,3-epoxybutane[f]	43	578	0.802	0.821	1.753	1.235	0.287	0.631
m-xylene[e]	91	873	3.342	3.596	2.731	2.602	0.215	0.305
p-xylene[d]	106	855	3.515	3.641	3.759	3.631	0.068	0.680
1-dodecyne[e]	67	1207	3.159	1.936	1.600	1.966	0.263	0.166
Heptane[c]	100	699	3.678	2.343	3.622	3.569	0.193	0.032
Hexane	57	600	4.010	3.554	4.108	3.530	0.179	0.568
Pentane[c]	41	501	4.043	4.160	4.066	4.008	0.030	0.322
Toluene[c]	92	768	3.948	4.027	3.938	3.915	0.032	0.639
Sulfur-containing								
Carbon disulfide	76	537	3.538	3.674	3.689	3.543	0.026	0.051
Dimethyl sulfide[c]	62	519	3.868	3.896	3.769	3.711	0.032	0.131
Nitrogen and sulfur-contaning								
2-acetyl-2-thiazoline	101	1112	2.509	2.123	3.192	2.524	0.248	0.511
Furan								
2-pentylfuran[c]	81	991	3.501	3.516	3.303	3.883	0.208	0.813

LRI = linear retention index.
[a,b]Means with different letters within the same row are statistically different ($P < 0.05$); Mass spectrum identified using Mainlib/NIST/Wiley 7 Mass Spectral Database; LRI in agreement with Kondjoyan and Berdagué (1996) and NIST Mass Spec Data Center (2011); LRI: in agreement with linear retention index, obtained with DB5 column: [c]Vasta *et al.* (2007); [d]Vasta *et al.* (2011); LRI: in agreement with linear retention index obtained with other column; [e]OV-101; [f]methyl silicon; [g]BPX-5; [h]SE-30.
[A]Values (log_{10} specific ion peak area units) are means of eight lambs per dietary treatment.
[B]MS mass fragment of which area was integrated.
[C]The dietary treatments were: a barley- and oat-based concentrate with a low level of lipids and a high content of non-structural carbohydrate (diet C); concentrate containing 20% on a dry matter basis (DM) of linseed (diet L); a concentrate containing 35% DM olive cake (diet OC); a diet containing a mixture of linseed (10% DM) and olive cake (17% on DM; diet OCL).

oxidizable substrates and the antioxidants (Luciano *et al.*, 2009 and 2013). In this trial the increased unsaturation degree of meat lipids did not probably cause extensive lipid oxidation, which could be due to the presence of antioxidants in the feeds, specifically in olive cake (Luciano *et al.*, 2013). This is consistent with the fact that the total aldehydes in raw meat was negatively correlated with the tocopherol concentration in the muscle (−0.348, $P = 0.05$), which may be an indication that the tocopherol reduced the rate of aldehyde-producing lipid oxidation process.

The 2-butanone and 3-hydroxy-2-butanone (acetoin) were the ketones detected in both the raw and cooked meat. None of the ketone was significantly affected by the dietary treatment; however in cooked meat the concentration of

ketones was positively correlated with C18:3 *cis*-9 *trans*-12 *cis*-15 (0.365, $P = 0.040$) and C18:3 *trans*-9 *trans*-12 *cis*-15 (0.359, $P = 0.043$). Some studies showed a relationship between 3-hydroxy-2-butanone and fat-enriched diets (Elmore *et al*., 2000 and 2005), while other authors reported no relationship (Vasta *et al*., 2010). This variation of the findings could be due to the different cooking and extraction conditions, particularly the temperature that has an impact on the volatile profile in the samples being analysed (Vasta *et al*., 2010). The 2,3-octanedione, which has been suggested to originate from n-6 fatty acids oxidation (Meynier *et al*., 1998), was detected in cooked meat only. The concentration of 2,3-octanedione was observed to increase with the increase in extraction temperature, which may indicate that 2,3-octanedione could be derived from heat-induced reactions (Vasta *et al*., 2010). This could explain why in the present study 2,3-octanedione was absent in the raw meat samples.

The n-3 fatty acids are probable precursors of benzene (Elmore *et al*., 2005). Nute *et al.* (2007) found that addition of linseed oil into lamb diets increased the benzene concentration in the muscle phospholipids. On the contrary, in the current study, no effect of the dietary treatment was observed with regards to the formation of benzene. The lack of effects in the production of n-3 PUFA-derived volatiles could be explained considering that, although the linseed-supplemented feed contained high amounts of C18:3 n-3, the tocopherols in the diet that were deposited in the lamb tissues (Luciano *et al*., 2013) were probably enough to overcome the increased unsaturation in the muscle fatty acids. Similarly, in cooked meat, none of the formed hydrocarbon was significantly influenced by the dietary treatment. The presence of hydrocarbons such as hexane, heptane and pentane in the cooked meat and their absence in the raw meat could be due to the reduced oxidative stability of the cooked samples. In fact a positive correlation was found between hydrocarbons and the fatty acids in the *longgissimus dorsi* muscle such as C18:1 *cis*-9 (0.375, $P = 0.034$), C18:3 *cis*-6, *cis*-9, *cis*-12 (0.357, $P = 0.045$), C20:3 *cis*-8 *cis*-11 *cis*-14 (0.352, $P = 0.048$), and C22:5 *cis*-4 *cis*-7 *cis*-10 *cis*-13 *cis*-16 (0.503, $P = 0.003$) and C22:4 *cis*-7, *cis*-10, *cis*-13, *cis*-16 (0.348, $P = 0.05$), but not in raw meat.

Regarding the alcohols, both 2-phenoxyethanol and 1-octen-3-ol were detected in raw and cooked meat. In the raw meat, the 2-phenoxyethanol was found to be significantly affected by the dietary treatments, where OCL meat had significantly lower concentration than either C ($P = 0.006$) and OC ($P = 0.015$) meats. The 2-phenoxyethanol has been reported in cooked Irish organic and conventional beef meats (Machiels *et al*., 2003), and 1-octen-3-ol is frequently reported among the lipid oxidation-derived VOCs in meat and meat products. The 1-pentanol was present only in cooked meat and was significantly affected by the dietary treatment, being higher in C meat in comparison with OC meat ($P = 0.038$). This compound was previously reported in cooked lamb meat (Elmore *et al*., 2000) and cooked beef muscle (Wettasinghe *et al*., 2001), and could be derived from the reduction of pentanal, which is an oxidation product of linoleic acid (C18:2 n-6). This is consistent with the results of the compositional analysis of the feeds used in this study in which the linoleic acid content of C diet was more than double when compared with OC diet (Mele *et al*., 2014). Even so, no significant differences were found in the levels of volatiles, which may be due to the protective effect of the antioxidants derived from the feeds. Indeed, the concentration of volatile alcohols in cooked meat tended to be negatively correlated with the tocopherol concentration in the muscle (−0.328, $P = 0.067$).

In the raw meat, 3-methylpentanoic acid and nonanoic acid were significantly affected by the dietary treatment, while in the cooked meat, no acids were detected. The concentration of 3-methylpentanoic acid was lower in treatments L and OCL as compared with treatment C ($P = 0.002$ and $P = 0.001$, respectively). Similarly, treatments L and OCL resulted in lower concentrations of 3-methylpentanoic acid compared with treatment OC ($P = 0.001$). On the other hand, the concentration of nonanoic acid in treatment L was lower than in both treatments C ($P = 0.026$) and OC ($P = 0.027$) and was comparable with treatment OCL. The branched chain fatty acids are, in general, responsible for the flavour of goat and sheep meat (Vasta and Priolo, 2006) and were found to be significantly higher in intramuscular fat from lambs in the C group (Mele *et al*., 2014). The 3-methylpentanoic acid is one among the many volatile compounds found in sheep tissues fed either fresh grass or concentrates (Young *et al*., 1997). Nonanoic acid was identified in the LM muscle of lambs fed different diets (Vasta *et al*., 2010). The dietary carbohydrates are fermented in the rumen producing propionate, which in excessive quantities is used in the synthesis of odd-chain fatty acids in the adipose tissue (Priolo *et al*., 2001). Branched and odd chain fatty acids may also be produced directly in the rumen by microorganisms, starting from branched amino acids and propionate, respectively (Mele *et al*., 2008). The lower levels of 3-methylpentanoic acid and nonanoic acid in meat from lambs fed the linseed-containing diets could be a consequence of the changes in the rumen microorganism metabolism. Previous studies, indeed, reported that linseed supplementation in the diet of dairy ewes may induce a decrease in the content of odd and branched chain fatty acids in milk (Mele *et al*., 2011).

The 3-butenoic acid ethyl ester, carbon disulfide and dimethyl sulfide were unaffected by the dietary treatment. Similarly, 2-acetyl-2-thiazoline and 2-pentylfuran, which were both detected in the cooked meat only, were not affected by the dietary treatment. According to Wettasinghe *et al.* (2001), the 3-butenoic acid ethyl ester, which was detected in the meat in the current study, may have little impact in the meat aroma. The carbon disulfide and dimethyl sulfide, on the other hand, are important intermediates of Maillard reaction in the formation of heterocyclic compounds (Mottram and Mottram, 2002). The 2-acetyl-2-thiazoline is considered to be a potent odorant in cooked meat patties (Kerler and Grosch, 1996), and the presence of this compound confirms the occurrence of the reaction between

lipid degradation and Maillard reaction products during cooking (Elmore *et al.*, 1997). The 2-pentylfuran could be derived from the oxidation of C18:2 n-6 and other n-6 fatty acids (Elmore *et al.*, 2005). However, no correlation between 2-pentylfuran and any of the n-6 fatty acids was observed. The mild cooking and extraction temperature used here could explain why, unlike other studies, only one furan was detected in this study and the lack of evidence between the occurrence of these compounds and their precursors.

Conclusions

In spite of the strong effect of the dietary treatment on the intramuscular fatty acid composition and on the oxidative stability of the meat, the supplementation of lamb diets with linseed and olive cake, in combination or not, had minimal impact on the production of volatile compounds derived from lipid oxidation both in the raw and cooked lamb meat, except for the branched and odd chain fatty acids and alcohol. The higher concentration of volatile compounds in cooked meat indicated that cooking induced the formation of volatile compounds in meat, most of which derive from fatty acid oxidation. On the basis of the volatile compounds profile, it can be concluded the feeding lambs with diets including high proportions of olive cake and linseed in replacement of the conventional ingredients did not produce appreciable changes in the appearance of VOCs in lamb meat. Sensory evaluation of meat would be certainly of interest to explore in depth the effect of these diets upon meat sensory properties.

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