



Feeding lambs hazelnut skin and linseed decreases meat lipid oxidation during *in vitro* digestion

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ARTICLE INFO

Keywords:

Lamb meat
Phenolic compounds
Mass spectrometry
Lipid hydroperoxides
Metabolites
Antioxidants
Oxidative stability

ABSTRACT

The present study aimed to evaluate the effect of the inclusion in lamb diet of hazelnut skin (H diet), extruded linseed (L diet), or a combination thereof (HL diet) on the oxidative stability of cooked and *in vitro* digested lamb meat compared to a basal diet (C diet). A significant decrease of 46.1 % and 40.9 % in lipid hydroperoxides was attended after *in vitro* digestion in lamb meat from the L and H diets with respect to C diet. Moreover, the HL diet was the most effective in decreasing the TBA-RS value after intestinal digestion (23.3 % decrease compared to C diet). Five different phenolic- and four tocopherol-derived metabolites were identified whose amount was greater in meat from supplemented diets. Furthermore, the inclusion of hazelnut skin in the lamb diet resulted in higher amount of endogenous antioxidants (carnosine and reduced glutathione) in raw meat.

1. Introduction

Nowadays, meat is considered important from a nutritional point-of-view being rich in high biological value proteins and essential amino acids as well as in fatty acids, vitamins, and micronutrients such as iron (Geiker et al., 2021; Giromini & Givens, 2022; McAfee et al., 2010). Despite this, meat intake (especially red and processed meat intake) has been recently associated with an increased risk of the onset of non-communicable and chronic diseases such as cancer and cardiovascular diseases (Giromini & Givens, 2022; Grosso et al., 2022; Shi, Huang, Schooling, & Zhao, 2023).

The link between red meat consumption and the onset of chronic diseases can be explained by the so-called “heme hypothesis” (Hemeryck, Rombouts, De Paepe, & Vanhaecke, 2018). According to this hypothesis, the simultaneous presence in red meat of heme, oxygen, and polyunsaturated fatty acids (PUFAs) promotes an oxidative stress condition that stimulates lipid peroxidation during cooking and especially during red meat gastro-intestinal digestion, resulting in the formation of toxic compounds such as lipid hydroperoxides and genotoxic aldehydes (the so-called advanced lipoxidation end-products, ALEs)

(Lorrain, Dangles, Loonis, Armand, & Dufour, 2012; Oueslati, de La Pomélie, Santé-Lhoutellier, & Gatellier, 2016; Papuc, Goran, Predescu, & Nicorescu, 2017; Van Hecke et al., 2015). ALEs, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), generated during gastro-intestinal digestion can be absorbed and interact with LDL and promote the progression of atherosclerosis and cardiovascular diseases (Bolea et al., 2021; Negre-Salvayre et al., 2010; Papuc et al., 2017). Post-prandial modification induced by ALEs may be prevented by phenolic compounds in the diet (Gorelik, Kanner, Schurr, & Kohen, 2013; Gorelik, Ligumsky, Kohen, & Kanner, 2008a). Lipid hydroperoxides and ALEs may also promote colon cancer development by forming specific DNA adducts acting as carcinogenic and genotoxic compounds (Angeli et al., 2011; Guéraud et al., 2015; Hemeryck et al., 2018; Van Hecke et al., 2015).

Various strategies have been proposed to mitigate the extent of the oxidative phenomena affecting lipids in meat during gastro-intestinal digestion. These strategies included the addition of antioxidant-rich extracts/foods to meat products, the consumption of meat together with antioxidant-rich vegetable foods/seasoning, and, more recently, the inclusion of antioxidant-rich vegetables/extracts in animal diets (Li

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<https://doi.org/10.1016/j.foodchem.2025.144266>

Received 10 January 2025; Received in revised form 4 April 2025; Accepted 7 April 2025

Available online 8 April 2025

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et al., 2010; Martini, Tagliacucchi, Minelli, & Lo Fiego, 2020; Minelli et al., 2020; Sirri et al., 2018). The latter strategy is particularly intriguing as it allows not only the improvement of meat quality but also the use of by-products from the food industry in animal feeding.

From a circular economy perspective, the replacement of standard feed crops with food industry waste products may increase the sustainability of the livestock and food sectors by decreasing the environmental impact of these industries.

Hazelnut skin is the most important by-product for several food industries and its disposal is a serious problem economically and environmentally and its re-use is strongly encouraged (Ceylan, Adrar, Bolling, & Capanoglu, 2023). Hazelnut skin is rich in unsaturated fatty acids (mainly oleic acid), dietary fiber, tocopherols, and phenolic compounds (mainly monomeric and oligomeric flavanols) (Del Rio, Calani, Dall'Asta, & Brighenti, 2011; Priolo et al., 2021). Hazelnut skin has been used in livestock nutrition as an ingredient for ewes, lambs, cows, and rabbits (Campione et al., 2020; Candellone, Peiretti, Binello, Bergagna, & Meineri, 2019; Priolo et al., 2021; Renna et al., 2020). In particular, in the lamb diet, the inclusion of hazelnut skin had no effect on animal growth performances, modulated the microbiota composition of rumen digesta, increased the amount of PUFAs as well as of tocopherols in meat, whose presence delayed lipid oxidation during meat storage (Daghio et al., 2021; Granit et al., 2001; Menci et al., 2023; Priolo et al., 2021). Hazelnut skin was also used as an ingredient in pork burger formulation to delay lipid oxidation during refrigerated storage (D'Ambra, Minelli, & Lo Fiego, 2023). However, it has not yet been explored whether the inhibitory effect on lipid oxidation can be maintained also during the gastro-intestinal digestion of meat.

Linseed is another widely used supplement in animal nutrition normally administered to increase the amount of PUFAs in meat since it is an important source of ω -3 fatty acids (α -linolenic acid) (Bernardi, Bertol, Pflanzner, Sgarbieri, & Pollonio, 2016; Minelli, D'Ambra, Maccchioni, & Lo Fiego, 2023). Moreover, linseed is also a rich source of phenolic compounds, mainly lignans, able to inhibit lipid oxidation (Socrier et al., 2019). Increasing PUFAs consumption in humans is highly recommended due to the health benefits associated especially with ω -3 fatty acids, however, PUFAs are more prone to oxidative reactions (Shahidi & Ambigaipalan, 2018).

In a recent study, Musati et al. (2024) found that partial replacement of maize with linseed and hazelnut skin in lamb diet improved nutritional quality of meat, enriching the intramuscular fat with health promoting fatty acids, such as rumenic, vaccenic, α -linolenic, and long-chain ω -3 fatty acids. Moreover, during a shelf-life study over 7 days of refrigerated storage, meat from lambs fed linseed and hazelnut skin, which provided antioxidants (e.g., vitamin E and phenolic compounds), showed a better oxidative stability despite its higher content of pro-oxidant molecules (PUFAs) (Musati et al., 2025). Therefore, in the light of the above results, the aim of this study was to understand the effects of the inclusion of hazelnut skin and linseed, both alone or in combination, in lamb diet on lipid oxidation during meat cooking and *in vitro* gastro-intestinal digestion. The hypothesis is that the antioxidant compounds present in the hazelnut skin and linseed may be absorbed by lamb during digestion and prevent the further oxidative phenomena in meat. Indeed, the phenolic profiles of the given diets as well as the appearance of exogenous and endogenous antioxidant metabolites in raw meat samples from the different treatments were evaluated by high-resolution mass spectrometry. This research is a continuation of the work previously published in Musati et al. (2024).

2. Materials and methods

2.1. Materials

All the reagents and enzymes for the *in vitro* gastro-intestinal digestion and analytical determinations were obtained from Sigma (Milan, Italy). Solvents for extraction and mass spectrometry analysis were

purchased from BioRad (Hercules, CA, USA).

2.2. Lamb diets and meat sampling

The experiment took place in the experimental farm of the University of Catania (37°24' 35.3" N, 15°03' 34.9" E) with forty 60-days-old male lambs (*Valle del Belice* × *Comisana* breed) randomly assigned to 4 groups ($n = 10$) as described in Musati et al. (2024). The control group (diet C) received a conventional maize-barley-based diet whereas in treated groups part of corn was replaced with hazelnut skin (diet H; 15 % hazelnut skin) or linseed (diet L; 8 % linseed) or a combination thereof (diet HL; 7.5 % hazelnut skin and 4 % linseed). The composition of the different diets has been already reported in Musati et al. (2024). All the experimental procedures were conducted in accordance with the European Union welfare guidelines (Directive 2010/63/EU; Council Regulation 1099/2009) and approved by "Organismo Preposto al Benessere degli Animali (OPBA)" of the University of Catania (protocol nr. 82,427).

Longissimus thoracis and lumborum (LTL) muscles were collected from each carcass, vacuum packed, and aged at 4 °C for 24 h as described in Musati et al. (2024). For the present study, six meat samples for each treatment were randomly collected for further experiments.

2.3. High-resolution mass spectrometry profiling of phenolic compounds in feedstuffs

Phenolic compounds were extracted from the different feedstuffs following the protocol reported in Nissen, Cattivelli, Casciano, Gianotti, and Tagliacucchi (2022). Briefly, 1.5 g of feedstuff were mixed with 5 mL of methanol/water/formic acid solution (70:28:2, v/v), homogenized, and incubated for 30 min at 37 °C. After extraction, the mixtures were subjected to centrifugation for 20 min (3000 ×g, 4 °C) and the collected supernatants were injected into the high-resolution mass spectrometry system.

Phenolic compounds were firstly separated by ultra-high performance liquid chromatography (UHPLC) (Ultimate 3000 separation module; Thermo Fisher Scientific, San Jose, CA, USA) through a C18 column (Acquity UPLC HSS C18 Reversed phase, 2.1 × 100 mm, 1.8 μ m particle size, Waters, Milan, Italy) before entering the high-resolution mass spectrometry system consisting of a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The chromatographic and mass spectrometry conditions as well as the full protocol were described in Martini et al. (2020). Quantification was carried out by building external calibration curves with the available standard compounds as reported in supplementary Table S1.

2.4. Tocopherols quantification in lamb diets and raw meat samples

The vitamin E (α - and γ -tocopherols) content of lamb diets was quantified following the method of Rufino-Moya, Joy, Lobón, Bertolín, and Blanco (2020). Briefly, fat-soluble vitamins were extracted from 200 mg of feedstuffs using 300 μ L methanol: acetone: petroleum ether (1:1:1, v/v/v). After centrifugating the samples, the supernatant was collected (the operation was repeated three times). The collected supernatants were dried and dissolved in methanol. The concentration of tocopherols in lamb meat was determined according to Bertolín, Joy, Rufino-Moya, Lobón, and Blanco (2018) with adaptations as follows. Two and a half g of samples were dissolved with 7.5 mL of 10 % potassium hydroxide in ethanol:water and incubated in an orbital shaker overnight. Ascorbic acid was added to prevent any oxidation process. Tocopherols were extracted using 5 mL of 9:1 hexane:ethyl acetate with 25 mg/L of butyl-hydroxytoluene (BHT), and then the samples were centrifugated. The collected supernatants were dried, and residues were dissolved with methanol. Quantification of α - and γ -tocopherols from feed and meat was performed using UHPLC analysis. Instrument,

Table 1

Quantitative data of phenolic compounds identified in the different feedstuffs. Data are reported as mg/100 g of feedstuff. C diet: Control diet; L diet: Linseed supplemented diet; H diet: Hazelnut skin supplemented diet; HL diet: Hazelnut skin and linseed supplemented diet.

Compound	C diet	L diet	H diet	HL diet
Hydroxybenzoic acid isomer 1	0.50 ± 0.02	0.48 ± 0.01	0.38 ± 0.09	0.49 ± 0.03
Hydroxybenzoic acid isomer 2	2.18 ± 0.06	5.54 ± 0.17	14.02 ± 0.28	7.38 ± 0.15
Hydroxybenzoic acid isomer 3	2.37 ± 0.13	2.31 ± 0.11	2.21 ± 0.07	2.02 ± 0.01
Hydroxybenzoic acid isomer 4	3.86 ± 0.16	3.47 ± 0.04	2.79 ± 0.08	3.32 ± 0.06
Dihydroxybenzoic acid isomer 1	0.17 ± 0.07	0.91 ± 0.12	1.97 ± 0.03	1.02 ± 0.05
Protocatechuic acid	3.81 ± 0.17	6.95 ± 0.08	15.64 ± 0.37	7.82 ± 0.02
Dihydroxybenzoic acid isomer 2	0.18 ± 0.11	0.59 ± 0.02	2.39 ± 0.01	0.75 ± 0.16
Gentisic acid	n.d.	n.d.	0.78 ± 0.17	0.22 ± 0.09
Hydroxy-methoxybenzoic acid isomer 1	3.98 ± 0.14	3.44 ± 0.13	4.15 ± 0.13	3.62 ± 0.15
Vanillic acid	5.77 ± 0.01	5.00 ± 0.03	4.14 ± 0.50	3.99 ± 0.04
Hydroxy-methoxybenzoic acid isomer 2	5.08 ± 0.27	4.47 ± 0.01	3.83 ± 0.06	3.72 ± 0.01
Hydroxy-methoxybenzoic acid isomer 3	1.23 ± 0.17	7.55 ± 0.13	17.41 ± 0.53	9.58 ± 0.03
Gallic acid	4.83 ± 0.35	6.23 ± 0.18	8.37 ± 0.13	4.83 ± 0.12
Dimethoxy-hydroxybenzoic acid isomer 1	4.62 ± 0.05	3.51 ± 0.03	3.60 ± 0.08	3.86 ± 0.21
Syringic acid	5.18 ± 0.07	4.41 ± 0.24	1.85 ± 0.15	1.21 ± 0.01
Dimethoxy-hydroxybenzoic acid isomer 2	1.21 ± 0.04	0.96 ± 0.01	0.84 ± 0.02	1.13 ± 0.34
Hydroxybenzoic acid-O-hexoside isomer 1	1.98 ± 0.13	1.93 ± 0.06	1.46 ± 0.06	1.97 ± 0.01
Hydroxybenzoic acid-O-hexoside isomer 2	2.49 ± 0.02	2.12 ± 0.02	1.99 ± 0.08	2.29 ± 0.01
Dihydroxybenzoic acid-O-hexoside isomer 1	0.29 ± 0.03	0.26 ± 0.01	0.18 ± 0.01	0.23 ± 0.01
Dihydroxybenzoic acid-O-hexoside isomer 2	3.01 ± 0.02	3.07 ± 0.09	2.98 ± 0.05	3.31 ± 0.01
Dihydroxybenzoic acid-O-hexoside isomer 3	1.24 ± 0.07	1.22 ± 0.03	1.16 ± 0.03	1.48 ± 0.07
Dihydroxybenzoic acid-O-hexoside isomer 4	0.64 ± 0.01	0.36 ± 0.01	0.46 ± 0.01	0.51 ± 0.04
Hydroxy-methoxybenzoic acid-O-hexoside	42.17 ± 1.03	33.12 ± 0.94	36.61 ± 2.35	36.69 ± 0.36
Gallic acid-O-hexoside	0.49 ± 0.01	0.40 ± 0.02	0.13 ± 0.01	0.14 ± 0.01
Syringic acid-O-hexoside isomer 1	30.80 ± 1.18	24.20 ± 1.56	25.60 ± 0.95	28.77 ± 2.24
Syringic acid-O-hexoside isomer 2	n.d.	3.05 ± 0.08	8.57 ± 0.19	4.21 ± 0.26
Hydroxybenzoic acid-O-hexoside-pentoside	1.21 ± 0.02	0.81 ± 0.02	0.86 ± 0.06	0.87 ± 0.02
Vanillic acid-O-hexoside-pentoside	19.68 ± 0.90	17.44 ± 0.46	15.42 ± 0.84	17.11 ± 0.46
Hydroxybenzoic acid-O-hexoside-hexoside	0.32 ± 0.01	0.22 ± 0.01	0.22 ± 0.02	1.49 ± 1.75
Total hydroxybenzoic acids	149.29 ± 5.24	144.02 ± 4.59	180.01 ± 7.21	154.02 ± 6.70
<i>p</i> -Coumaric acid	15.77 ± 0.71	11.70 ± 0.01	10.38 ± 0.08	11.12 ± 0.10
<i>m</i> -Coumaric acid	0.96 ± 0.02	0.66 ± 0.05	0.52 ± 0.11	0.63 ± 0.05
Caffeic acid	1.63 ± 0.06	1.19 ± 0.03	0.96 ± 0.01	0.88 ± 0.01
Ferulic acid	6.46 ± 0.04	5.41 ± 0.01	4.75 ± 0.10	4.90 ± 0.01

Table 1 (continued)

Compound	C diet	L diet	H diet	HL diet
Isoferulic acid	1.11 ± 0.01	0.83 ± 0.01	0.77 ± 0.03	0.87 ± 0.11
Dimethoxy-hydroxycinnamic acid	1.18 ± 0.03	1.00 ± 0.06	1.05 ± 0.01	1.07 ± 0.06
Coumaroyl-glycerol	19.13 ± 0.58	12.55 ± 0.70	7.07 ± 0.26	7.21 ± 0.23
Caffeoyl-glycerol isomer 1	0.73 ± 0.05	0.46 ± 0.01	0.32 ± 0.01	0.28 ± 0.02
Caffeoyl-glycerol isomer 2	7.56 ± 0.69	4.35 ± 0.23	2.79 ± 0.10	2.79 ± 0.07
Feruloyl-tyramine	1.82 ± 0.05	1.52 ± 0.07	1.27 ± 0.03	1.47 ± 0.03
3-O-Coumaroylquinic acid	0.35 ± 0.01	0.31 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
5-O-Coumaroylquinic acid	0.19 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
Caffeic acid-O-hexoside isomer 1	0.21 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Caffeic acid-O-hexoside isomer 2	0.20 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	0.15 ± 0.01
5-O-Caffeoylquinic acid	0.88 ± 0.11	0.83 ± 0.02	0.66 ± 0.01	0.51 ± 0.01
3-O-Caffeoylquinic acid <i>trans</i>	17.38 ± 0.11	17.62 ± 1.06	14.11 ± 0.34	8.63 ± 0.10
4-O-Caffeoylquinic acid	1.44 ± 0.10	1.30 ± 0.08	1.12 ± 0.11	0.76 ± 0.04
3-O-Caffeoylquinic acid <i>cis</i>	0.58 ± 0.05	0.53 ± 0.06	0.30 ± 0.04	0.31 ± 0.04
Ferulic acid-O-hexoside isomer 1	0.38 ± 0.01	0.43 ± 0.02	0.37 ± 0.03	0.43 ± 0.01
Ferulic acid-O-hexoside isomer 2	0.90 ± 0.01	0.77 ± 0.06	0.70 ± 0.04	0.72 ± 0.04
3-O-Feruloylquinic acid	0.88 ± 0.07	0.74 ± 0.03	0.63 ± 0.01	0.70 ± 0.01
5-O-Feruloylquinic acid	0.89 ± 0.07	0.84 ± 0.06	0.74 ± 0.01	0.86 ± 0.03
Dimethoxy-hydroxycinnamic acid-O-hexoside isomer 1	0.75 ± 0.01	0.77 ± 0.01	0.99 ± 0.06	0.82 ± 0.02
Dimethoxy-hydroxycinnamic acid-O-hexoside isomer 2	0.52 ± 0.01	0.42 ± 0.01	0.39 ± 0.03	0.42 ± 0.01
3,5-O-Dicaffeoylquinic acid	1.48 ± 0.12	1.30 ± 0.06	1.37 ± 0.05	1.20 ± 0.06
3,4-O-Dicaffeoylquinic acid	1.21 ± 0.18	1.14 ± 0.10	1.13 ± 0.02	0.80 ± 0.03
Total hydroxycinnamic acids	84.62 ± 3.08	67.17 ± 2.77	53.18 ± 1.47	48.13 ± 1.06
Epicatechin	3.39 ± 0.06	4.49 ± 0.22	7.42 ± 0.03	4.10 ± 0.08
Catechin	0.20 ± 0.01	0.42 ± 0.03	1.22 ± 0.01	0.60 ± 0.01
Epigallocatechin	2.93 ± 0.24	1.99 ± 0.01	0.83 ± 0.01	0.60 ± 0.02
Epicatechin-3-O-gallate	0.33 ± 0.02	0.55 ± 0.02	1.06 ± 0.03	0.57 ± 0.01
(Epi)catechin-O-hexoside	0.68 ± 0.02	0.60 ± 0.05	0.48 ± 0.02	0.62 ± 0.06
Procyanidin B2	1.61 ± 0.08	3.99 ± 0.11	13.92 ± 0.09	5.38 ± 0.15
Procyanidin dimer B type isomer 1	15.53 ± 0.55	20.88 ± 1.11	39.00 ± 0.79	22.97 ± 0.02
Procyanidin B1	0.79 ± 0.05	1.38 ± 0.05	3.42 ± 0.12	1.61 ± 0.29
(Epi)catechin-(<i>epi</i>)gallo catechin isomer 1	12.71 ± 0.43	13.21 ± 0.39	19.97 ± 0.07	13.85 ± 0.42
(Epi)catechin-(<i>epi</i>)gallo catechin isomer 2	0.72 ± 0.05	0.97 ± 0.02	2.05 ± 0.02	0.99 ± 0.13
(Epi)catechin-O-hexoside-hexoside	0.16 ± 0.02	0.14 ± 0.01	0.12 ± 0.01	0.15 ± 0.01
Procyanidin dimer B type gallate isomer 1	0.22 ± 0.01	0.97 ± 0.08	3.51 ± 0.02	1.48 ± 0.07
Procyanidin trimer B type isomer 1	n.d.	0.65 ± 0.01	2.96 ± 0.13	1.08 ± 0.01
Procyanidin trimer B type isomer 2	1.50 ± 0.11	2.95 ± 0.04	7.64 ± 0.45	3.87 ± 0.10

(continued on next page)

Table 1 (continued)

Compound	C diet	L diet	H diet	HL diet
Total flavan-3-ols	40.77 ± 1.65	53.16 ± 2.14	100.60 ± 1.69	57.89 ± 1.32
Hydroxy-trimethoxyflavanone	12.08 ± 0.06	9.98 ± 0.14	9.10 ± 0.22	9.84 ± 0.42
Naringenin-C-hexoside	1.77 ± 0.01	1.29 ± 0.06	1.41 ± 0.04	1.18 ± 0.04
Naringenin-O-hexoside isomer 1	0.26 ± 0.01	0.24 ± 0.01	0.29 ± 0.03	0.25 ± 0.01
Naringenin-O-hexoside isomer 2	0.36 ± 0.01	0.33 ± 0.01	0.41 ± 0.01	0.35 ± 0.01
Naringenin-O-hexoside isomer 3	0.35 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01
Naringenin-O-hexoside isomer 4	0.46 ± 0.01	0.30 ± 0.01	0.32 ± 0.01	0.30 ± 0.01
Naringenin-O-hexoside isomer 5	0.19 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
Tetra-hydroxyflavanone-O-hexoside isomer 1	1.76 ± 0.08	1.60 ± 0.07	1.69 ± 0.04	1.54 ± 0.10
Tetra-hydroxyflavanone-O-hexoside isomer 2	0.34 ± 0.01	0.18 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Total flavanones	17.57 ± 0.21	14.36 ± 0.34	13.73 ± 0.38	13.96 ± 0.58
Luteolin	0.48 ± 0.01	0.45 ± 0.01	0.47 ± 0.02	0.52 ± 0.01
Luteolin-C-hexoside	0.39 ± 0.05	0.39 ± 0.02	0.32 ± 0.01	0.38 ± 0.03
Luteolin-O-hexoside isomer 1	0.33 ± 0.03	0.18 ± 0.01	0.22 ± 0.03	0.30 ± 0.05
Luteolin-O-hexoside isomer 2	0.42 ± 0.01	0.39 ± 0.02	0.35 ± 0.03	0.44 ± 0.01
Luteolin-7-O-glucoside	0.29 ± 0.01	0.18 ± 0.01	0.21 ± 0.02	0.22 ± 0.03
Luteolin-7-O-glucuronide	0.24 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.25 ± 0.01
Apigenin-C-hexoside-C-pentoside	6.91 ± 0.15	6.39 ± 0.36	5.75 ± 0.19	7.82 ± 0.09
Apigenin-O-hexoside-pentoside	0.63 ± 0.01	0.55 ± 0.05	0.52 ± 0.01	0.55 ± 0.02
Total flavones	9.70 ± 0.26	8.73 ± 0.48	8.04 ± 0.32	10.49 ± 0.24
Quercetin	0.28 ± 0.01	0.53 ± 0.02	1.16 ± 0.01	0.71 ± 0.02
Quercetin-3-O-rhamnoside	0.13 ± 0.01	1.94 ± 0.04	4.90 ± 0.03	2.58 ± 0.01
Quercetin-3-O-glucoside	0.43 ± 0.01	0.77 ± 0.03	0.91 ± 0.02	0.41 ± 0.01
Quercetin-3-O-rutinoside	0.30 ± 0.01	0.32 ± 0.01	0.34 ± 0.01	0.31 ± 0.01
Isorhamnetin-3-O-rutinoside	0.14 ± 0.01	0.18 ± 0.01	0.27 ± 0.02	0.19 ± 0.01
Total flavonols	1.27 ± 0.04	3.74 ± 0.10	7.59 ± 0.08	4.21 ± 0.04
Dihydroxyphenylacetic acid	3.11 ± 0.34	16.33 ± 0.32	45.91 ± 10.02	24.36 ± 0.44
Total hydroxyphenylacetic acids	3.11 ± 0.34	16.33 ± 0.32	45.91 ± 10.02	24.36 ± 0.44
Matairesinol-O-hexoside	n.d.	0.40 ± 0.01	n.d.	0.45 ± 0.01
Secoisolaricresinol-O-hexoside	n.d.	0.28 ± 0.01	n.d.	0.30 ± 0.01
Total lignans	n.d.	0.68 ± 0.02	n.d.	0.75 ± 0.02
Total phenolic content	306.33 ± 10.81	308.18 ± 10.76	409.07 ± 21.27	313.82 ± 10.40

n.d. means that the compound was not detected in the sample.

and 49.1 % of total phenolic compounds in C, L, H, and HL diets, respectively (Fig. 1B and Table 1). The total amount of hydroxybenzoic acids was not significantly different among diets C, L, and HL ($P > 0.05$), whereas a significantly higher amount was found in the feedstuff from diet H ($P < 0.05$) (Fig. 1B and Table 1).

The second most representative class of phenolic compounds in feedstuffs from C and L diets were hydroxycinnamic acids, which represented 27.6 % and 21.8 % of total phenolic compounds, respectively, followed by flavanols which accounted for 13.3 % and 17.3 % of total phenolic compounds, respectively (Fig. 1C and D and Table 1). On the contrary, in the hazelnut skin-supplemented feedstuffs, flavanols were the second most important class of phenolic compounds representing 24.6 % and 18.5 % of total phenolic compounds in H and HL diets, respectively (Fig. 1D and Table 1).

Additionally, small amounts of flavanones, flavones, and flavonols were identified in the feedstuffs from the different diets (Fig. 1E and F and Table 1). While the amount of flavanones was significantly higher ($P < 0.05$) in feedstuff from the C diet, the concentration of flavonols was significantly higher ($P < 0.05$) in feedstuffs from supplemented diets, especially diet H (Fig. 1E and F and Table 1). Moreover, feedstuffs from supplemented diets also displayed significantly higher amounts ($P < 0.05$) of dihydroxyphenylacetic acid with respect to the feedstuff from the C diet (Fig. 1F and Table 1). Finally, two linseed-derived lignans, *i.e.* matairesinol-O-hexoside and secoisolaricresinol-O-hexoside were identified exclusively in linseed-supplemented diets (Fig. 1F and Table 1).

Regarding the tocopherol content, all the dietary treatments increased α -tocopherol and γ -tocopherol content (Table 2). The highest increase was observed in the H diet, followed by HL diet and L diet.

3.2. Effect of the different dietary treatments on lipid peroxidation in raw and cooked lamb meat

The amount of total lipid hydroperoxides in raw lamb meat from the C diet was $8.50 \pm 4.04 \mu\text{mol H}_2\text{O}_2/100 \text{ g}$ of meat (Fig. 2). The lipid hydroperoxides value more than doubled in lamb meat from the C diet after cooking. No significant differences ($P > 0.05$) were found between the amount of lipid hydroperoxides in raw and cooked lamb meat samples from the different diets. Differently, the amount of TBA-RS was significantly lower ($P < 0.05$) in both raw and cooked lamb meat samples from H, L and HL diets compared to the control diet (Fig. 2). However, there were no significant differences ($P > 0.05$) in the TBA-RS values among the raw and cooked lamb meat samples from the supplemented diets.

3.3. Effect of the different dietary treatments on lipid peroxidation after *in vitro* gastric and intestinal digestion of lamb meat

In vitro gastro-intestinal digestion of cooked lamb meat from the C diet resulted in an enhancement of the oxidative processes affecting lipids (Fig. 3). After gastric digestion, the amount of formed lipid hydroperoxides increased by about 12 times reaching the value of $228.93 \pm 5.92 \mu\text{mol H}_2\text{O}_2/100 \text{ g}$ of meat. However, at the end of the *in vitro* intestinal phase of the digestion the amount of lipid hydroperoxides decreased by 1.8 times. As reported in Fig. 3, lamb meat samples from the L, H, and HL diets displayed significantly lower amounts of lipid hydroperoxides both after gastric and intestinal digestion. The highest decrease in lipid hydroperoxide levels was found for the lamb meat samples from the HL diet with a recorded decrease of 56.4 % and 74.5 % after gastric and intestinal digestion, respectively. No significant differences were observed between lamb meat from L and H diets at the end of the gastric (33.6 % and 22.3 % decrease, respectively) and intestinal digestion (46.1 % and 40.9 % decrease, respectively).

The behaviour of the TBA-RS values in lamb meat from the C diet during *in vitro* gastro-intestinal digestion was quite different as compared to the trend observed in the case of lipid hydroperoxides

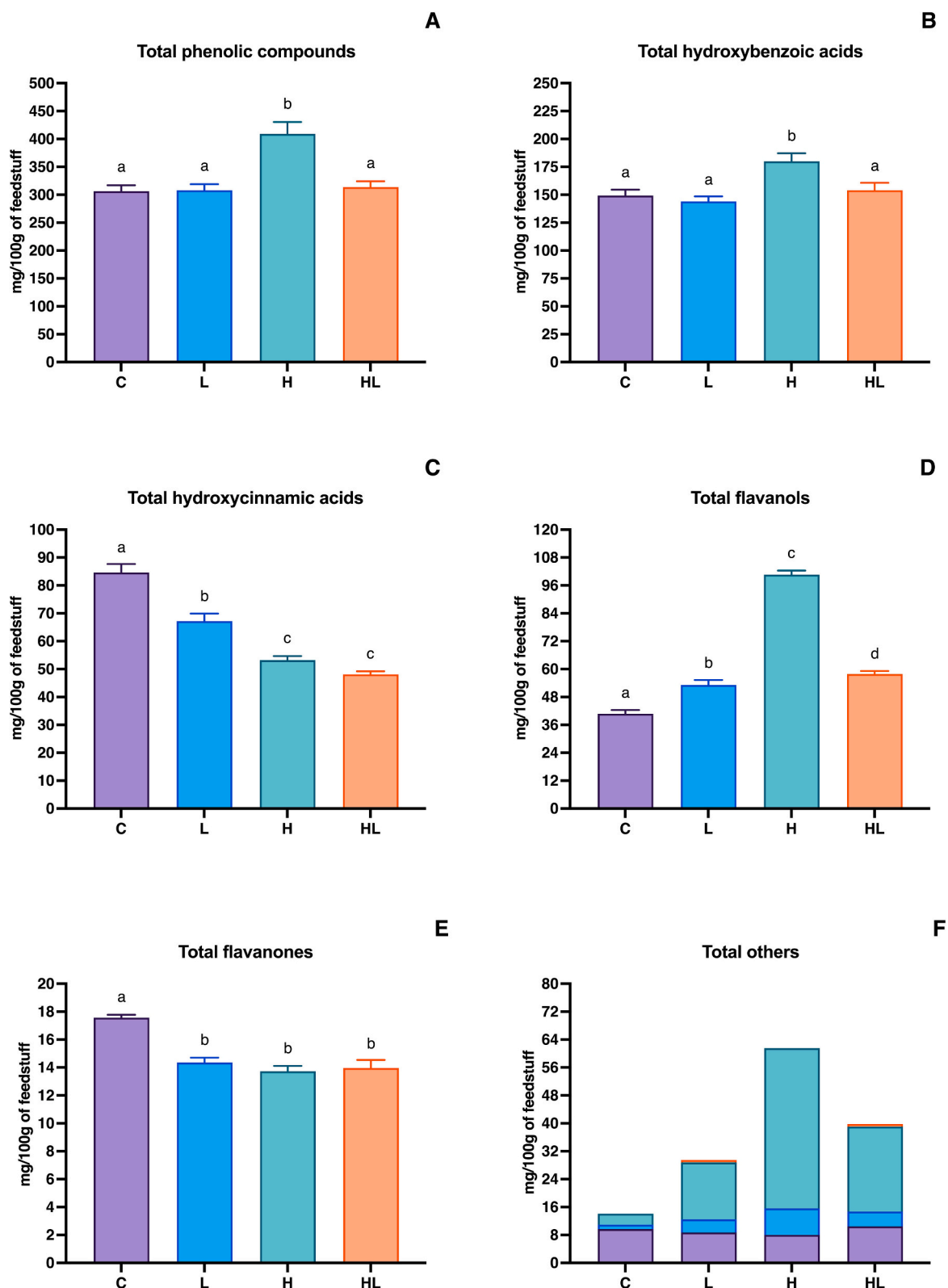


Fig. 1. Quantitative data on the phenolic compounds identified in the different feedstuffs grouped by classes. Abbreviations are C: control diet; L: linseed supplemented diet; H: hazelnut skin supplemented diet; HL: hazelnut skin and linseed supplemented diet. (A) Total phenolic compounds as sum of individual phenolic compounds quantified by mass spectrometry; (B) Total hydroxybenzoic acids as sum of individual hydroxybenzoic acids quantified by mass spectrometry; (C) Total hydroxycinnamic acids as sum of individual hydroxycinnamic acids quantified by mass spectrometry; (D) Total flavanols as sum of individual flavanols quantified by mass spectrometry; (E) Total flavanones as sum of individual flavanones quantified by mass spectrometry; (F) Total others include: total flavones (purple colour), total flavonols (light blue colour), total hydroxyphenylacetic acids (light green colour) and total lignans (orange colour) as sum of individual flavones, flavonols, hydroxyphenylacetic acids and lignans quantified by mass spectrometry. Different letters in the panels A, B, C, D and E indicate that the values are significantly different ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Effect of dietary treatment on the vitamin E content of experimental diets. C diet: Control diet; L diet: Linseed supplemented diet; H diet: Hazelnut skin supplemented diet; HL diet: Hazelnut skin and linseed supplemented diet.

	Dietary treatment			
	C diet	H diet	L diet	HL diet
α -tocopherol (mg/kg DM)	6.43	43.2	13.9	24.6
γ -tocopherol (mg/kg DM)	15.7	57.8	32.3	46.0
Total tocopherols (mg/kg DM)	22.1	101	46.2	70.6

(Fig. 3). The TBA-RS value slightly but non significantly declined after gastric digestion as compared to the cooked meat to then experience a 2.3-times increase at the end of the intestinal digestion in concurrence with the decrease in lipid hydroperoxides.

Data in Fig. 3 shows that TBA-RS concentration after gastric digestion was significantly reduced by including the combination of hazelnut skin and extruded linseed in the lamb diet ($4.86 \pm 1.10 \mu\text{mol MDA}/100 \text{ g}$ of meat) respect to the basal diet ($7.85 \pm 1.25 \mu\text{mol MDA}/100 \text{ g}$ of meat). The same trend was also observed in lamb meat from the diets L and H (6.53 ± 0.56 and $5.95 \pm 0.50 \mu\text{mol MDA}/100 \text{ g}$ of meat, respectively) although the difference with the C diet was not significant ($P > 0.05$). After intestinal digestion, all the dietary treatments resulted in lower TBA-RS values in lamb meat although only the TBA-RS value of meat from the diet HL ($14.11 \pm 1.14 \mu\text{mol MDA}/100 \text{ g}$ of meat) was significantly lower than the TBA-RS value of meat from the C diet ($18.39 \pm 1.29 \mu\text{mol MDA}/100 \text{ g}$ of meat). Therefore, the HL diet was the most effective in decreasing the TBA-RS after gastric and intestinal digestion with a reduction of 38.1 % and 23.3 %, respectively.

3.4. Identification of antioxidant compounds in raw meat samples

No parent phenolic compounds were identified in the raw meat from the different dietary treatments, whereas both α -tocopherol and γ -tocopherol were detected in all the meat samples, although in different amounts (Table 3). No significant differences in tocopherol concentration were found between the meat samples C diet and L diet, while meat samples from hazelnut-supplemented diets exhibited significantly higher levels of tocopherols compared to meat samples from the C diet (Table 3). In particular, the concentration of α -tocopherol, γ -tocopherol, and total tocopherols was 2.1-times, 4.7-times and 2.4-times higher in raw meat from H diet compared to C diet, respectively.

High-resolution mass spectrometry experiments carried out on raw lamb meat, revealed the presence of 5 phenolic compound metabolites and 4 tocopherol metabolites (Table 4).

These metabolites were found in all the analysed meat samples, however, the semi-quantitative analysis revealed some differences related to the diet composition (Fig. 4A). In particular, 5-(hydroxymethoxyphenyl)- γ -valerolactone and the isomer 2 of hydroxyphenylvaleric acid were detected in significantly higher amounts in meat samples from H diet, pinpointing a specific contribution of hazelnut skin flavanols in determining the metabolite profiles of meat. One additional phenolic metabolite, hydroxyphenyl-propionic acid was detected only in meat samples from L and HL diet and therefore generated by the gut microbiota metabolism of the phenolic compounds contained in linseed.

Concerning the tocopherol metabolites, high-resolution mass spectrometry revealed the presence of 4 tocopherol metabolites (two isomers of δ -(2'-carboxyethyl)-6-hydroxychroman, δ -CEHC, and two isomers of α -(2'-carboxyethyl)-6-hydroxychroman, α -CEHC) in all the meat samples, although still with some differences related to the dietary treatments (Fig. 4B). Notably, samples from the H diet were the richest in the

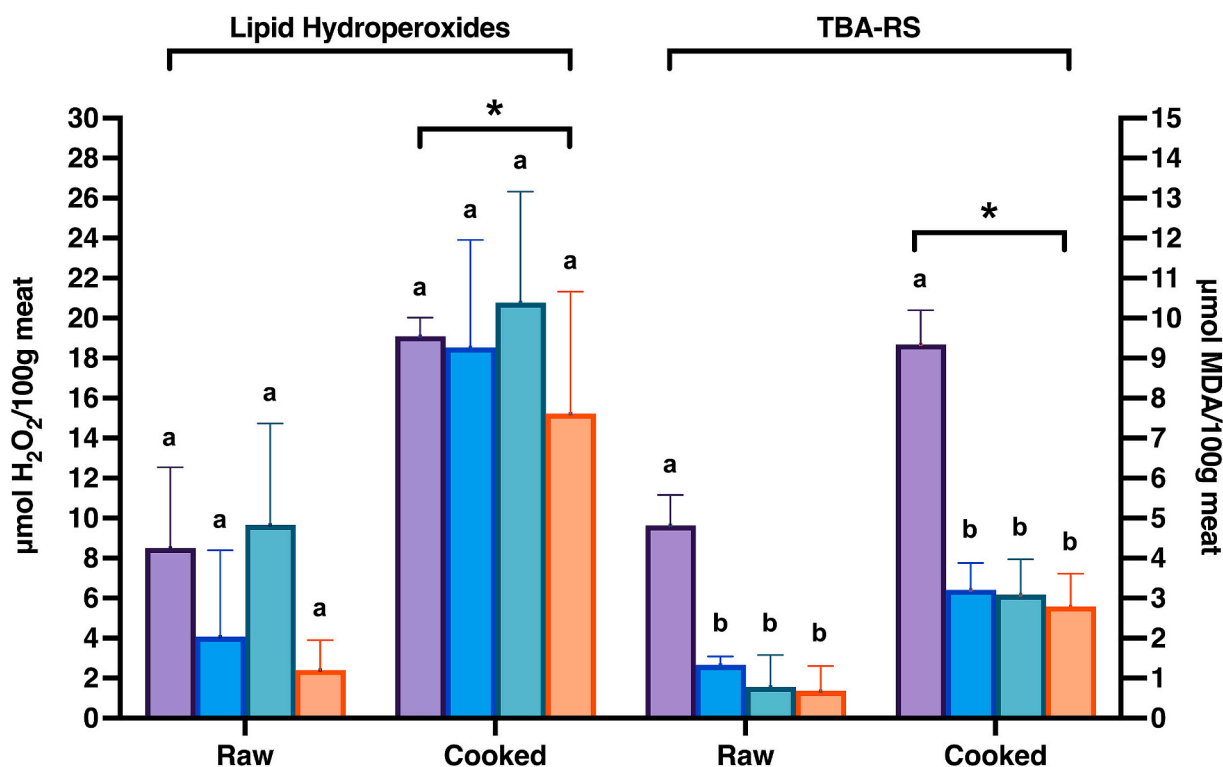


Fig. 2. Effect of the lamb diets on the amount of lipid hydroperoxides (A) and lipid oxidation end-products (B) in raw and cooked lamb meat. Analysis was carried out on raw and cooked meat. Different colours identified the different diets. Purple bars: control diet; light blue bars: linseed supplemented diet; light green bars: hazelnut skin supplemented diet; orange bars: hazelnut skin and linseed supplemented diet. Lipid oxidation end-products were quantified as thiobarbituric acid-reactive substances (TBA-RS). Left y-axis: lipid hydroperoxides. Right y-axis: TBA-RS. MDA: malondialdehyde. Different letters indicate significant differences among treatments ($P < 0.05$). *indicates significant differences between raw and cooked samples in the same treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

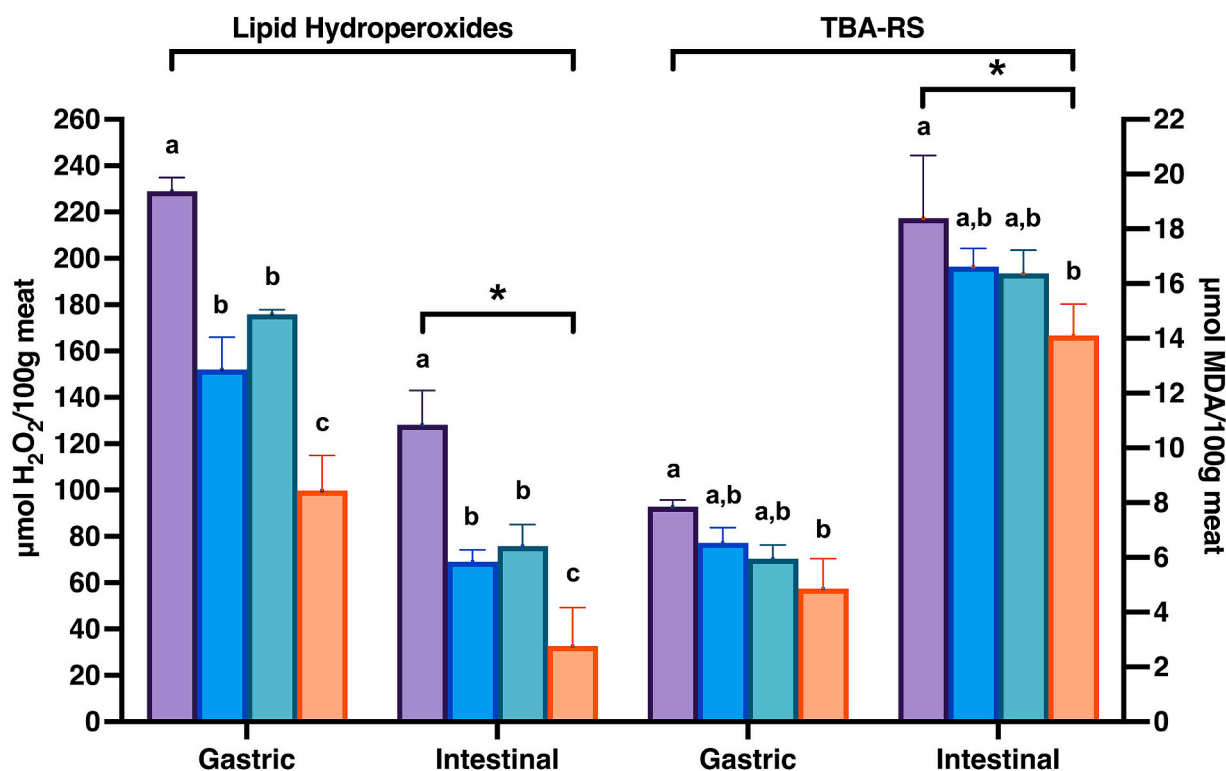


Fig. 3. Effect of the lamb diets on the amount of lipid hydroperoxides (A) and lipid oxidation end-products (B) after gastric and intestinal digestion. Analysis was carried out on meat subjected to *in vitro* gastric and intestinal digestion. Different colours identified the different diets. Purple bars: control diet; light blue bars: linseed supplemented diet; light green bars: hazelnut skin supplemented diet; orange bars: hazelnut skin and linseed supplemented diet. Lipid oxidation end-products were quantified as thiobarbituric acid-reactive substances (TBA-RS). Left y-axis: lipid hydroperoxides. Right y-axis: TBA-RS. MDA: malondialdehyde. MDA: malondialdehyde. Different letters indicate significant differences among treatments ($P < 0.05$). *indicates significant differences between gastric and intestinal samples in the same treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Effect of dietary treatment on the vitamin E content of meat.^{a,b,c}

	Dietary treatment ¹				SED ²	P-value
	C	H	L	HL		
α-tocopherol (mg/kg meat)	0.672 ^{bc}	1.41 ^a	0.650 ^c	1.04 ^{ab}	0.141	<0.001
γ-tocopherol (mg/kg meat)	0.074 ^c	0.351 ^a	0.180 ^{bc}	0.301 ^{ab}	0.051	<0.001
Total tocopherols (mg/kg meat)	0.746 ^b	1.76 ^a	0.830 ^b	1.34 ^a	0.185	<0.001

^{a,b,c} Within a row, different superscripts indicate significant differences ($P < 0.05$).

¹ C: Control diet; H: Hazelnut skin diet; L: linseed diet; HL: Hazelnut skin + Linseed diet.

² SED: standard error of the difference.

two isomers of δ-CEHC, with isomer 2 that was found in an amount 6.5 times higher in meat samples from the H diet as compared to meat samples from the C diet. Moreover, the isomer 2 of α-CEHC was found in higher amounts in meat from H and HL diets with respect to meat samples from C and L diets, although there were no significant differences between the treatments.

Regarding the effect of the different dietary treatments on the amount of endogenous antioxidant peptides in lamb meat, a significant increase ($P < 0.05$) in the relative amounts of carnosine was observed in meat samples from dietary treatments H and HL as compared to the control meat (Fig. 3C). In particular, the relative amount of carnosine increased by 1.40 and 1.42 times in meat samples from treatments H and HL, respectively, with respect to the control meat sample. On the other hand, the relative amount of reduced glutathione in meat samples from

H and HL diets increased by 1.63 and 1.51 times, respectively, in comparison with the C diet, although there were no significant differences between the treatments. Furthermore, no significant differences were found in the levels of anserine among the different dietary treatments (Fig. 4C).

4. Discussion

Frequent red meat intake has been associated with an increased risk of developing some chronic diseases such as atherosclerosis, cardiovascular diseases, and colon cancer (Giromini et al., 2022). The association between red meat intake and the onset of chronic diseases is based on the oxidative phenomena that occur on lipids during cooking and gastro-intestinal digestion of meat (Hemeryck et al., 2018; Kanner & Lapidot, 2001). In particular, the stomach represents an optimal environment for these oxidative reactions being rich in oxygen, having an acidic pH, and favouring the release from the meat of catalysts such as heme and free iron (Kanner & Lapidot, 2001). During gastric digestion, polyunsaturated fatty acids present in meat undergo oxidative reactions leading to the accumulation of lipid hydroperoxides, which are stable at low gastric pH values (Kanner & Lapidot, 2001). Next, the mild alkaline pH of the small intestine favours the breakdown of lipid hydroperoxides generating low molecular weight reactive carbonyls such as MDA (Gorelik et al., 2013). The generated carbonyl compounds may be responsible for the supposed toxic effects of red meat consumption. At the intestinal level, these oxidation products may behave as cytotoxic and genotoxic compounds that react with the DNA, forming adducts and damaging the DNA itself (Hemeryck et al., 2018; Niedernhofer, Daniels, Rouzer, Greene, & Marnett, 2003; Van Hecke et al., 2015). Angeli et al. (2011) found that incubating colon cancer SW480 cells with linoleic acid hydroperoxides and haemoglobin led to an increase in intracellular

Table 4

Mass spectral data and retention times (RT) of metabolites identified in raw lamb meat from different diets.

Compound ^a	Molecular formula	RT (min)	[M-H] ⁻ (m/z)	MS ² ion fragments (m/z)
<i>Tocopherol metabolites</i>				
δ-CEHC isomer 1	C ₁₅ H ₂₁ O ₃	24.58	249.1497	205.1591
δ-CEHC isomer 2	C ₁₅ H ₂₁ O ₃	26.76	249.1497	205.1591
α-CEHC isomer 1	C ₁₆ H ₂₁ O ₄	25.49	277.1447	233.1546, 205.1594
α-CEHC isomer 2	C ₁₆ H ₂₁ O ₄	26.29	277.1447	233.1546, 205.1594
<i>Polyphenols metabolites</i>				
Hydroxy-phenylpropionic acid	C ₉ H ₁₀ O ₃	11.14	165.0549	121.0648
5-(hydroxy-methoxyphenyl)-γ-valerolactone	C ₁₂ H ₁₄ O ₄	20.90	221.0819	206.0585
Hydroxyphenyl-valeric acid isomer 1	C ₁₁ H ₁₄ O ₃	22.33	193.0872	177.0556, 149.0593
Hydroxyphenyl-valeric acid isomer 2	C ₁₁ H ₁₄ O ₃	24.08	193.0872	177.0556, 149.0593
Methoxyphenyl-valeric acid	C ₁₂ H ₁₆ O ₃	22.41	207.1026	192.0792
<i>Endogenous peptides</i>				
Carnosine	C ₉ H ₁₄ N ₄ O ₃	0.85	225.0990	154.0622, 110.0711, 179.0556
Anserine	C ₁₀ H ₁₆ N ₄ O ₃	0.95	239.1148	168.0769, 58.0285, 151.0503, 87.0550
Reduced glutathione	C ₁₀ H ₁₇ N ₃ O ₆ S	1.55	306.0764	143.0452, 128.0342, 179.0455, 160.0063, 210.0877, 254.0788, 272.0891

^a CEHC means carboxyethyl-hydroxychroman.

oxidative stress and MDA concentration, resulting in the accumulation of specific DNA adducts. Reactive carbonyls may also be absorbed at the intestinal level determining raised plasma MDA levels (Gorelik, Ligumsky, Kohen, & Kanner, 2008b). The increased concentration of plasmatic MDA following a meal rich in meat was linked to an increase in the levels of MDA-modified LDL in humans (Gorelik et al., 2013). MDA-modified LDL may have a pivotal role in the progression of atherosclerosis, as modified and oxidized LDL have been detected in atherosclerotic lesions (Palinski et al., 1989).

The results presented in this study demonstrated that the inclusion in the lamb diet of hazelnut skin and linseed delayed meat lipid oxidation during cooking and *in vitro* gastro-intestinal digestion. An increase in lipid oxidation products, especially TBA-RS, was observed during meat cooking. This increase was more pronounced in the meat samples from the C diet compared to the meat samples from the integrated diets. Previous studies found that the inclusion in the animal diet of antioxidant-rich vegetable feeds or by-products, such as hazelnut skin or almond hulls in lamb diet or grape skin and oregano in pig diet, decreased the TBA-RS values in raw meat in comparison with a control diet, without affecting lipid hydroperoxides levels (Martini et al., 2020; Menci et al., 2023; Scerra et al., 2023). Similarly, the dietary addition of pomegranate by-product, almond hulls, and rosemary extract to lamb reduced TBA-RS formation during meat cooking compared to the control

meat (Natalello et al., 2020; Scerra et al., 2023; Serrano, Ortuño, & Bañón, 2014). Differently from our data, in previous studies, supplementation of lamb diet with extruded linseed did not result in a decrease in TBA-RS value in raw meat (De la Fuente-Vázquez et al., 2014; Urrutia et al., 2016).

Therefore, the inclusion of both hazelnut skin and extruded linseed or their combination in the lamb diet decreased the TBA-RS levels in raw lamb meat and enhanced the oxidative stability of meat during cooking.

Meat lipid oxidation was further enhanced by several folds during gastric digestion. In the meat samples from the C diet, a 12-fold increase in lipid hydroperoxides was observed at the end of the gastric digestion, although this was not accompanied by an increase in the concentration of TBA-RS. However, intestinal digestion resulted in a decrease in the amount of lipid hydroperoxides with a concomitant increase in the amount of TBA-RS. Similar behaviour was already seen during *in vitro* gastro-intestinal of turkey meat and high-fat beef (Han et al., 2022; Martini et al., 2018). The data indicated that lipid hydroperoxides were continuously formed and remained stable during gastric digestion without any degradation to the end-products. However, during the intestinal phase of the digestion, lipid hydroperoxides were unstable and degraded into the advanced lipoxidation end-products (*i.e.*, TBA-RS). All the dietary treatments resulted in lower values in both lipid hydroperoxides and TBA-RS during *in vitro* gastric and intestinal digestion compared to the basal diet. In a previous study, it was established that the formation of MDA during *in vitro* gastric digestion of turkey meat was strongly associated with an increase in the concentration of MDA in the bloodstream following the consumption of meat by human volunteers. Thereby, the results reported in this study may be relevant from a physiological point-of-view.

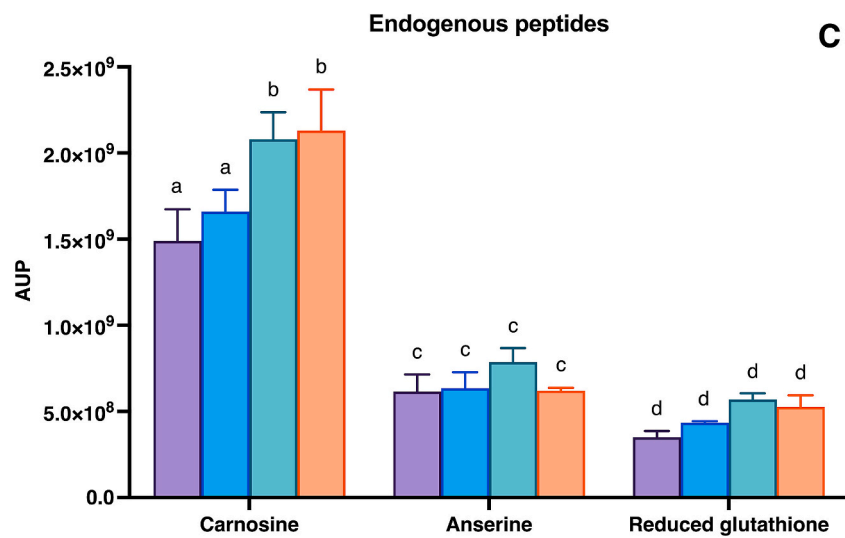
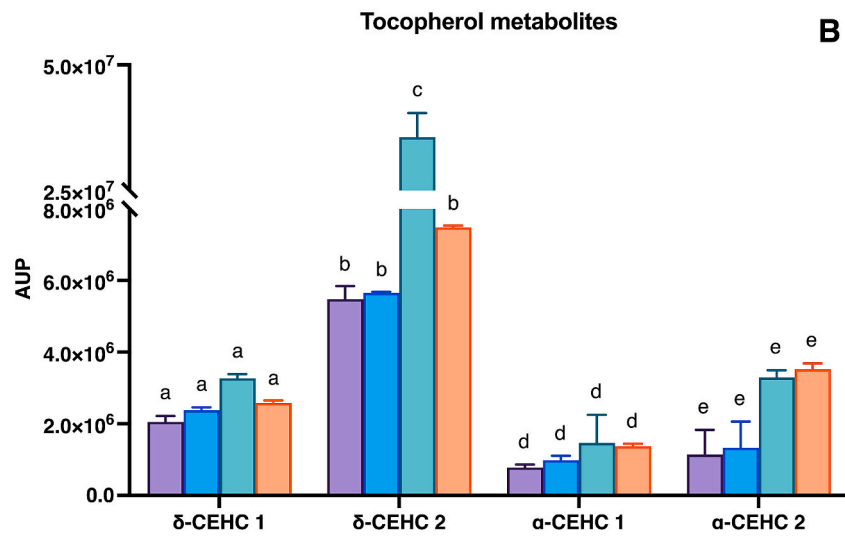
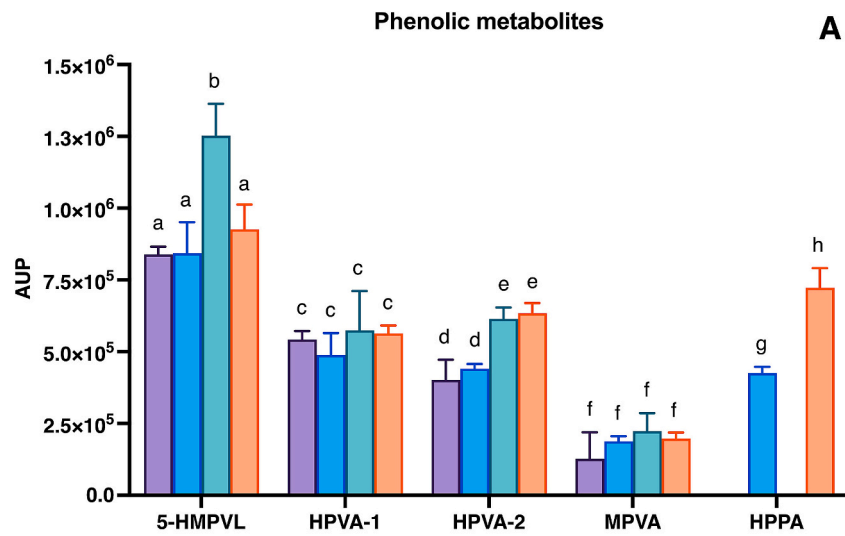
Both hazelnut skin and linseed were rich in phenolic compounds, and their inclusion in the feedstuff brought about different quantitative and qualitative phenolic profiles in diets. In particular, hazelnut skin was found to be rich in flavanols, whose concentration was about 2.5-times higher in the H diet compared to the C diet. Previously, it has been demonstrated that hazelnut skin from different varieties was rich in flavanols, especially procyanidins (Del Rio et al., 2011).

Phenolic compounds are generally considered poorly absorbable molecules at the intestinal level. The majority of phenolic compounds supplied with the diet are instead metabolized by gut microbiota, releasing short phenolic acids (*i.e.*, hydroxyphenylpropionic acids, hydroxyphenylacetic acids, and hydroxybenzoic acids) and, in the case of flavanols, hydroxyphenylvalerolactones and hydroxyphenylvaleric acids (Carregosa et al., 2022; Di Pede et al., 2023). Gut metabolites of phenolic compounds are then absorbed, reaching the liver, where they can be metabolized in phase II-conjugated (mainly by glucuronidation, methylation, and/or sulphation) before entering the bloodstream (Carregosa et al., 2022; Di Pede et al., 2023).

Similarly, tocopherols can be metabolized in the liver by consecutive ω-hydroxylation (catalysed by the cytochrome P450 CYP4F2) and oxidation followed by a series of β-oxidation cycles that leads to the terminal metabolites (2'-carboxyethyl)-6-hydroxychromans (CEHCs) (Jiang, 2022).

Among the identified phenolic compounds metabolites, 4 were gut microbiota metabolites of flavanols, namely 5-(hydroxy-methoxyphenyl)-γ-valerolactone, methoxyphenyl-valeric acid, and two isomers of hydroxyphenyl-valeric acid (Di Pede et al., 2023). These metabolites were identified in meat samples from all the dietary treatments, although in higher amounts in H and HL samples. The ubiquitous presence of flavanols metabolites in all the assessed dietary treatments was related to the presence of barley (rich in flavanols) as a staple food of the various diets (Idehen, Tang, & Sang, 2017).

Indeed, one additional metabolite, hydroxyphenyl-propionic acid, was detected only in meat samples from the linseed-supplemented diet. Accordingly, Landete (2022) found that gut microbial metabolism of linseed phenolic compounds converged in the formation of hydroxyphenylpropionic and hydroxyphenylacetic acids. Both



(caption on next page)

Fig. 4. Occurrence of the main phenolic compounds and tocopherol metabolites and endogenous antioxidant peptides in raw meat samples expressed as area under the peak (AUP). Different colours identified the different diets. Purple bars: control diet; light blue bars: linseed supplemented diet; light green bars: hazelnut skin supplemented diet; orange bars: hazelnut skin and linseed supplemented diet. (A) Phenolic compounds metabolites detected in raw meat samples from the different dietary treatments. 5-HMPVL: 5-(hydroxy-methoxyphenyl)- γ -valerolactone; HPVA-1: hydroxyphenyl-valeric acid isomer 1; HPVA-2: hydroxyphenyl-valeric acid isomer 2; HPPA: hydroxy-phenylpropionic acid. (B) Tocopherol metabolites detected in raw meat samples from the different dietary treatments. δ -CEHC-1: δ -carboxyethyl-hydroxychroman isomer 1; δ -CEHC-2: δ -carboxyethyl-hydroxychroman isomer 2; α -CEHC-1: α -carboxyethyl-hydroxychroman isomer 1; α -CEHC-2: α -carboxyethyl-hydroxychroman isomer 2. (C) Endogenous antioxidant peptides detected in raw meat samples from the different dietary treatments. Different letters indicate that the values are significantly different ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydroxyphenylvalerolactones and hydroxyphenylvaleric acids displayed antioxidant activity and can be partially responsible for the inhibition of the oxidative reactions occurring during cooking and gastrointestinal digestion of meat from the hazelnut-supplemented diets (Campos, Stehle, & Simon, 2019; Unno, Tamemoto, Yayabe, & Kakuda, 2003).

Furthermore, meat from supplemented diets contained higher amounts of tocopherols and tocopherol metabolites. Tocopherols are well-known antioxidant compounds able to inhibit lipid peroxidation in meat (Granit et al., 2001). Moreover, tocopherol metabolites maintain the intact chromanol ring, which is the moiety in tocopherols able to scavenge reactive oxygen.

In addition to antioxidant compounds from the diet, meat also contains endogenous antioxidant compounds, the most important of which are carnosine and its methylated derivative anserine and reduced glutathione (Kulczyński, Sidor, & Gramza-Michałowska, 2019). Carnosine and, to a lesser extent, anserine possessed antioxidant properties and were able to scavenge free radicals, chelate metals, and delay lipid peroxidation (Wu, Shiau, Chen, & Chiou, 2003). Furthermore, carnosine was found able to inhibit lipid peroxidation during cooking and *in vitro* digestion of pork in a concentration-dependent manner (Li et al., 2021). On the other hand, reduced glutathione had established scavenger ability against several reactive oxygen species, such as superoxide anion and hydroxyl radicals, and was also able to inhibit lipid peroxidation (Kulczyński et al., 2019). The increased levels of carnosine and glutathione in meat samples from H and HL diets can be due to the highest amount of proteins in these diets compared to the C diet, which can provide the highest amounts of the amino acids necessary for the biosynthesis of these two peptides. Previous studies found that the biosynthesis of carnosine and glutathione can be induced by dietary supplementation with alanine and cysteine, which are the rate-limiting factors for carnosine and glutathione synthesis, respectively (Blancquaert et al., 2017; Goshovska et al., 2021). Otherwise, exogenous antioxidants present in hazelnut skin may have protected endogenous antioxidant peptides by preserving them from oxidation.

A considerably higher level of phenolic metabolites was found in meat from lambs fed with hazelnut skin, linseed, or a combination of both compared to the control diet. Most of these metabolites originated from lamb microbiota colonic metabolism of flavanols. Moreover, higher concentrations of tocopherols and tocopherol-derived metabolites were detected in meat from lamb fed with hazelnut skin-supplemented feedstuffs. In addition, the inclusion of hazelnut skin in the lamb diet resulted in the accumulation of specific endogenous antioxidants in meat.

Therefore, specific dietary regimes can modify the profile of exogenous and endogenous antioxidants in lamb meat, ultimately resulting in an inhibitory effect towards lipid peroxidation both during cooking and digestion of the meat itself.

5. Conclusion

Our results highlighted that the inclusion in lamb diet of hazelnut skin and extruded linseed displayed a protective effect in meat against lipid peroxidation mitigating and hindering the formation of toxic compounds during cooking and *in vitro* gastro-intestinal digestion. Oxidation is also the main factor involved in the deterioration of quality

and shelf life of meat. Therefore, the improvement in lamb meat oxidative stability by hazelnut skin may have an impact, not only from a health point of view, but also in increasing the shelf-life and the quality of the meat itself.

The demonstrated inhibitory effects against lipid oxidation could be attributable both to the presence of specific gut microbial metabolites of phenolic compounds as well as of tocopherols and related metabolites in meat and to the highest amount of endogenous antioxidant peptides as a consequence of the different dietary treatments.

From a circular economy and health perspective, the inclusion of by-products from the food industry, such as hazelnut skin, in the lamb diet allows not only to minimize food waste and disposal costs but also to improve meat quality as well as human health by decreasing lipid oxidative phenomena and the production of toxic compounds during cooking and digestion.

CRedit authorship contribution statement

Alice Cattivelli: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Melissa Zannini:** Writing – review & editing, Investigation, Formal analysis. **Katia D’Ambra:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Roberta Trovato:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Giovanna Minelli:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization. **Martino Musati:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Data curation. **Giuseppe Luciano:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Alessandro Priolo:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Antonio Natalello:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Angela Conte:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition. **Davide Tagliacucchi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Domenico Pietro Lo Fiego:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Financial support

This research was funded by PRIN 2020 National Project (Grant N. 2020244EWW).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the Fondazione Cassa di Risparmio di Modena for funding the UHPLC-ESI-Q Exactive Hybrid Quadrupole-

Orbitrap Mass Spectrometer system at the Centro Interdipartimentale Grandi Strumenti (CIGS).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2025.144266>.

Data availability

Data will be made available on request.

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