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# Meat oxidative stability in lambs given almond skin to partially replace maize

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ABSTRACT

The effects of feeding lambs diets containing two levels of almond skin on meat oxidative stability were investigated. Male lambs ($n = 30$, 2 months old, body weight 12.7 ± 2.07 kg) Valle del Belice × Pinzirita were assigned to 3 dietary treatments and fed *ad libitum* for 56 days with: a concentrate-based diet (CON) or CON containing 140 (A14) or 280 (A28) g/kg of dried almond skin in replacement of maize. Vitamins and hydrophilic antioxidant capacity were assessed in fresh meat, while colour and lipid oxidation were evaluated over 7 days of refrigerated storage. Data from fresh meat were statistically analysed using one-way ANOVA, whereas shelf-life trial data were evaluated using a mixed-effects model. Almond skin inclusion affected the antioxidant capacity of meat, showing different results: Folin-Ciocalteu and ferric reducing antioxidant power (FRAP) assays showed a decreased reducing activity of the extract, while an improved radical scavenging activity was observed with the Trolox equivalent antioxidant capacity (TEAC) assay. Ferrous ion chelating activity (FICA) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays showed no differences. A reduction of the total tocopherol content was observed in A28 treatment compared to the others. Neither the colour nor the lipid oxidation was influenced by the dietary treatment, except for the TBARS (Thiobarbituric Acid Reactive Substances) values in raw meat, which tended to increase in A28 treatment. The study evidenced that 140 g/kg dietary almond skin in lambs may be a successful strategy for replacing maize without detrimental effects on meat oxidative stability.

## 1. Introduction

A key objective pursued by food scientists and livestock researchers is the improvement of meat quality traits. This is particularly true given concerns about consumer health, environmental sustainability, and animal welfare. Over the past decades, a decrease in the consumption of animal-derived products with a high proportion of saturated fatty acids (SFA) toward polyunsaturated fatty acids (PUFA) has been observed (Lenighan et al., 2019). According to the guidelines of the World Health Organization, increasing the consumption of PUFA is fundamental for reducing the incidence of cardiovascular disease events (World Health Organization, 2023). However, it is known that PUFA are an easily oxidizable substrate, and their oxidation susceptibility increases in relation to both the chain length and the number of double bonds (Barden & Decker, 2016). The oxidation of lipidic substrate gives rise to the formation of undesirable flavours and odours in meat, which may worsen the palatability of the product, and toxic compounds (*i.e.* aldehydes, ketones) may arise, posing a risk to consumers (Domínguez et al., 2019). In light of the above, the enhancement of the fatty acid (FA) composition of meat, in terms of PUFA content, requires a concurrent increase in its antioxidant capacity to avoid the shortening of shelf-life and the loss of nutritional value (Luciano et al., 2013). A successful approach for enhancing the antioxidant compounds levels in muscle is through dietary intake (Ponnampalam et al., 2022). Moreover, dietary supplementation of antioxidants has proven greater efficacy in mitigating meat lipid oxidation than *post mortem* use as a preservative (Govaris et al., 2004). Many feeding strategies have been proposed to improve meat quality and its antioxidant content, such as grazing (van Vliet et al., 2021), dietary inclusion of plant extracts (Leal et al., 2019), and using agro-industrial by-products (Pinotti et al., 2023). The latter may also represent a solution for alternative feeds for reducing the environmental impact of the livestock sector using human-inedible

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matrices. Indeed, some by-products contain high levels of bioactive compounds like vitamins and polyphenols, which may be involved in the enhancement of muscle antioxidant capacity (Salami et al., 2019).

Within the available agro-industrial by-products in the Mediterranean region, those from almond (*Prunus dulcis* Mill.) have gained interest in response to the worldwide increase in production and their content in bioactive compounds (Nayik & Gull, 2020). Almond production represents 26% of the world's tree nut production, with a total yield of 1.46 million metric tons of fruits in the biennium 2023/2024 (International Nut Council, 2024). The industrial transformation of almonds generates several by-products consisting of: *i)* hull, the fibrous mesocarp which covers the in-shell fruit that is dry, leathery and astringent; *ii)* shell, the ligneous endocarp covering the almond kernel; *iii)* skin, the brown leathery peel surrounding the almond kernel (Musati et al., 2023). Particularly, the almond skin (AS) can be obtained by two industrial processes: blanching or roasting of the whole kernel (Garrido et al., 2008). Despite AS accounts for only 4–8% of the total in shell fruit weight, large quantities are produced by the industries (Smeriglio et al., 2016). The AS are mainly composed of fibre, so they can be a suitable feedstuff for ruminants that can utilise these human-inedible fibrous biomasses as an energy source (Musati et al., 2024). Moreover, AS is characterised by a high proportion of polyphenols, mainly flavonoids (Garrido et al., 2008). Musati et al. (2024) evaluated the impact of three increasing inclusion levels (70, 140 and 210 g/kg) of AS in the ovine diet on ruminal biohydrogenation and fermentation under *in vitro* conditions. A reduction in the proportion of SFA and an increase in monounsaturated fatty acids (MUFA) in ruminal digesta were observed for both 140 and 210 g/kg of AS inclusion compared to the control diet, with no detrimental effect on rumen fermentation parameters. These effects might be due to the bioactive molecules, such as polyphenols, contained in AS. Furthermore, the polyphenols, including flavonoids, are known to inhibit lipid oxidation through radical scavenging, chelate metals and reduce tocopherol radicals (Esfahlan et al., 2010). To this purpose, we hypothesised that the variety of bioactive compounds present in AS, such as lipophilic vitamins and polyphenols, could delay the oxidative processes of meat without detrimental effects on fresh meat quality parameters. Fat-soluble vitamins, water holding capacity (WHC), and hydrophilic antioxidant capacity were analysed, together with colour stability and lipid oxidation during refrigerated storage for 7 days.

## 2. Methods and materials

### 2.1. Almond skin by-product

The AS were obtained from a local almond factory (Avola, Sicily, Italy). To separate the skin from the kernel, the whole kernels were immersed in warm water (blanching). The resulting skin was collected and immediately dried in a greenhouse, using the sunlight as the sole energy source, for approximately 24 h at 40 °C to prevent undesirable fermentations.

### 2.2. Animal management and treatments

The study was approved by the "Organismo Preposto al Benessere degli Animali (OPBA)" of the University of Catania (approval No. 54766). The animals were reared in the university's facilities and handled by specialised workers, following the guidelines of the European Union. Briefly, thirty Valle del Belice × Pinzirita male lambs were divided into three dietary treatments balanced for bodyweight (12.7 ± 2.07 kg) and allocated to individual pens. After 5 days of gradual adaptation to the experimental diets, lambs were fed *ad libitum* for 56 days with one of the following diets: the control (CON) group received a maize-based concentrate diet; the other two groups were fed with the CON diet with 140 (A14) and 280 (A28) g/kg AS in replacement of maize. The diets' ingredients and chemical composition are shown in Table 1.

**Table 1**
Ingredients and chemical composition of the experimental diets and almond skin.

| | Almond skin | Experimental diets[1] | | |
|---|---|---|---|---|
| | | CON | A14 | A28 |
| *Ingredients (g/kg DM)* | | | | |
| Maize | | 430 | 290 | 150 |
| Alfalfa hay | | 300 | 300 | 300 |
| Soybean meal | | 220 | 220 | 220 |
| Molasses cane | | 30 | 30 | 30 |
| Mineral mix | | 20 | 20 | 20 |
| Almond skin | | – | 140 | 280 |
| *Chemical composition (g/kg DM)* | | | | |
| DM (g/kg as fed) | 931 | 907 | 914 | 907 |
| Ash | 49.6 | 79.8 | 127 | 108 |
| Crude protein | 125 | 193 | 202 | 194 |
| Crude fat | 111 | 19.5 | 39.7 | 54.2 |
| aNDF | 458 | 218 | 249 | 300 |
| ADF | 372 | 154 | 185 | 259 |
| ADL | 217 | 136 | 124 | 165 |
| Metabolizable energy[2] (MJ/kg DM) | – | 9.12 | 8.74 | 8.32 |
| Total polyphenols (mg TAeq/g DM) | 13.9 | 4.43 | 5.60 | 7.44 |
| Total tannins (mg TAeq/g DM) | 11.8 | 1.78 | 3.25 | 4.85 |
| Total tocopherols (μg/g DM) | 108 | 43.9 | 57.6 | 71.0 |
| α-tocopherol (% of total tocopherols) | 68.8 | 26.1 | 31.9 | 38.5 |
| γ-tocopherol (% of total tocopherols) | 29.5 | 68.6 | 63.8 | 53.3 |
| δ-tocopherol (% of total tocopherols) | 1.66 | 5.25 | 4.34 | 8.23 |
| *Fatty acids (% of total fatty acids)* | | | | |
| C16:0 | 9.97 | 21.5 | 11.9 | 12.0 |
| C18:0 | 2.47 | 8.56 | 3.37 | 4.81 |
| C18:1 *c*9 | 46.6 | 22.6 | 39.8 | 35.7 |
| C18:1 *c*11 | 1.26 | 0.810 | 1.18 | 1.31 |
| C18:2 *c*9*c*12 | 37.2 | 39.0 | 39.1 | 40.1 |
| C18:3 *c*9*c*12*c*15 | 0.760 | 5.04 | 2.59 | 3.52 |

Abbreviations: ADF: acid detergent fibre; aNDF: amylase-treated neutral detergent fibre; ADL: acid detergent lignin; DM: dry matter; TAeq: tannic acid equivalents.
[1] CON: control diet; A14: 14% almond skin diet; A28: 28% almond skin diet.
[2] Metabolizable energy estimated with CPM-Dairy Beta v3 software.

### 2.3. Feed composition analysis

An aliquot of AS was collected after sun drying. Samples of the experimental diets were collected three times during the trial (at the beginning, at the middle and at the end) and stored at −30 °C in a vacuum bag. Samples were prepared by mixing equal aliquots of the three diet subsamples mentioned above to conduct the analysis. Dry matter, ash, crude protein and crude fat were analysed following AOAC, 1995 methods (930.15, 942.05, 984.13 and 920.39, respectively), whereas the fibre fractions (amylase-treated neutral detergent fibre aNDF; acid detergent fibre ADF; acid detergent lignin ADL) were processed as indicated in a previous study (Van Soest et al., 1991).

The extraction of total polyphenols and tannins from feed samples (200 mg) was performed using 70% (*v/v*) acetone. Following the method described in the prior study (Makkar et al., 1993), total extractable phenolic compounds were determined using the Folin-Ciocalteu reagent (1 *N*). Quantification was carried out using a calibration curve prepared with tannic acid (TA) standards. The FA content of feeds was determined as detailed in our previous study (Musati, Bertino, et al., 2025). Feedstuffs were analysed for the tocopherol content following a developed method (Rufino-Moya et al., 2020). Shortly, vitamins contained in 200 mg of ground feedstuff were extracted with 300 μL of a solvent mixture consisting of methanol, acetone, and petroleum ether in a 1:1:1 (*v/v/v*) ratio. After centrifugation, the resulting supernatant was collected,

evaporated under a nitrogen stream and subsequently reconstituted in 1 mL of methanol. Fat-soluble compounds were quantified using a liquid chromatography system equipped with a C18 column (Zorbax, Supelco; 25 cm × 4.6 mm; particle size: 5 μm), following the chromatographic conditions described elsewhere (Natalello et al., 2022). Analytes were quantified by comparison with the injections of pure standards.

### 2.4. Slaughter procedure and samplings

At the conclusion of the experimental period, the animals were weighed and moved to the commercial slaughterhouse facility, where they were stunned and slaughtered. The weights of the hot carcasses were recorded immediately after the evisceration procedure. The carcasses were stored for 24 h at 4 °C, after which the weight and the pH were measured. The pH meter was adjusted using two calibration standards. The *longissimus thoracis et lumborum* (LTL) muscle was removed from the carcass, vacuum-packed and stored at −80 °C for subsequent analysis, except for the aliquot used to conduct the shelf-life study (colour and lipid oxidation), which was maintained at 4 °C.

### 2.5. Lipidic profile of meat

Total intramuscular lipids were extracted following a developed method (Folch et al., 1957), with modifications by Carlson (1985). Briefly, the extracted lipids were trans-esterified as indicated in Alves et al. (2015), using the nonadecanoic acid (C19:0) as internal standard. The fatty acids methyl esters (FAME) were analysed by a gas chromatograph equipped with a flame ionisation detector, and coupled with a capillary column (SP-2560, 100 m × 0.25 mm, 0.20 μm film thickness, Supelco). Helium was used as a gas carrier. Each FAME was identified by comparison to a commercially available standard mix (Supelco). In this work, only the principal FA classes are reported, as mg/100 g of meat, as these are the most relevant for explaining the oxidative stability of meat in accordance with the aim of the study. Lipid oxidation susceptibility of FA was also assessed by determining the total highly peroxidizable PUFA (HP-PUFA; with at least 3 double bonds), as proposed in a previous study (Priolo et al., 2021).

### 2.6. Water-soluble antioxidant activity of meat

The meat antioxidant activity was assessed in its hydrophilic fraction using five assays: Trolox equivalent antioxidant capacity (TEAC) (Re et al., 1999), ferric reducing antioxidant power (FRAP) (Benzie & Strain, 1996), Folin-Ciocalteu (Makkar et al., 1993), ferrous ion chelating activity (FICA) (Yen & Wu, 1999), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Yen & Wu, 1999). The extraction procedures are deeply described in another study (Musati, Bertino, et al., 2025).

### 2.7. Tocopherols and cholesterol in meat

Fat-soluble vitamins (α-tocopherol, γ-tocopherol, δ-tocopherol, and retinol) and cholesterol in LTL were extracted and analysed following the procedure described elsewhere (Bertolín et al., 2018) with adaptations. An aliquot of 2.5 g of meat was minced and mixed with 0.2 g of ascorbic acid and 7.5 mL of 10% potassium hydroxide (KOH) in ethanol: water (1:1, *v/v*) and incubated in an orbital shaker overnight. Lipophilic fraction was extracted using 5 mL of a solvent mixture composed of hexane and ethyl acetate (9:1, *v/v*), supplemented with 25 mg/L of butylated hydroxytoluene (BHT). The organic phase was subsequently subjected to centrifugation, and the resulting supernatant was carefully collected. These operations were repeated twice, and the obtained supernatants were combined and dried under a nitrogen stream. The residues were solubilised in methanol and filtered (PTFE, 0.22 μm). The compounds were quantified using a liquid chromatography system set as described above in paragraph 2.3.

### 2.8. Drip loss

Drip loss was evaluated by using a raw meat cube (approximately 1.5 cm/side) weighing ~3.5 g ($W_0$) in duplicate for each sample. Each piece of meat was put inside an inflated plastic bag and maintained in the centre using a nylon thread, avoiding contact with the bag walls. The samples thus prepared were stored at 4 °C for 48 h and 96 h. At the end of the storage periods, the samples were removed from the bags, gently blotted dry and weighed, obtaining the weights referred to the two storage periods identified as $W_1$. Drip loss was expressed as the sample's loss of weight over the two periods, expressed as a percentage of the initial weight, using the following formula:

$$\textit{Drip loss}\ (\%) = \frac{W_0 - W_1}{W_0} \times 100$$

### 2.9. Centrifugal loss

Centrifugal loss was measured as follows to estimate the WHC of raw meat. Meat samples, approximately 10 × 10 × 15 mm and 2 g of weight ($W_0$), were prepared by wrapping with filter paper. The samples were then put in 50 mL conical centrifuge tubes, previously prepared with adsorbent cotton at the bottom of the tubes. The samples were centrifuged for 20 min at 1500 ×*g* at 20 °C. At the end of the process, the weight of the meat ($W_1$) was measured again and recorded. The samples were treated in duplicate. The centrifugal loss was calculated using the following equation:

$$\textit{Centrifugal loss}\ (\%) = \frac{W_0 - W_1}{W_0} \times 100$$

### 2.10. Cooking loss

The cooking loss was determined by weighing approximately 35 g of raw meat samples, maintaining the same thickness of 1.5 cm for all samples ($W_0$). Then, each sample was vacuum-packed using a heat-resistant plastic bag and placed in a water bath maintained at 70 °C for 30 min for cooking. At the end of the process, the samples were extracted and cooled at room temperature. The external moisture was removed using blotting paper, and then the samples were weighed again ($W_1$). All the measurements were performed in duplicate. The cooking loss was calculated using the following equation:

$$\textit{Cooking loss}\ (\%) = \frac{W_0 - W_1}{W_0} \times 100$$

### 2.11. Oxidative stability of meat

Oxidative stability was assessed in raw and cooked meat throughout aerobic refrigerated storage. Six slices (1.5 cm thickness) were cut from the left LTL muscle of each animal. Three slices were placed in plastic trays, overwrapped with household cling film and stored at 4 °C in the absence of light. Each slide was employed to assess the colour stability and the primary (hydroperoxides) and secondary (2-thiobarbituric acid reactive substances; TBARS) lipid oxidation products at day 0 (2 h for blooming), day 4, and day 7 of refrigerated storage. The other three slices were cooked as specified above in paragraph 2.10. At the end of the cooking time, samples were rapidly chilled and used for secondary lipid oxidation analysis on days 0, 2 and 4 of conservation in the same conditions as the raw meat. The colour stability was assessed on raw meat using a portable spectrophotometer. The colour descriptors $L^*$ (lightness), $a^*$ (redness), $b^*$ (yellowness), $C^*$ (saturation) and $h_{ab}$ (hue angle) were recorded in the CIELAB colour space and calculated as the average value on three measurements on non-overlapping areas. Reflectance spectra in the 400–700 nm wavelength range were collected to assess metmyoglobin formation (Krzywicki, 1979). Metmyoglobin accumulation was further estimated using the ratio of scattering

coefficients at 572 and 525 nm (Stewart et al., 1965). After colour measurements, the samples were frozen at −80 °C for the subsequent analysis. The hydroperoxide content in raw meat was determined with reference to a previous study (Maqsood et al., 2012) with some modifications to the original method as detailed elsewhere (Menci et al., 2023). The TBARS analyses were conducted on both raw and cooked meat following a standard procedure (Siu & Draper, 1978) recently modified (Natalello et al., 2020).

### 2.12. Statistical analyses

Data on animal performances, FA, lipophilic vitamins, WHC and antioxidant capacity of meat were analysed by one-way ANOVA with SPSS software, using the dietary treatment as a fixed factor and considering the individual animal as the experimental unit. A generalised mixed model was used for the colour and lipid oxidation data to test the effects of the dietary treatment, time of storage, and their interaction, with the individual animal as a random factor. Significance was set at $P \leq 0.05$, with the Tukey *post hoc* test applied for multiple comparisons; values with $0.050 < P \leq 0.100$ were interpreted as trends.

## 3. Results

### 3.1. Experimental diets, animal performances and intakes

The formulation and the chemical composition of the experimental diets and the AS are reported in Table 1. In diets containing AS, only one ingredient was replaced, the maize. The increment of the AS doses resulted in a higher content of fibre (aNDF and ADF), lipids and total tocopherols. In particular, the vitamin E content was 57.6 and 71.0 μg/g of dry matter (DM) in A14 and A28, respectively, compared to 43.9 μg/g of the CON group. The γ-tocopherol was the most abundant vitamin E isomer in all treatments. However, in the dietary treatment, the γ-tocopherol proportion gradually decreased from 68.6% to 53.3% increasing the doses of AS, and a concurrent increase of α-tocopherol was observed. Contrarily, in AS a prevalence of the α isomer was observed. The FA composition of the diets was affected by the inclusion of AS as follows: the content of palmitic (C16:0) and stearic acid (C18:0) was reduced in both AS treatments compared to CON. The AS diets were enriched in oleic acid (C18:1 *c*9). The performance parameters of the lambs, listed in Table 2, including final body weight, carcass weight, average daily gain, voluntary feed intake and feed conversion ratio, were not influenced by the dietary treatment ($P > 0.05$). Despite slight differences in polyphenol and tannin levels among the diets, the intakes of polyphenols, tannins and total tocopherols were affected by the dietary treatment as follows: C < A14 < A28 ($P < 0.001$).

**Table 2**
Effect of dietary treatment on animal performance and intakes.

| | Dietary treatment[1] | | | SEM | *P-value* |
|---|---|---|---|---|---|
| | CON | A14 | A28 | | |
| *Performances* | | | | | |
| DMI (g/day) | 841 | 928 | 952 | 28.4 | 0.251 |
| ADG (g/day) | 252 | 278 | 269 | 9.1 | 0.511 |
| FCR (g DMI/g ADG) | 3.41 | 3.36 | 3.56 | 0.067 | 0.461 |
| Final body weight (kg) | 27.1 | 28.4 | 27.3 | 0.699 | 0.725 |
| Hot carcass weight (kg) | 12.1 | 12.9 | 12.6 | 0.367 | 0.687 |
| Cold carcass weight (kg) | 12.0 | 12.8 | 12.4 | 0.366 | 0.687 |
| Chilling loss (%) | 1.50 | 1.37 | 1.85 | 0.146 | 0.392 |
| *Intakes* | | | | | |
| Polyphenols (g TAeq/day) | $3.73^{c}$ | $5.20^{b}$ | $7.09^{a}$ | 0.312 | <0.001 |
| Tannins (g TAeq/day) | $1.50^{c}$ | $3.02^{b}$ | $4.62^{a}$ | 0.262 | <0.001 |
| Total tocopherols (mg/day) | $37.0^{c}$ | $53.5^{b}$ | $67.6^{a}$ | 2.91 | <0.001 |
| α-tocopherol (mg/day) | $9.64^{c}$ | $17.1^{b}$ | $26.0^{a}$ | 1.40 | <0.001 |
| γ-tocopherol (mg/day) | $25.4^{b}$ | $34.1^{a}$ | $36.0^{a}$ | 1.31 | <0.001 |
| δ-tocopherol (mg/day) | $1.94^{b}$ | $2.32^{b}$ | $5.57^{a}$ | 0.329 | <0.001 |

Abbreviations: DMI: dry matter intake; ADG: average daily gain; FCR: feed conversion ratio; TAeq: tannic acid equivalents; SEM: standard error of the mean.
[a,b,c] Within a row, different superscripts indicate significant differences ($P \leq 0.05$).
[1] CON: Control diet; A14: 14% Almond skin diet; A28: 28% Almond skin diet.

### 3.2. Fatty acids, vitamins, antioxidant capacity and water holding capacity of meat

The effects of the dietary treatment on FA, vitamins, antioxidant capacity and WHC of meat are summarised in Table 3. Meat pH was not influenced by the experimental diets, together with the WHC evaluated in different ways ($P > 0.05$). The intramuscular fat (IMF) content was higher in A28 compared to the CON ($P = 0.034$), as well as the content of PUFA and n-6 PUFA ($P = 0.039$ and 0.007, respectively). Consequently, since the n-3 PUFA content was the same for all dietary treatments, the n-6/n-3 PUFA ratio increased following the order C < A14 < A28 ($P < 0.001$). Although a greater amount of PUFA in meat from the A28 group, no differences were observed in HP-PUFA content. The experimental diets did not affect the SFA neither MUFA contents ($P > 0.05$). Regarding vitamin E, the main isomer for all treatments was the α-tocopherol. The CON and A14 groups had similar total tocopherol

**Table 3**
Effect of dietary treatment on the ultimate pH, water holding capacity, intramuscular fat, cholesterol, vitamins and antioxidant capacity of meat.

| | Dietary treatment[1] | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | CON | A14 | A28 | | |
| Ultimate meat pH | 5.87 | 5.78 | 5.83 | 0.034 | 0.552 |
| *Water holding capacity (%)* | | | | | |
| Centrifugal loss | 16.2 | 15.5 | 14.5 | 0.433 | 0.274 |
| Drip loss 48 h | 3.09 | 2.98 | 3.00 | 0.111 | 0.922 |
| Drip loss 96 h | 5.74 | 5.65 | 5.24 | 0.145 | 0.333 |
| Cooking loss | 26.2 | 27.7 | 26.2 | 0.527 | 0.400 |
| IMF (g/100 g meat) | $1.48^{b}$ | $2.12^{ab}$ | $2.36^{a}$ | 0.147 | 0.034 |
| *Fatty acid (mg/100 g meat)* | | | | | |
| Saturated | 534 | 769 | 731 | 66.9 | 0.317 |
| Monounsaturated | 589 | 785 | 779 | 69.7 | 0.443 |
| Polyunsaturated | $185^{b}$ | $238^{ab}$ | $268^{a}$ | 13.8 | 0.039 |
| n-6 PUFA | $150^{b}$ | $210^{ab}$ | $244^{a}$ | 13.0 | 0.007 |
| n-3 PUFA | 35.0 | 30.9 | 26.6 | 1.64 | 0.111 |
| n-6/n-3 ratio | $4.28^{c}$ | $6.88^{b}$ | $9.40^{a}$ | 0.431 | <0.001 |
| HP-PUFA | 76.7 | 74.6 | 69.3 | 2.71 | 0.529 |
| Cholesterol (g/kg) | 0.710 | 0.695 | 0.703 | 0.018 | 0.949 |
| *Fat-soluble vitamins (mg/kg)* | | | | | |
| Total tocopherols | $1.16^{a}$ | $0.937^{a}$ | $0.585^{b}$ | 0.066 | <0.001 |
| α-tocopherol | $0.851^{a}$ | $0.670^{a}$ | $0.416^{b}$ | 0.051 | 0.001 |
| γ-tocopherol | $0.298^{a}$ | $0.253^{a}$ | $0.156^{b}$ | 0.017 | 0.001 |
| δ-tocopherol | 0.014 | 0.013 | 0.014 | <0.001 | 0.368 |
| Retinol | 0.153 | 0.147 | 0.150 | 0.005 | 0.890 |
| HP-PUFA/vitamin E[2] | $2.83^{b}$ | $2.91^{ab}$ | $3.08^{a}$ | 0.035 | 0.005 |
| IMF/vitamin E[2] | $4.11^{b}$ | $4.36^{ab}$ | $4.59^{a}$ | 0.056 | <0.001 |
| *Antioxidant capacity (mg/g)* | | | | | |
| FICA (EDTA eq) | 0.751 | 0.739 | 0.732 | 0.016 | 0.885 |
| Folin-Ciocalteu (TA eq) | $0.620^{a}$ | $0.562^{b}$ | $0.566^{b}$ | 0.009 | 0.013 |
| FRAP ($Fe^{2+}$ eq) | $27.2^{a}$ | $21.6^{b}$ | $22.7^{b}$ | 0.554 | <0.001 |
| TEAC (Trolox eq) | $44.9^{b}$ | $55.4^{a}$ | $56.9^{a}$ | 1.76 | 0.006 |
| DPPH (Trolox eq) | 0.760 | 0.750 | 0.760 | 0.037 | 0.914 |

Abbreviations: DPPH: 1,1-diphenyl-2-picrylhydrazyl; EDTA: ethylenediaminetetraacetic acid; FICA: ferrous ion chelating activity; FRAP: ferric reducing antioxidant power; HP-PUFA: highly peroxidizable polyunsaturated fatty acid; PUFA: polyunsaturated fatty acid; TA eq: tannic acid equivalents; TEAC: Trolox equivalent antioxidant capacity; SEM: standard error of the mean; IMF: intramuscular fat.
[a,b,c] Within a row, different superscripts indicate significant differences ($P \leq 0.05$).
[1] CON: control diet; A14: 14% almond skin diet; A28: 28% almond skin diet.
[2] Calculated as the ratio between HP-PUFA or IMF and vitamin E (total tocopherols), both expressed in mg/g meat. Since the original data did not meet normality according to the Anderson–Darling test, a logarithmic transformation was applied, and the table reports the resulting LOG10 values.

contents, whereas A28 showed a concentration that was about half compared to CON ($P < 0.001$). Consequently, the HP-PUFA to vitamin E ratio was higher in A28 compared to CON ($P = 0.005$). Cholesterol and retinol levels in meat remained unaffected by the dietary treatment ($P > 0.05$). Considering the antioxidant capacity assays, the diets containing AS (A14 and A28) lowered the reducing activity measured with Folin-Ciocalteu and FRAP tests compared to CON ($P = 0.013$ and $P < 0.001$, respectively). The TEAC values were higher in AS treatments compared to CON, showing an improved capacity of radical scavenging of the extract ($P = 0.006$). The antioxidant capacity of meat measured with FICA and DPPH assays showed no significant differences among the treatments.

### 3.3. Oxidative stability of meat

The data on oxidative stability are summarised in Table 4. No significant effect of the dietary treatment on colour parameters was observed ($P > 0.05$), conversely, storage time significantly altered all colour parameters ($P < 0.001$). Specifically, $C^*$ peaked on day 4 of storage and was lowest at days 0 and 7. The colour descriptor $b^*$ had the highest value on day 4, followed by day 7 and day 0 ($P < 0.001$), while that of $a^*$ had the highest value on day 4, followed by day 0 and day 7 ($P < 0.001$). An increase was observed in the metmyoglobin percentages and hue angle during storage time, while the index obtained as the ratio between scattering coefficient at 572 nm and 525 nm (KS572/KS525) decreased ($P < 0.001$). Concerning the lipid oxidation, the dietary treatment did not affect the primary products measured as hydroperoxides ($P > 0.05$), while the time of storage tended to reach the maximum value on day 4 of storage ($P = 0.094$). Moreover, an interaction between diet and time was found for hydroperoxides, but no significant differences were stressed in pairwise analysis after adjustment for multiple comparisons using Tukey's correction ($P > 0.05$). The TBARS values increased over storage time in both raw and cooked meat ($P < 0.001$), while experimental diets did not affect the lipid oxidation ($P > 0.05$). Only in raw meat was observed a trend toward significance ($P = 0.073$) for the accumulation of TBARS between CON and A28 ($P = 0.090$; data not shown).

## 4. Discussion

As far as we know, this is the first study examining the effects of dietary AS on the oxidative stability of meat. Previous studies have explored other almond by-products, such as the hull, and observed an improvement in the meat oxidative stability. Indeed, Scerra et al. (2022) showed a clear reduction in lamb lipid oxidation throughout a 7-day storage period with the dietary inclusion of almond hulls up to 30% DM. Other authors have reported contrasting results using the almond hull, in which lipid oxidation of fresh and cooked meat assumed different values (Cachucho et al., 2025). Further studies have investigated the effects of the dietary inclusion of skin from different nuts, such as hazelnut and pistachio, on meat oxidative stability (Musati, Bella, et al., 2025, Musati, Bertino, et al., 2025). These studies, although different from our diets in terms of polyphenols and tocopherols, suggest that the bioactive compounds present in the almond hull and nut skin may have contributed to the meat oxidative stability. The AS contains many antioxidant compounds, such as hydroxybenzoic and hydroxycinnamic acids, aldehydes, anthocyanidin and procyanidin, as they can act as protection for the oil-rich kernel from oxidation due to exogenous sources (Esfahlan et al., 2010) and some of these may have antioxidant capacity in the feed or be transferred to the meat. Apart the meat quality, the use of locally available agro-industrial by-products could be important for several environmental and economic aspects. Feed represents 60–70% of livestock production costs. Implementing by-products in feed formulation could reduce costs and improve profitability, especially in dry regions like the Mediterranean area, in which the main feedstuffs are imported from distant sources (Salami et al., 2019). In our study, the maize, an imported and high water-demanding crop, was reduced by up to 65% compared to CON diet, mitigating the environmental and economic impact of the feed on livestock production.

In the present study, the diets formulated with AS, although estimated metabolizable energy was comparable for all treatments, contained more than two-fold the fat than the CON diet, leading probably to greater accumulation of IMF, PUFA and n-6 PUFA in muscle. On the one hand, considering the similar content of HP-PUFA in all treatments, we could expect the same trend in lipid oxidation. On the other hand, a greater amount of IMF, PUFA and n-6 PUFA in A28 could lead to an increase in lipid oxidation; since it is known that the meat oxidative stability is influenced by the fat content and FA composition, and the oxidation susceptibility is linked to the degree of fat unsaturation (Domínguez et al., 2019).

The meat oxidative process is driven by pro-oxidant and antioxidant factors that interact with each other (Bekhit et al., 2013). Regarding the latter class of compounds, vitamin E is widely recognised as the most effective antioxidant in meat (Bellés et al., 2019). Diets containing AS

**Table 4**
Effect of the dietary treatment and time of storage on colour and lipid oxidation parameters of meat.

| | Dietary treatment (D)[1] | | | Time (T)[2] | | | SEM[3] | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CON | A14 | A28 | 0 | 1 | 2 | | D | T | D × T |
| *Colour stability of raw meat* | | | | | | | | | | |
| $a^*$(redness) | 13.6 | 14.0 | 14.1 | $14.1^{b}$ | $14.8^{a}$ | $12.9^{c}$ | 0.173 | 0.548 | <0.001 | 0.365 |
| $b^*$(yellowness) | 11.9 | 12.2 | 12.0 | $9.85^{c}$ | $14.0^{a}$ | $12.3^{b}$ | 0.243 | 0.822 | <0.001 | 0.299 |
| $C^*$ (saturation) | 18.1 | 18.7 | 18.6 | $17.2^{b}$ | $20.4^{a}$ | $17.8^{b}$ | 0.259 | 0.755 | <0.001 | 0.365 |
| $h_{ab}$ (Hue angle; deg) | 40.9 | 40.8 | 39.9 | $34.8^{b}$ | $43.4^{a}$ | $43.4^{a}$ | 0.469 | 0.234 | <0.001 | 0.043* |
| KS572/KS525[4] | 0.888 | 0.890 | 0.896 | $0.955^{a}$ | $0.872^{b}$ | $0.847^{c}$ | 0.006 | 0.766 | <0.001 | 0.659 |
| MetMyoglobin (%) | 51.0 | 50.8 | 50.2 | $44.2^{c}$ | $52.2^{b}$ | $55.6^{a}$ | 0.575 | 0.633 | <0.001 | 0.655 |
| *Lipid oxidation (mg/kg meat)* | | | | | | | | | | |
| Hydroperoxide of raw meat | 7.29 | 4.89 | 6.99 | 5.65 | 7.88 | 5.65 | 0.631 | 0.413 | 0.094 | 0.003* |
| TBARS[5] values | | | | | | | | | | |
| Raw meat | $0.677^{y}$ | $1.21^{xy}$ | $1.28^{x}$ | $0.383^{c}$ | $1.08^{b}$ | $1.71^{a}$ | 0.100 | 0.073 | <0.001 | 0.083 |
| Cooked meat | 3.82 | 3.90 | 3.98 | $2.55^{c}$ | $3.96^{b}$ | $5.19^{a}$ | 0.139 | 0.822 | <0.001 | 0.546 |

[a,b,c] Within a row, different superscripts indicate significant differences between storage time ($P \leq 0.05$).
[x,y] Within a row, different superscripts indicate a tendency between dietary treatment ($0.05 < P \leq 0.10$)
[1] CON: control diet; A14: 14% almond skin diet; A28: 28% almond skin diet.
[2] Times of storage 0, 1 and 2 correspond to: days 0, 4, 7 (raw meat); days 0, 2, 4 (cooked meat).
[3] SEM: standard error of the mean.
[4] Ratio between scattering coefficient at 572 nm and 525 nm.
[5] TBARS: thiobarbituric acid reactive substances (mg of malondialdehyde per kg of meat).
* In the pairwise analysis, no significant differences ($P > 0.05$) were found after adjustment for multiple comparisons using Tukey's correction.

exhibited a higher total tocopherol content than the CON and, consequently, resulted in a greater intake of vitamin E by the animals fed almond-based diets. These values are comparable to a previous study (Musati, Bertino, et al., 2025), in which lambs were fed with diets containing hazelnut skin, alone or in combination with extruded linseed. In that study (Musati, Bertino, et al., 2025), the total tocopherol content of these diets was 70 and 100 mg/kg of dry matter, while the daily intake was 63 and 87 mg/day, for the hazelnut combined with linseed and the hazelnut alone groups, respectively. The obtained meat from lambs given hazelnut skin had around two-fold vitamin E content compared to the control group (Musati, Bertino, et al., 2025). However, in the present study, the accumulation of vitamin E in meat was reduced (−50%) by the inclusion of 280 g/kg AS compared to CON, following a trend inversely proportional to the accumulation of IMF. The reduction of total tocopherol content could be explained by a dilution effect exerted by the increased IMF in meat from AS treatment. Indeed, considering the higher tocopherol content in the AS diets and the greater intakes, an increased concentration of tocopherols in meat from AS treatments was expected. The dilution effect is confirmed by the ratio IMF/vitamin E, which is higher in A28 compared to CON, with a strong statistical significance. Consequently, higher IMF resulted in a greater amount of oxidised FA, which reacts with tocopherol, reducing its active form, no longer detectable, into the oxidised one (Faustman et al., 1999). Additional efforts should be made to pay more attention to the oxidised forms of α-tocopherol (*e.g.*, α-tocopherolquinone, α-tocopherolhydroquinone, 5,6-epoxy-α-tocopherolquinone and 2,3-epoxy-α-tocopherolquinone), which may have formed in the muscle but not been identified with our analysis. In addition, the reduction of vitamin E in meat might be partially justified by the high concentrations and the type of PUFA in the diet, which can inhibit the absorption of vitamin E (McDowell, 2000). In the study of Chikunya et al. (2004) in which sheep fed with different PUFA sources and the same level of vitamin E supplementation (500 mg/kg DM), the authors observed a reduction of vitamin E in blood plasma depending on the PUFA sources. However, further studies are necessary to clarify the reduction of tocopherols in meat despite their greater amount in the diets.

Lipid oxidation is the second degradation process, after microbial spoilage, which is responsible for the deterioration of animal-derived foods during conservation, generating off-flavours and odours (Bhat et al., 2023). This process led to the formation of two classes of compounds, the primary products, represented by hydroperoxides, and the secondary products, including the aldehydes (Ross & Smith, 2006). In the present study, no differences were discovered in hydroperoxide content between diets. Hydroperoxide content showed only an increasing trend on day 4 of storage and a decrease on day 7. Accordingly to other studies (*e.g.*, Manheem et al., 2023), accumulation of hydroperoxides reflects the lipid oxidation pattern, where these compounds are rapidly converted into secondary compounds due to their low stability (Ross & Smith, 2006). Regarding the secondary products of lipid oxidation, malondialdehyde is one of the major representative compounds, and it is quantified with the TBARS test (Wang et al., 2019). In the present study, we evaluated the accumulation of TBARS both on raw and cooked meat. As expected, the TBARS values increased over time, peaked on day 7 of refrigerated conservation for raw meat and day 4 for cooked one.

For determining the pro-oxidant/antioxidant balance, the ratio between the most oxidizable substrate (HP-PUFA) and the strongest antioxidant of meat (vitamin E) was calculated (Musati, Bertino, et al., 2025). Our results showed a significantly higher HP-PUFA to vitamin E ratio in A28 than CON. This finding would suggest a greater susceptibility to lipid oxidation of A28 meat, and in the meantime, CON meat would exhibit greater resistance to oxidation. Despite the ratio mentioned above and the PUFA content, only a tendency to increase was observed in TBARS values of raw meat in A28 compared to CON ($P = 0.090$; data not shown). Contrary to the expectations suggested by the HP-PUFA/vitamin E ratio, no differences in TBARS accumulation were observed in cooked meat between the dietary treatment. This effect should be amplified in cooked meat, where a higher availability of oxidised substrate and more pronounced pro-oxidant conditions are present. The lack of differences in TBARS values of cooked meat in our study may suggest that vitamin E could have played a role in reducing the oxidation of lipid substrate, modifying its form into the oxidised one, no longer detectable as an active form (Faustman et al., 1999), or other factors, such as the phenolic compounds, may have prevented the lipid oxidation processes. Indeed, feeding lambs with almond hulls up to 30% DM reduced the lipid oxidation in both raw and cooked meat (Scerra et al., 2022). The authors attributed this effect to the phenolic compounds contained in the diets (Scerra et al., 2022). Moreover, as observed by Cachucho et al. (2025), feeding lambs up to 18% of almond hull did not modify the meat content of pro-oxidant FA, including HP-PUFA, and also the TBARS production in raw meat was comparable between the control group and the almond hull group with 18% of inclusion. While in cooked meat, the lambs fed 18% of almond hull showed a reduction in lipid oxidation compared to the control, attributed to the increased consumption of tocopherols and tannins.

Several hydrophilic compounds can modulate the stabilisation of radical species. Therefore, the study of antioxidant properties is generally characterised by using different tests that may represent a wider and complete spectrum (Csepregi et al., 2016). Among these, five of the most used assays were selected to be included in the present study. Although two of them (FICA and DPPH assays) showed no effects among the dietary treatment, the Folin-Ciocalteu and the FRAP ones had lower values in AS groups compared to CON, showing a less reduction activity of the extract, *i.e.* the extracts with minor reducing capacity donate fewer electrons to the oxidant reagent. In contrast, the TEAC assay showed an increase in radical scavenging activity of the meat hydrophilic extract in AS groups compared to CON. The different results among the three assays could be justified because they are based on different mechanisms. Both the Folin-Ciocalteu test and the FRAP test are based on redox reactions. The former is based on the reduction of phosphomolybdic-phosphotungstic acid complexes under alkaline conditions and reflects the overall reducing capacity of the sample and is sensitive to a wide range of non-phenolic compounds (*e.g.*, ascorbic acid or proteins). The FRAP assay specifically measures the reduction of the $Fe^{3+}$–TPTZ complex to $Fe^{2+}$–TPTZ under acidic conditions, detecting mainly compounds with electron-donating activity without taking radical quenching mechanisms into account (Prior et al., 2005). The TEAC assay evaluates the antioxidant capacity of a sample based on its ability to scavenge the $ABTS^{+}$ radical cation, comparing the inhibition of radical absorbance to that of the standard antioxidant Trolox. The assay reflects the hydrogen- or electron-donating activity of antioxidants but does not differentiate between specific classes of compounds or capture all radical quenching mechanisms (Prior et al., 2005; Re et al., 1999). We do not have a clear explanation regarding the contrasting results between the two assays that express the radical scavenging activity (*i.e.* TEAC and DPPH).

In contrast with the present study, previous articles reported consistent trends along the three mentioned assays (*i.e.* Folin-Ciocalteu, FRAP and TEAC). For instance, previous studies have shown that feeding lambs with quebracho tannins improves meat antioxidant capacity, measured with the three assays (Luciano et al., 2011), while Jerónimo et al. (2020) observed that dietary supplementation of *Cistus ladanifer* L. tannins in lambs did not influence all the assays as before. Moreover, in a recent study in which lambs were fed with hazelnut skin alone or in combination with extruded linseed, despite a higher intake of polyphenols and tannins, no differences were observed in the hydrophilic antioxidant capacity of meat, measured with the same assays used in the present study (Musati, Bertino, et al., 2025). Determining the antioxidant capacity of food matrices is challenging because no single analytical technique can adequately reflect all the antioxidant processes involved. Among the dietary water-soluble antioxidants, polyphenols play a crucial role in enhancing meat oxidative stability through

multiple complementary mechanisms (Álvarez-Rodríguez et al., 2022). Their ability to scavenge reactive species, chelate pro-oxidant molecules and stabilise heme proteins helps to slow down lipid oxidation (Álvarez-Rodríguez et al., 2022). The contrasting effects produced by the dietary polyphenols may be due to their complex chemical structure. Indeed, polyphenols are constituted by a diverse group of plant secondary metabolites encompassing phenolic acids, flavonoids, and tannins, with several bioactive properties (Santhiravel et al., 2022). In addition, dietary polyphenols are extensively transformed by ruminal microbiota into a variety of smaller metabolites before absorption, and only a limited proportion of intact parent compounds reach systemic circulation and subsequently muscle tissue. As a result, the phenolic profile in meat largely reflects ruminal and host biotransformation rather than direct transfer of dietary polyphenols (*e.g.*, hydrogenation, *O*-demethylation, and ring cleavage), and this limited transfer efficiency should be taken into account when interpreting effects on meat antioxidant status (Ahsin et al., 2025). Moreover, in agro-industrial by-products, polyphenols occur with a mix of other antioxidants, making it challenging to isolate each compound and quantify its effect on overall antioxidant capacity (Valenti et al., 2019).

An important quality trait of meat that directly influences the consumer's acceptance is the WHC, because it affects key sensory attributes (Warner, 2017). Higher WHC improves juiciness, tenderness and visual appearance. Consequently, products with greater WHC are generally perceived as higher quality and more acceptable to consumers (Warner, 2017). In the present study, the WHC was estimated by analysing the centrifugal loss, drip loss and cooking loss, but none of these parameters was affected by dietary treatment. This is consistent with the absence of differences in meat pH between dietary treatment. Indeed, the meat pH is widely recognised as the main factor affecting WHC, because it regulates the net charge of myofibrillar proteins, hence their ability to retain water (Huff-Lonergan & Lonergan, 2005). Moreover, WHC is strongly affected by *post-mortem* protein denaturation, myofibrillar shrinkage and sarcolemma integrity, all of which determine the amount of fluid release through drip or thermal losses (Warner, 2017). The lack of differences in WHC parameters suggests that the biochemical conditions of the muscle were not modified by the dietary treatment.

In the present study, no differences in colour parameters were observed between CON and AS groups. Since the meat colour also plays an important role in consumer preferences, the results obtained in the present study could be interpreted as positive. Additionally, all colour descriptors were affected by the storage period. In detail, redness ($a^*$) decreased between day 0 and 7 of conservation, while yellowness ($b^*$) and hue angle ($h_{ab}$) increased. These results are comparable to those obtained in a similar study (Scerra et al., 2022), in which lambs were fed with diets containing increasing doses of almond hulls up to 30%. Moreover, the proportion of metmyoglobin increased with storage time, reaching the maximum value on day 7 of storage, while the parameter KS572/KS525 decreased over conservation time accordingly. This trend is consistent with the browning of meat, due to the oxidation conditions of lipids. In fact, in the present study, the TBARS values increased over time, as well as the colour parameters (*i.e.* $b^*$ and $h_{ab}$).

## 5. Conclusion

This study showed that feeding lambs with AS, an agro-industrial by-product, can replace maize without affecting meat quality. Lambs receiving AS ingested more fat and bioactive molecules, including phenolic compounds and tocopherols. Unexpectedly, despite a higher intake of vitamin E, total meat tocopherols decreased by 50% in the highest level of AS inclusion (*i.e.*, 280 g/kg DM), suggesting a potential imbalance between HP-PUFA and vitamin E. No negative effects were found in hydroperoxide and TBARS production in raw and cooked meat after 7 or 4 days of refrigeration, respectively. However, lipid oxidation in raw meat tended to be higher in lambs fed 280 g/kg AS.

Antioxidant capacity of the hydrophilic fraction varied: Folin–Ciocalteu and FRAP assays indicated a decrease, while TEAC showed an increase, highlighting the need for further investigation. The WHC and colour were unaffected, indicating stable muscle biochemistry and visual quality. The inclusion of 280 g/kg of AS increased IMF and PUFA compared to CON.

In both cases of AS inclusion, the animal growth performance was comparable to that of the CON group, indicating that replacing maize with AS did not worsen diet palatability or energy conversion efficiency. To sum up, AS could be included up to 140 g/kg as a maize substitute, without any detrimental effects on meat quality, supporting a circular economy. Further studies are needed to elucidate the effect of 280 g/kg AS inclusion on vitamin E and lipid oxidation of raw meat.

## CRediT authorship contribution statement

**Antonino Bertino:** Writing – original draft, Visualization, Formal analysis, Data curation. **Martino Musati:** Writing – review & editing, Supervision, Formal analysis. **Giuseppe Luciano:** Writing – review & editing, Investigation, Conceptualization. **Fabrizio Mangano:** Writing – review & editing, Formal analysis. **Marco Sebastiano Cannone:** Writing – review & editing, Formal analysis. **Alessandro Priolo:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Luisa Biondi:** Writing – review & editing. **Guido Mangione:** Writing – review & editing, Formal analysis. **Antonio Natalello:** Writing – review & editing, Supervision, Investigation, Conceptualization.

## Ethics approval

Experimental procedures with animals were conducted in accordance with European Union (Council Directive 2010/63/EU) legislation for the protection of animals used for experimental and other scientific purposes and reviewed by the Research Ethics Committees of the University of Catania (protocol number: 54766).

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## Declaration of competing interest

The authors declared that they have no conflicts of interest to this work.

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## Data availability

The data used and/or analysed are available from the corresponding author on reasonable request.

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