

Effect of dietary treatment with olive oil by-product (olive cake) on physico-chemical, sensory and microbial characteristics of beef during storage

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Abstract

Several studies have demonstrated that the use of natural preservatives through animal diets could increase the shelf life of meat and meat products since many plant-derived substances show antioxidant and antimicrobial properties. The aim of this work was to study the effect of olive cake dietary supplementation on beef oxidative stability and antimicrobial activity during storage. Beef cattle were randomly divided into three homogeneous groups that were assigned to one of the three diets: a commercial unified based diet administered for 90 days until slaughter (CTR), CTR diet supplemented with 0.5% olive cake administered for 90 days until slaughter (OC1), and CTR diet supplemented with 0.5% olive cake and administered for 60 days followed by the administration of the CTR diet for 30 days until slaughter (OC2). Beefsteaks were overwrapped with oxygen-permeable packaging and analysed at four different storage times (zero, three, six and nine days). At the four sampling times considered from all of the samples, total viable count (TVC), *Enterobacteriaceae* counts, colour coordinates (CIE L*a*b* colour system), peroxide value (PV), thiobarbituric reactive substances (TBARS) determinations and descriptive sensory analyses were performed. No differences in TVC and *Enterobacteriaceae* count were detected among the groups over all of the sampling times considered. Differences were recorded among groups for PV, TBARS, colour and sensory analysis. The addition of olive cake in the animal diet had an effect on lipid oxidation reducing the level of PV, TBARS and retarding colour deterioration and the development of off odour in OC meat during storage.

Introduction

Lipid oxidation is one of the main factors limiting the quality and acceptability of meat because it is responsible for the development of several alterations in meat, as well as for the production of potentially toxic compounds (Zanardi *et al.*, 1998). Recently, particular attention has been paid to animal feed with natural antioxidants, aiming to guarantee the quality and safety of meat during storage (Descalzo and Sancho, 2008; Nieto *et al.*, 2010). Furthermore, lipid oxidation is an important matter of concern to retailers because its discolouration effects, as well as off-odour and off-flavour development, strongly affect the meat's shelf life (Luño *et al.*, 2000; Allen and Cornforth, 2010).

Among the different natural antioxidants used in animal nutrition (Insani *et al.*, 2008; Ranucci *et al.*, 2013, 2015; Branciarì *et al.*, 2015) olive oil by-products have been considered for their high content of phenolic substances (Novelli *et al.*, 2014). In particular, olive cake, a mixture of skins, pulp, woody end-carp and seeds, is characterised by a high content in phenolic antioxidants, such as hydroxytyrosol (3,4-DHPEA), tyrosol (*p*-HPEA) and secoiridoids derivatives, and in particular, the dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA or *p*-HPEA (3,4-DHPEA-EDA or *p*-HPEA-EDA), and verbascoside (Servili *et al.*, 2009). Furthermore the use of these bioactive compounds in food resulted in growth inhibition of different bacterial strains (Pereira *et al.*, 2006; Fasolato *et al.*, 2015). No data are currently available on the effects of dietary supplementation with olive cake on beef meat oxidative stability and microbial dynamics during storage.

The aim of this work was to evaluate the effect of dietary treatment with olive cake on beef oxidative stability during storage. The effect of antioxidants on microbial evolution was also studied.

Materials and Methods

The experiment was carried out using half-breed beef cattle from a flock located in the Apulia Region (southern Italy). The beef cattle were randomly divided into three homogeneous groups of six animals each, balanced for age and body condition score. The three groups were assigned to one of the three diets: a commercial unified based diet administered for 90 days until slaughter (CTR), CTR diet supplemented with 0.5% olive cake and administered for 90 days until slaughter (OC1), and a CTR diet supplemented with 0.5% olive cake and administered for 60 days followed by the

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administration of the CTR diet for 30 days until slaughter (OC2). The three diets were isoenergetic and isoproteic.

At the end of the experimental period, the animals were transported and slaughtered at a local slaughterhouse. Immediately after slaughter, the carcasses were promptly chilled to 7°C within 24 h and were then aged for 10 days at 0±2°C. After aging, the *Longissimus dorsi* was removed, cut into steaks 2.0 cm thick, and were randomly assigned to retail packs with two steaks each, overwrapped with oxygen-permeable packaging and stored at 4±1°C for nine days in refrigerated display case with white fluorescent lighting (Lumos® LED up light 4000K, 908 lx; OSRAM spa, Milan, Italy). Meat samples packed were analysed at zero, three, six, and nine days for colour, total viable count (TVC), *Enterobacteriaceae*, thiobarbituric reactive substances (TBARS), peroxide value (PV) and sensory attributes. At zero days of storage, the olive cake phenolic compound content in the meat was evaluated and an increase in hydroxytyrosol (3,4-DHPEA) content in OC1 and OC2 meat, with higher value in OC1, was observed (data not reported). For the microbial analysis, about 25 gr of samples were collected at zero, three, six and nine days. Samples were placed in sterile and buffered peptone water (CM1049; Oxoid, Basingstoke, UK), and homogenised in a stomacher apparatus (Stomacher 400 circulator; Seward Ltd, Norfolk, UK) for two minutes at room temperature. Serial decimal dilutions in buffered peptone water were prepared and submitted to the following microbiological analyses: TVC determined using Plate Count Agar (CM0325; Oxoid) incubated at 30°C for 48 h (UNI EN ISO 4833:2004; ISO, 2004b), *Enterobacteriaceae* (ISO 21528-2:2004; ISO,

2004a). After counting, the mean and standard deviation were calculated and data were reported as Log colony forming unit (CFU)/g.

A colour measurement was performed during each of the sampling intervals after a 30 min bloom period at refrigeration temperature using a CR400 Minolta chromameter (Minolta, Osaka, Japan) light source of D65 calibrated against a standard white tile. The results were expressed as redness (a^*), hue value ($\tan^{-1} b^*/a^*$) and saturation index, or chroma [$(a^{*2} + b^{*2})/2$], (CIE, 1986). For the evaluation of meat lipid oxidation, the peroxide value was determined during storage. The peroxide value (PV) was performed using Shantha and Decker's (1994) method slightly modified. Briefly fat from beef meat (8 g) was extracted with hexane-isopropanol (3/2 v/v). Of extracted lipids, 100 mg were mixed with 9.5 mL chloroform/methanol (7:1, v/v) and 50 mL of thiocyanate/Fe²⁺ solution and were then vortexed. The absorbance of the supernatant was measured at 500 nm using an Ultraspec 2100 pro UV/visible spectrometer (Amersham Pharmacia Biotech, Piscataway, NJ, USA). For the quantitative determination of PV, a Fe (III) standard calibration curve was constructed with samples containing 1-40 µg Fe (III). The standard calibration curve obtained was $y=0.0259 x-0.0068$ ($R^2=0.9999$) and PV was expressed as meq O₂ kg⁻¹. The secondary lipid oxidation products of meat were assessed using the test of TBARs, according to Tarladgis *et al.* (1960), and the values were expressed as mg malondialdehyde (MDA)/kg meat.

A descriptive sensory analysis was performed using a six-member panel, which was trained following the criteria of ISO 8586-1:1993 (ISO, 1993). The attributes were gener-

ated in three pre-testing sessions as described by Branciaro *et al.* (2014). For quantification of the intensity of the attributes discolouration, red colour and off-odour, the panellist used a five-point descriptive scale according to ISO 13299:2003 (ISO, 2003).

The data were analysed using the GLM procedure of SAS (2001). An ANOVA model was used with diet (CTR, OC1 and OC2) as the fixed factor. The differences of the means were analysed using a Tukey test and were considered to be significant when $P<0.05$.

Results and Discussion

No difference in TVC and *Enterobacteriaceae* was recorded among the different groups (Figure 1). The administration of OC-enriched diets to beef cattle did not seem to interfere with the microbial evolution of meat during storage. Some studies proved that this bioactive substance shows antimicrobial and antifungal activity *in vitro* or when directly added to food (Fasolato *et al.*, 2015;

Medina *et al.*, 2007; Brenes *et al.*, 2007). Furthermore, feeding animals with natural bioactive compounds (10% of distillate from rosemary leaves) (Nieto *et al.*, 2010) was thought to delay the growth of total aerobic bacteria in lamb meat. This antimicrobial effect was not observed in this study.

The addition of the olive cake in the animal diet had an effect on lipid oxidation, and subsequently on colour, during beef storage (Figures 2-4).

OC1 beef was significantly redder (highest a^* value) than were the other samples in all stages of storage ($P<0.05$). Furthermore, OC2 shows a higher a^* value than did CTR in all stages of storage except at nine days. The colour of the OC1 beef loin steaks was a stable red throughout storage (Figure 2). OC2 steaks were less red than were OC1 steaks, but were redder than were CTR till six days of storage.

The stability of saturation index and the hue-angle in OC meat samples confirmed the efficacy of the presence of OC polyphenol in meat in maintaining an attractive colour, which was evident in the OC1 sample (Figures 3 and 4). Differences between OC1 and CTR

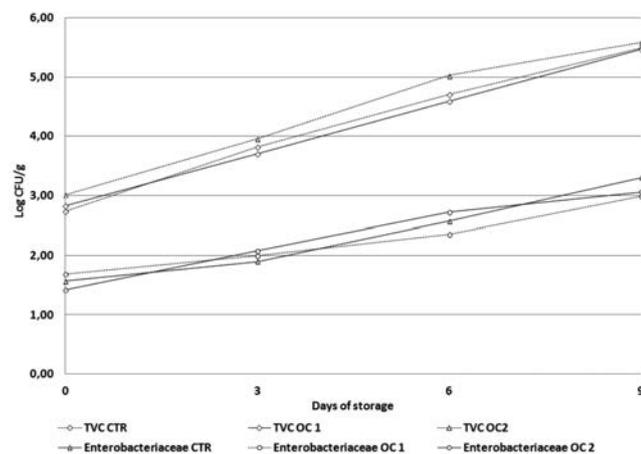


Figure 1. Microbial dynamics in beef steak during storage at $4\pm 1^\circ\text{C}$. TVC, total viable count; CTR, meat from cattle fed a standard diet; OC1, meat from cattle fed enriched diet with destoned olive cake for 90 days; OC2, meat from cattle fed enriched diet with destoned olive cake for 60 days.

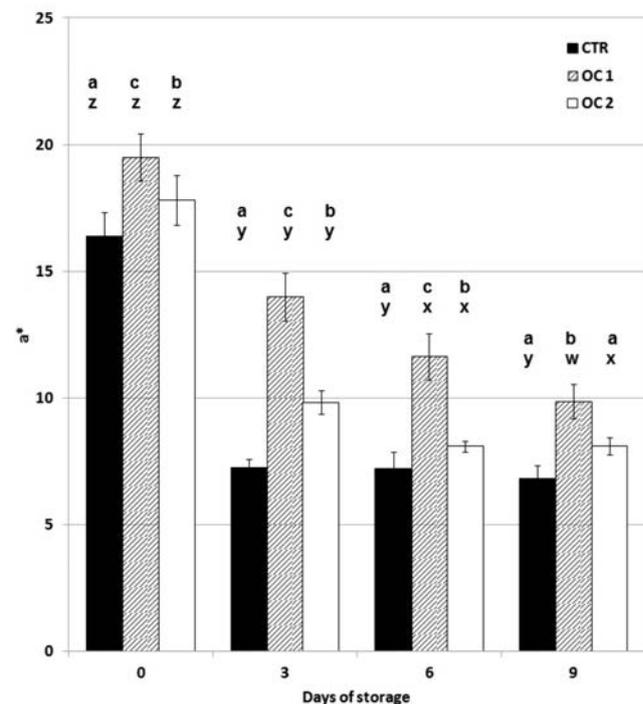


Figure 2. The effect of storage time on Commission Internationale de l'Eclairage a^* (redness) values of beef steak stored at $4\pm 1^\circ\text{C}$. CTR, meat from cattle fed a standard diet; OC1, meat from cattle fed enriched diet with destoned olive cake for 90 days; OC2, meat from cattle fed enriched diet with destoned olive cake for 60 days. Values are means plus standard deviation. ^{a-c} Within each day of storage, different superscripts indicate differences between dietary treatments ($P\leq 0.05$); ^{w-z} within each dietary treatment, different superscripts indicate differences between days of storage.

were also confirmed after nine days of storage. The panel also confirmed the stability of the colour in OC1 meat (Table 1), thus showing the effect of the polyphenol in retarding colour deterioration and extending the display life of fresh meat. The colour differences among the group may be due to the action of olive

polyphenols that act as radical quenchers and metal chelators, probably interfering with the oxidation cycle at the propagation step, preventing additional lipid radicals from forming (Allen and Cornforth, 2010; Choe and Min, 2009). Likely, polyphenol limits the initiation of lipid oxidation by binding metals, such as

iron and copper, stabilising them in an inactive or insoluble form. Meat colour can thus be preserved due to the antioxidant's ability to limit lipid oxidation, preventing the subsequent formation of reactive aldehydes and limiting iron-catalysed lipid oxidation (Allen and Cornforth, 2010; Dal Bosco *et al.*, 2012). The

Table 1. Mean sensory values of beef steaks.

Sensory values	Type of meat	Days of storage				SEM
		0	3	6	9	
Off odour ^o	CTR	1.0 ^{aA}	2.0 ^{bB}	3.3 ^{bC}	5.0 ^{bD}	0.328
	OC1	1.0 ^{aA}	1.0 ^{aA}	1.3 ^{aA}	3.0 ^{aB}	0.190
	OC2	1.0 ^{aA}	1.3 ^{aA}	3.0 ^{bB}	4.2 ^{bB}	0.287
Discolouration ^o	CTR	1.5 ^{aA}	2.8 ^{bA}	4.3 ^{bB}	5.0 ^{bB}	0.306
	OC1	1.0 ^{aA}	1.5 ^{aA}	2.5 ^{aB}	3.2 ^{aB}	0.221
	OC2	1.3 ^{aA}	2.5 ^{bB}	3.7 ^{bC}	5.0 ^{bD}	0.297
Red colour [#]	CTR	1.8 ^{bA}	3.3 ^{bB}	4.3 ^{cD}	5.0 ^{bD}	0.268
	OC1	1.0 ^{aA}	1.3 ^{aB}	2.0 ^{aBC}	2.7 ^{aC}	0.150
	OC2	1.5 ^{abA}	2.8 ^{bB}	3.7 ^{bC}	4.5 ^{bD}	0.258

SEM, standard error of mean; CTR, meat from cattle fed a standard diet; OC1, meat from cattle fed enriched diet with destoned olive cake for 90 days; OC2, meat from cattle fed enriched diet with destoned olive cake for 60 days. ^oScale: 1=none; 2=slight; 3=small; 4=moderate; 5=extreme. [#]Scale: 1 denoted extremely intense red colour while 5 denoted extremely weak red colour. ^{a-c}Values in the same column with different letters are significantly different ($P < 0.05$); ^{A-B}values in the same row with different letters are significantly different ($P < 0.05$).

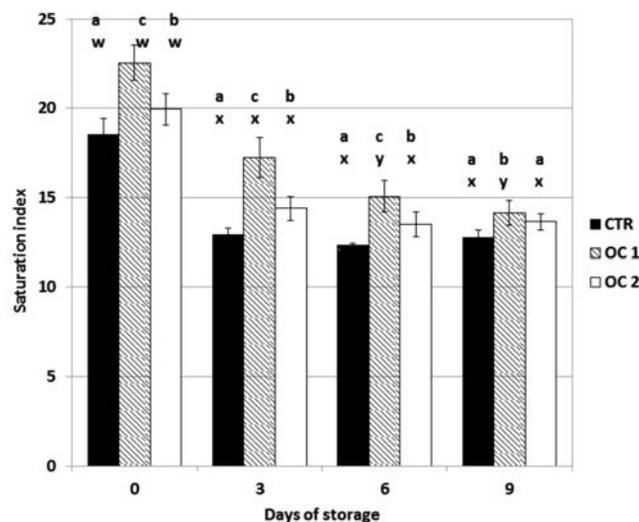


Figure 3. The effect of storage time on Saturation index of beef steak stored at $4 \pm 1^\circ\text{C}$. CTR, meat from cattle fed a standard diet; OC1, meat from cattle fed enriched diet with destoned olive cake for 90 days; OC2, meat from cattle fed enriched diet with destoned olive cake for 60 days. Values are means plus standard deviations. ^{a-c}Within each day of storage, different superscripts indicate differences between dietary treatments ($P \leq 0.05$); ^{w-z}within each dietary treatment, different superscripts indicate differences between days of storage.

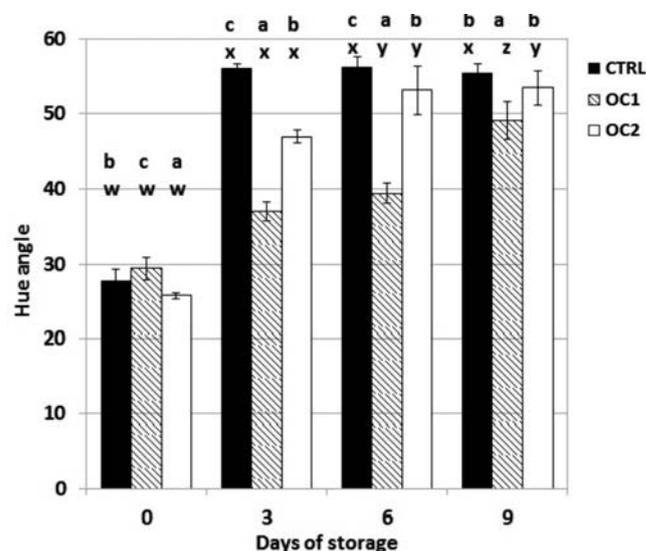


Figure 4. The effect of storage time on Hue angle of beef steak stored at $4 \pm 1^\circ\text{C}$. CTR, meat from cattle fed a standard diet; OC1, meat from cattle fed enriched diet with destoned olive cake for 90 days; OC2, meat from cattle fed enriched diet with destoned olive cake for 60 days. Values are means plus standard deviations. ^{a-c}Within each day of storage, different superscripts indicate differences between dietary treatments ($P \leq 0.05$); ^{w-z}within each dietary treatment, different superscripts indicate differences between days of storage.

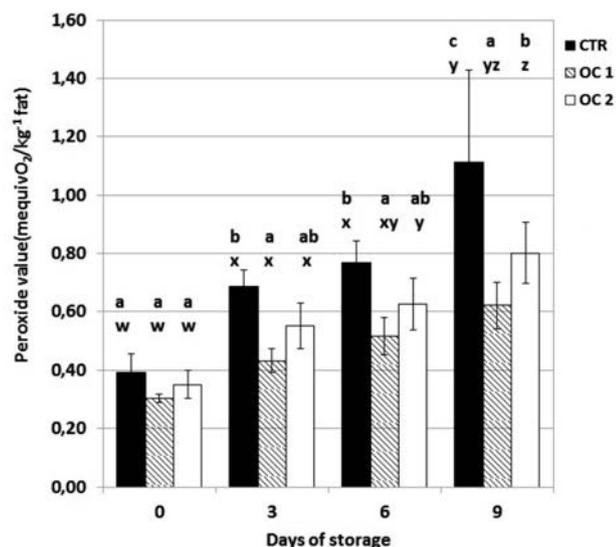


Figure 5. The effect of storage time on peroxide value (meq O₂·kg⁻¹ fat) of beef steak stored at 4±1°C. CTR, meat from cattle fed a standard diet; OC1, meat from cattle fed enriched diet with destoned olive cake for 90 days; OC2, meat from cattle fed enriched diet with destoned olive cake for 60 days. Values are means plus standard deviations. ^{a-c}Within each day of storage, different superscripts indicate differences between dietary treatments (P≤0.05); ^{w-z}within each dietary treatment, different superscripts indicate differences between days of storage.

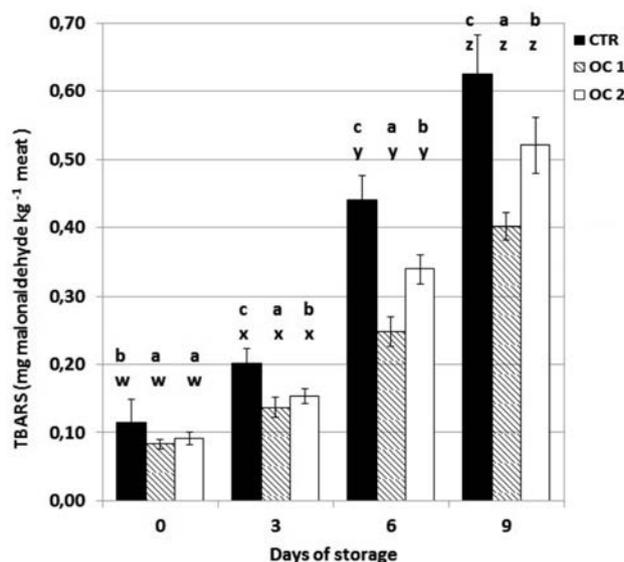


Figure 6. The effect of storage time on thiobarbituric reactive substances (mg MDA kg⁻¹ meat) of beef steak stored at 4±1°C. TBARS, thiobarbituric reactive substances; CTR, meat from cattle fed a standard diet; OC1, meat from cattle fed enriched diet with destoned olive cake for 90 days; OC2, meat from cattle fed enriched diet with destoned olive cake for 60 days. Values are means plus standard deviations. ^{a-c}Within each day of storage, different superscripts indicate differences between dietary treatments (P≤0.05); ^{w-z}within each dietary treatment, different superscripts indicate differences between days of storage.

time to develop off-odours (Table 1) was also influenced by phenolic compounds, thus confirming the results of other studies that showed that off-flavours of beef were due to aldehydes produced during lipid oxidation. The higher presence of polyphenol substances in animal tissues probably affects the stability of lipids during meat storage, resulting in the delayed development of off-odour (Descalzo *et al.*, 2008).

Phenolic compounds also positively contributed to the oxidative status of the meat of treated animals by reducing the level of PV and TBARS during storage in OC1 and OC2 meat, which was more evident in OC1 meat (Figures 5 and 6). Other authors have also reported the beneficial effects of olive polyphenols on the oxidative status of meat (Dal Bosco *et al.*, 2012; Luciano *et al.*, 2013), demonstrating their antioxidant effect in meat of animals fed olive phenolic compounds.

Conclusions

The results of the experiment demonstrated that olive polyphenols used in livestock diets could favourably extend the storage of meat through delaying lipid oxidation and preserving meat colour stability even though no antimicrobial effect was observed. Further

work will be needed to expand our understanding of the effect of these bioactive compounds in different packaging systems, influencing the shelf life of meat.

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