PAPER

EFFECTS OF DIETARY LINSEED AND SYNTHETIC OR NATURAL ANTIOXIDANTS ON SHELF-LIFE OF PORK

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ABSTRACT

The effects of including extruded linseed in pig diets supplemented with either polyphenol-rich red grape skin extract (3 g kg^{-1}) or synthetic antioxidants (200 mg kg^{-1} α-tocopheryl acetate plus 0.21 mg kg^{-1} of selenium) on shelf-life of pork stored in modified atmosphere packaging (MAP) at different oxygen concentrations (0 and 70%) were evaluated. Linseed reduced n-6/n-3 fatty acid ratio in lipids of backfat and loin. Color parameters, pH, weight losse, oxidative stability (TBARS), did not differ between antioxidants neither in raw, nor in cooked, nor in stored muscle. High oxygen concentration in MAP increased TBARS and ΔE, yielding redder meat.

Keywords: antioxidant, extruded linseed, fatty acid composition, grape skin, modified atmosphere packaging, pork

1. INTRODUCTION

In recent years, consumers' awareness of the cause-effect relationship between dietary fat composition and health has prompted research into modify omega-6/omega-3 polyunsaturated fatty acids (PUFA) ratio in meat and meat products (CORINO *et al*., 2008; RILEY *et al*., 2000). It is known that in Western countries omega-6 fatty acids are the majority of PUFA in the food supply, whereas the consumption of omega-3 fatty acids is very low (SIMOPOULOS, 2002).

Among the factors that can affect the deposition of lipids and their fatty acid (FA) composition in pig meat, diet composition and slaughter weight play a pivotal role (LO FIEGO *et al*., 2010; WOOD *et al*., 2008). Thus pork can be a source of omega-3 PUFA whether pigs are fed with diets containing linseed or its by-products, feeds rich in α-linolenic acid (C18:3n-3, ALA) (CORINO *et al*., 2014; GUILLEVIC *et al*., 2009b; HOZ *et al*., 2003; MUSELLA *et al*., 2009).

On the other hand, the enrichment in PUFA can negatively affect the oxidative stability of pork. Lipid oxidation impairs the acceptability of meat modifying sensory attributes and nutritional quality (JAKOBSEN and BERTELSEN, 2000). To prevent the oxidative phenomena, synthetic antioxidants are usually added in pig diets, vitamin E, as α-tocopheryl acetate, being among the most common (DUNSHEA *et al*., 2005). Recently, consumers have shown an ever-increasing interest in natural antioxidants obtained from plant sources (HAAK *et al*., 2008; LORENZO *et al*., 2014). Winery by-products are rich in phenolic compounds whose antioxidant activity has been widely studied (BRENES *et al*., 2016). Further, anti-inflammatory, anti-carcinogenic, cardioprotective and vasodilatory properties have been ascribed to polyphenols (TEIXEIRA *et al*., 2014). In the Po Valley, where Italian pig production is concentrated, the cultivation of the grapevine is also widespread. Wine industry, like many other sectors of the Italian agri-food industry, produces a lot of waste products with consequent environmental problems. For instance, wastes represent almost one third of the total of the grapes used in wineries, posing serious storage and disposal problems (TEIXEIRA *et al*., 2014). The commitment to sustainable agriculture requires the reduction or elimination of these wastes. In light of all this, stakeholders of the pig industry have shown a strong interest in the possibility of utilize these by-products in the feeding of pigs. Nonetheless, the effect of grape skin extract in pig feeding on pork quality still needs to be elucidated (BRENES *et al*., 2016).

Usually fresh meat is commercialized packed in expanded polystyrene trays wrapped with stretchable cling films, characterized by high oxygen transmission rate, in order to maintain the typical bright color. In these conditions, fresh meat has a very short shelf-life due to microbial proliferation and a rapid brown discoloration (due to metmyoglobin formation) that normally takes place before unacceptable bacterial growth has occurred. In order to further increase the shelf-life of meats modified atmospheres packaging (MAP), usually characterized by variations in the content of N_2, CO_2 and O_2 in the package, is extensively used (SPANOS *et al*., 2016). Modified atmosphere packaging in an atmosphere high in oxygen (up to 80%) extends display life, prolonging the stability of the oxymyoglobin red pigments, but it promotes lipid oxidation (MCMILLIN, 2017).

The aims of this study were to investigate the effect of inclusion of extruded linseed in pig diets on carcass lipid fatty acid composition and to evaluate the protective effect of either supranutritional doses of vitamin E and selenium (Se) or grape skin extracts on the quality and shelf-life of pork packaged in modified atmospheres with or without oxygen in the inner gaseous atmosphere, during refrigerated storage.

2. MATERIALS AND METHODS

2.1. Animals and diets

All the experimental procedures were in accordance to the Italian legislation (D.Lgs 4 Marzo 2014 n. 26 art. 2 punto F).
Twelve castrated male Italian Large White pigs, evenly divided according to weight into three groups of four subjects each, were housed in 9 m^2 concrete floored pens. Starting from 79.4±7.4 kg body weight (BW) and till slaughtering, at 135.4±9.7 kg BW, the subjects were fed restricted for 70 days on 8.5% of metabolic weight ($BW^{0.75}$) either on a barley-soya bean meal feed (C group) or the same feed where 5% barley was substituted with 5% extruded linseed. Linseed diets were supplemented either with supranutritional levels of synthetic antioxidants (200 ppm of α-tocopheryl acetate and 0.21 ppm of Se (LE group) or with 3 g kg^{-1} feed of red grape skin extract (Enocianina Fornaciari, Reggio Emilia, Italy) (LGSE group), providing 29.8 ppm of polyphenols (expressed as gallic acid equivalent). Grape skin extract is a natural product used in the food industry as a supplement, nutraceutical or food coloring, included in finished products at concentrations ranging from 2 to 4g kg^{-1}, according to the manufacturer's suggestions, that we complied with.
The diets were isoenergetic and isoproteic and with the same lysine/digestible energy ratio. Water was always available through nipple drinkers and the diet was distributed in liquid form (water:feed ratio 3:1). Grape skin extract was diluted in the water of the diet. Composition of experimental diets and their fatty acid proportions are shown in Table 1.

2.2. Slaughtering and sampling

The pigs were slaughtered on the same day in a commercial slaughterhouse, where the subjects were electrically stunned, in agreement with the Council Regulation (EC) No 1099/2009 on the protection of animals at the time of the killing.
After slaughtering, each carcass was graded using Fat-o-Meater, at level of 3/4 last rib, at 8 cm from the splitting line of the carcass (D.M. Mipaaf October 24, 2018 - GU n. 298, December 24, 2018) and dissected in commercial cuts. At dissection the whole left *Longissimus thoracis et lumborum* (LTL) muscle and a sample of subcutaneous adipose tissue at the last rib level, were removed from each carcass. The samples were stored at +4°C and sent to the laboratory for subsequent analyses.
At the lab, 24 h *post mortem*, individual LTL muscles were sliced and, on a subsample taken at the last rib, some physical parameters and oxidative stability were determined as described below. A second subsample of LTL muscle, destined to chemical analyses, was vacuum packed (Elegen, Scandiano, Reggio Emilia, Italy) and stored at -20°C. Eventually, six slices for each LTL muscle were packaged in modified atmosphere for the shelf-life study. Individual backfat samples were vacuum packed and stored at -20°C for the subsequent fatty acid composition determination.

2.3. Physical parameters of raw and cooked muscle

The values of pH were measured at 24 h *post mortem* using a portable Crison pH-meter equipped with a Xerolite electrode (Crison Instruments, Alella, Spain). The pH probe was calibrated using two buffers (pH 4.0 and 7.0).

Table 1. Composition of experimental diets (as fed basis).

		Diet[a]		
		C	LE	LGSE
Ingredients				
Extruded linseed	%	0.0	5.0	5.0
Barley	%	89.5	84.4	84.5
Solvent extracted soybean meal	%	7.0	7.0	7.0
L-Lysine	%	0.3	0.3	0.3
L-Threonine	%	0.1	0.1	0.1
Calcium carbonate	%	1.2	0.8	1.2
Dicalcium phosphate	%	1.0	1.0	1.0
Sodium chloride	%	0.4	0.4	0.4
Minerals and vitamins premix[b]	%	0.5	0.5	0.5
Vitamin E + selenium[c]	%	0.0	0.5	0.0
Red grape skin extract	%	0.0	0.0	0.3
Calculated nutrient composition[d]				
Digestible energy (DE)	Kcal/kg	3082	3151	3154
Calcium	%	0.78	0.76	0.80
Phosphorus	%	0.52	0.53	0.53
Digestible phosphorus	%	0.24	0.25	0.25
Lysine	%	0.85	0.87	0.87
Digestible lysine	%	0.73	0.75	0.75
Lysine/DE ratio	g/Mcal	2.75	2.75	2.75
Analyzed composition [e]				
Dry matter	%	90.5	90.6	90.7
Crude protein	% as fed	13.2	13.7	13.6
Crude fat	"	1.6	3.5	3.5
Crude fiber	"	5.0	5.0	4.9
ADF	"	7.0	6.8	7.0
NDF	"	20.6	19.6	20.8
ADL	"	1.3	1.2	1.4
Crude ash	"	5.2	5.1	5.0
FAs[f] composition (% of total FAs)				
C 14:0 (myristic)		0.59	0.26	0.32
C 16:0 (palmitic)		27.53	15.94	16.70
C 16:1 (palmitoleic)		0.08	0.02	0.02
C 18:0 (stearic)		1.30	2.86	2.79
C 18:1n-9 (oleic)		12.47	16.10	15.96
C 18:2n-6 (linoleic)		52.16	35.50	35.44
C 18:3n-3 (α-linolenic)		5.81	29.24	28.65
C 20:1 (eicosenoic)		0.06	0.04	0.11

[a]C, control (Extruded linseed, 0 g kg^{-1}); LE, (Extruded linseed, 50 g kg^{-1}, vitamin E (α-tocopheryl acetate), 200 mg and Se 0.21 mg kg^{-1}); LGSE, (Extruded linseed, 50 g kg^{-1}, red grape skin extract 3 g kg^{-1}). [b]The vitamins and minerals of the diet provided by premix (kg^{-1}): vitamin A, 15,000 UI; vitamin D3, 2000 IU; vitamin E (α-tocopheryl acetate), 50 mg; vitamin K, 2.5 mg; vitamin B1, 2.0 mg; vitamin B2, 5.0 mg; calcium D-pantothenate, 15.0 mg; niacin, 25.0 mg; vitamin B12, 0.036 mg; vitamin B6, 4.0 mg; folic acid, 1.0 mg; biotin, 0.15 mg; choline chloride, 346.0 mg; Zn (ZnO), 100.0 mg; Cu ($CuSO_4$), 15.0 mg; Mn (MnO), 25.0 mg; Fe ($FeSO_4$), 150.0 mg; I ($Ca(IO_3)_2$), 1.5 mg; Co ($CoCO_3$), 0.4 mg; Se (Na_2SeO_3), 0.1 mg. [c]Providing vitamin E (α-tocopheryl acetate) 200 mg and Se 0.21 mg kg^{-1} as fed, supported on $CaCO_3$. [d]According to Sauvant *et al.* (2004). [e]According to the Association of Official Analytical Chemists (1995). [f]Fatty acids

The sample surface color was determined using a Minolta CM-600d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan) with a window diameter of 8 mm and D65 as illuminant source. Before color measuring, the spectrophotometer was calibrated against a white plate supplied by the manufacturer. Color measurements complied the CIELAB color system, where the three fundamental coordinates are L*- "lightness", a*- "redness", b*- "yellowness" values. Further, Chroma (C), referred to as saturation index and color intensity, was calculated as: $(a^{*2}+b^{*2})^{0.5}$, and hue angle (h), spectral color, was calculated as follows: $\tan^{-1}(b^*/a^*)$. The average values were the mean of three measurements in different areas of the surface of the sample, avoiding the zones of visible fat. Drip loss was determined according to HONIKEL (1998), slightly modified. A slice of fresh muscle (about 100 g) was hooked and then suspended in an inflated bag, ensuring that the sample did not make contact with the bag. The weight loss percentage after 48 h of storage at 4°C was calculated. Cooking loss was calculated as weight difference between raw and cooked samples. In brief, slices of about 2.5 cm thickness, weighing approximately 100 g, placed in vacuum plastic bags, were put in water-bath till the core temperature reached 70°C. The internal temperature of samples was controlled during cooking with a temperature probe. The samples were weighed after cooling. Cooking losses were expressed as a percentage of the initial sample weight.
The Warner–Bratzler shear force (WBSF) was determined on the cooked samples. The samples were cut, parallel to the longitudinal orientation of the muscle fibres according to HONIKEL (1998) method, into six cylindrical cores (Ø 1.50 cm). Each core was sheared with a WBSF device attached to a Zwick Z50 kN Testing Machine (model BT1-FB050TN, Zwick Roell, Kennesaw, GA USA) with a 1kN load cell equipped with the V-shaped blade with a triangular hole of 60° at a speed of 250 mm/min. The peak force (average value of 6 measurements for each sample) was expressed as kg.

2.4. Chemical composition of muscle

After thawing, samples of LTL muscle were analyzed in duplicate to determine the moisture, ether extract with previous acid hydrolysis, and crude protein, according to the methods of the Association of Analytical Chemists (AOAC, 1995). Results were expressed as percentage of wet matter.

2.5. Fatty acid profile of backfat and muscle

Fatty acid (FA) profile of lipids in thawed samples of subcutaneous adipose tissue and LTL muscle was determined using a TRACE™GC Ultra (Thermo Electron Corporation, Rodano, Milano, Italy) equipped with a Flame Ionization Detector, a PTV injector, and a TR-FAME Column (Thermo Scientific, Rodano, Milano), 30 m long, 0.25 mm i.d., 0.2 µm film thickness. Total lipids were extracted from the samples of subcutaneous adipose tissue (IUPAC, 1979) and from LTL muscle (FOLCH *et al*., 1957). Then, an aliquot of 25 mg was subjected to methylation by means of a methanolic solution of potassium hydroxide (KOH 2N) according to FICARRA *et al*. (2010), using tridecanoic acid (C13:0) (Larodan Fine Chemicals AB, Malmö, Sweden) as internal standard. The injection of the fatty acid methyl ester sample (1 µL) was performed in split mode with a split flow of 10 mL/min, operating at a constant flow of 1 mL/min of helium as carrier gas. The temperature of injector and detector was kept at 240°C. The temperature program used for the analysis started from 140°C, was maintained for 2 min, then increased to 250°C, at a rate of 4°C min^{-1}, and kept at this temperature for 5 min. The peaks of the fatty acid methyl esters

(FAMEs) were recorded and integrated using Chrom-Card software (vers. 2.3.3, Thermo Electron Corporation, Rodano, Milano, Italy) and identified by comparison with the retention times of standard solutions with known quantities of various methyl esters (Supelco® 37 Component FAME mix, PUFA standard n.2, Animal Source, Supelco, Bellafonte, PA, USA and single FAMEs standard, Larodan Fine Chemicals AB, Malmö, Sweden). For quantification purposes, the response factor was calculated, and the method of the internal standard was used. The amount of each FAME in the sample was expressed as FAME relative percentage with respect to the total amount of FAMEs. Iodine value (IV) of backfat was calculated adopting the equation proposed by LO FIEGO *et al*. (2016): IV = 85.703 + [C14:0] x 2.740 - [C16:0] x 1.085 - [C18:0] x 0.710 + [C18:2n-6] x 0.986.

2.6. Oxidative stability of raw and cooked muscle

Oxidative stability was evaluated by the 2-thiobarbituric acid reactive substances (TBARS) measurements according to SIU and DRAPER (1978). In detail, each sample of muscle was minced and an aliquot of 2.5 g was homogenized in 12.5 mL of distilled water at 9500 rpm for 2 min, using an Ultra-Turrax tissue homogenizer (IKA, Germany), and then vortexed for 1 min at high speed. Samples were centrifuged for 20 min at 2000 rpm at 4°C with 12.5 mL of 10% trichloroacetic acid (TCA) (Sigma-Aldrich, Milan, Italy) and the supernatant decanted through a paper filter (Whatman 541). Four mL of clear filtrate were transferred into 15 mL pyrex screw cap test tubes and added of 1 mL of 0.06M 2-thiobarbituric acid (TBA). A distilled water-TCA-TBA reagent blank was prepared and treated like the samples. The samples were heated in a water bath at 80°C for 90 min and then cooled. Absorbance at 532 nm was measured against a blank sample on two replicates of each sample on a Jasco spectrophotometer (Model V550, UV/VIS, Tokyo, Japan) immediately after cooling. TBARS were expressed as mg of malondialdehyde (MDA) per kg of muscle using tetraethoxypropane (TEP) (Sigma-Aldrich, Milan, Italy) as a standard.

2.7. Packaging and Shelf-life study

From each loin, 6 slices (approximately 2-2.5 cm thick), designated for the evaluation of the shelf-life in two different modified atmospheres, were individually weighed and packed in a total of 72 high barrier trays lidded with a PET/EVOH/PE film (AERPACK System, kindly supplied by Coopbox Group, Italy). The whole package oxygen transmission rate (OTR) was < 0.1 cm^3 day^{-1} (air, 25°C). MAP was performed using a semiautomatic vacuum compensation thermosealing machine (Ca.Ve.Co, Italy). Two different gaseous mixtures were used: 70%N_2/30%CO_2 (N_2) and 70%O_2/30%CO_2 (O_2). Gas composition of the headspace was analysed before opening packages using a HWD-GC equipped with a concentric CRT I column (6ft x 1/4"; outer column: activated molecular sieve; inner column: porous polymer mixture) (Alltech Italia, S.r.l, Italy). Gascromatographic conditions: gas carrier helium (65 mL min^{-1}); temperature 55°C; analysis time 5 min. A septum was glued onto the surface of the lid and a 50 mL gas aliquot was withdrawn with a gastight syringe and injected in the gascromatograph. The calibration was performed by injecting separately pure gases as external standard (supplied by Sapio; pureness 99.9) and calculating the response factor for each one.
All samples packaged in modified atmosphere were stored in the dark at 3±1°C for a maximum of 8 days. Twenty-four trays (12 subjects x 2 different modified atmospheres) were removed from the refrigerators at day 4, 6 and 8 of storage, weighed to calculate weight losses and then submitted to color, pH and TBARS determination, as described

above (Sections 2.3 and 2.6). Color measurements were carried out after allowing a 30 min blooming, following pack opening. Moreover, overall color variation (ΔE) was calculated as $(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}$ where ΔL^*, Δa^* and Δb^* are the difference between time 0 (at 24 h *post mortem*) and the values L^*, a^* and b^* respectively at 4 (ΔE_{0_4}), 6 (ΔE_{0_6}) and 8 (ΔE_{0_8}) days of refrigerated storage.

2.8. Statistical analysis

Statistical analysis was performed by means of analysis of variance using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). The statistical model for performance, carcass characteristics, qualitative characteristics and chemical composition of fresh LTL muscle, fatty acid composition of subcutaneous adipose tissue and LTL muscle, used dietary treatments (DT) (C, LE and LGSE) as independent variables.
The data from samples stored in MAP were statistically analyzed within storage day including in the model also the packaging atmosphere (P) (O_2 or N_2) and the interaction DTxP. The interaction was never statistically significant (P>0.05) and was thus eliminated from the model.
The two degrees of freedom of dietary treatments were *a priori* splitted into two orthogonal contrasts comparing, respectively, control group *vs* average of extruded linseed groups (C *vs* LE+LGSE/2) and antioxidant supplemented groups between them (LE *vs* LGSE).

3. RESULTS

3.1. Performance and carcass characteristics

No effect of dietary treatments (DT) was found on farm performances (average daily gain 0.8±0.1 kg, slaughter weight 135.4±9.7 kg) and carcass characteristics (carcass weight 113.7±8.3 kg, dressing percentage 83.9±1.2, backfat thickness 24.3±3.3 mm and lean meat content 54.3±2.1%) (data not reported in Tables). GUILLEVIC *et al*. (2009a) and HAAK *et al*. (2008), feeding linseed to finishing light weight pigs, could not detect either any difference in on farm and abattoir performances.

3.2. Fresh meat quality

The effect of DT on qualitative characteristics of meat is reported in Table 2.
According to CORINO *et al*. (2014), muscle pH is not affected by linseed feeding. Our results confirm the inferences of their meta-analysis. The control group showed a slightly higher b* and Chroma values (P <0.05), denoting a greater yellowness and color intensity than linseed groups. CORINO *et al*. (2008) did not find significant differences related to the values of b* in pig fed with linseed. While our Chroma values agree with data reported by JUÁREZ *et al*. (2001), but there was no obvious explanation for this trend, therefore it requires further investigation. No difference was found between LE and LGSE groups. Further, drip and cooking losses, oxidative stability in raw and cooked muscle, shear force and chemical composition (moisture, crude protein and lipid contents of LTL muscle) were affected neither by linseed nor by the antioxidants. CORINO *et al*. (2008) and HAAK *et al*. (2008) found that linseed inclusion did not influence these qualitative traits in meat of light pigs either. Moreover, BOLER *et al*. (2009) reported that vitamin E reduces lipid

oxidation but has no effect on any carcass characteristics and loin quality. Eventually, the lack of effects of linseed feeding on chemical parameters of the muscle (moisture, crude protein and lipid contents) confirms the findings of HOZ *et al*. (2003) on tenderloin.

Table 2. Qualitative characteristics and chemical composition of *Longissimus thoracis et lumborum* muscle from pigs fed with the experimental diets.

Items	Dietary treatment[a]			P-value		R-MSE[b]
	C (n=4)	LE (n=4)	LGSE (n=4)	C *vs* (LE+LGSE)/2	LE *vs* LGSE	
pH *post mortem* (24h)	5.58	5.53	5.59	0.679	0.236	0.067
L*	55.83	56.26	55.70	0.878	0.616	1.526
a*	1.97	0.74	0.56	0.127	0.847	1.284
b*	12.88	12.08	11.18	0.044	0.177	0.870
Chroma	13.13	12.15	11.20	0.049	0.232	1.049
Hue angle	81.98	86.75	87.17	0.142	0.908	5.047
Drip loss (%)	3.46	3.13	3.39	0.763	0.731	1.032
Cooking loss (%)	17.05	19.78	16.98	0.358	0.110	2.236
TBARS[c] (raw muscle)	0.045	0.069	0.091	0.134	0.396	0.034
TBARS[c] (cooked muscle)	0.386	0.405	0.279	0.698	0.341	0.178
Shear force (Kg)	4.99	4.87	4.85	0.669	0.957	0.471
Chemical composition (%)						
Moisture	72.30	71.96	72.04	0.544	0.896	0.785
Crude protein	23.39	23.16	23.44	0.845	0.575	0.688
Lipids	1.36	1.74	1.27	0.571	0.145	0.418

[a]C, control (Extruded linseed, 0 g kg^{-1}); LE, (Extruded linseed, 50 g kg^{-1}; vitamin E (α-tocopheryl acetate), 200 mg and Se 0.21 mg kg^{-1}); LGSE, (Extruded linseed, 50 g kg^{-1}; red grape skin extract 3 g kg^{-1}).

[b]Root Mean Square Error.

[c]TBARS (thiobarbituric acid reactive substances) expressed in mg of malondialdehyde (MDA) per kilogram of muscle.

3.3. Adipose tissue and intramuscular fatty acid composition

Table 3 shows lipid content and fatty acid composition of subcutaneous adipose tissue.
No differences attributable to extruded linseed dietary inclusion or type of antioxidant were found for lipid content of backfat. The proportion of total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids was not affected by the inclusion of linseed in the diet, in agreement with MUSELLA *et al*. (2009) who detected no difference in the percentage of main FA classes in ham covering trimmed fat between control and linseed-fed pigs.
The total content of n-6 PUFA, although tendentially lower in linseed fed subjects, was not significantly influenced by dietary treatments (P>0.05) either. This is likely due to the limited number of experimental units. Overall, among n-6 PUFA and MUFA, only γ-linolenic (C18:3n-6) and heptadecenoic acids (C17:1) were affected by dietary linseed inclusion, which brought about a significant (P<0.05) reduction of their proportions. Conversely, n-3 PUFA increased significantly (P<0.01) with linseed dietary inclusion. This confirms the findings of RILEY *et al*. (2000). The increase of the proportion of the total n-3 PUFA is ascribable to the higher proportions (P<0.01) of α-linolenic (ALA, C18:3n-3) and eicosatrienoic (C20:3n-3) acids which trebled and docosapentaenoic acid (DPA, C22:5n-3)

that doubled, whereas eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) remained unchanged. GUILLEVIC *et al*. (2009b) could not find any correlation between ALA intake and the proportion of DHA in adipose tissues either. RILEY *et al*. (2000) hypothesized that the limited accumulation of longer chain PUFA in adipose tissues could be due to scant capacity for the synthesis of these products starting from dietary ALA.

Table 3. Lipid content (%) and fatty acid profile (% of total fatty acids) of backfat from pigs fed with the experimental diets.

Items	Dietary treatment[(a)]			P-value		R-MSE[(b)]
	C (n=4)	LE (n=4)	LGSE (n=4)	C *vs* LE+LGSE/2	LE *vs* LGSE	
Lipids	86.14	86.56	87.55	0.752	0.765	4.560
Fatty acids:						
C 10:0 (capric)	0.13	0.10	0.10	0.407	0.988	0.053
C 12:0 (lauric)	0.07	0.05	0.07	0.591	0.328	0.027
C 14:0 (myristic)	1.16	1.15	1.18	0.975	0.659	0.093
C 16:0 (palmitic)	24.66	24.59	24.39	0.735	0.731	0.785
C 17:0 (heptadecanoic)	0.35	0.32	0.30	0.338	0.673	0.055
C 18:0 (stearic)	15.32	14.95	15.47	0.843	0.437	0.896
C 20:0 (eicosanoic)	0.15	0.26	0.22	0.103	0.501	0.076
C 16:1 (palmitoleic)	1.62	1.51	1.57	0.553	0.674	0.208
C 17:1 (heptadecenoic)	0.32	0.26	0.24	0.016	0.517	0.040
C 18:1n-7 (vaccenic)	1.51	1.39	1.65	0.547	0.420	0.281
C 18:1n-9 (oleic)	39.47	38.08	39.30	0.956	0.220	2.040
C 20:1 (eicosenoic)	0.67	0.83	0.81	0.075	0.828	0.126
C 18:2n-6 (linoleic)	12.64	11.60	10.46	0.236	0.458	2.070
C 18:3n-3 (α-linolenic)	0.84	3.26	2.74	0.000	0.103	0.406
C 18:3n-6 (γ-linolenic)	0.09	0.05	0.07	0.026	0.152	0.022
C 20:2n-6 (eicosadienoic)	0.42	0.54	0.50	0.196	0.605	0.116
C 20:3n-3 (eicosatrienoic)	0.12	0.43	0.43	0.000	0.906	0.089
C 20:4n-6 (arachidonic)	0.30	0.25	0.26	0.323	0.859	0.070
C 20:5n-3 (eicosapentaenoic)	0.00	0.15	0.00	0.486	0.254	0.170
C 22:2n-6 (docosadienoic)	0.00	0.00	0.00	0.377	0.466	0.004
C 22:4n-6 (docosatetraenoic)	0.07	0.05	0.04	0.127	0.434	0.027
C 22:5n-3 (docosapentaenoic)	0.09	0.18	0.20	0.000	0.583	0.028
C 22:6n-3 (docosahexaenoic)	0.02	0.02	0.02	0.864	0.510	0.016
Total Saturated	41.83	41.40	41.72	0.719	0.717	1.185
Total Monounsaturated	43.58	42.06	43.57	0.587	0.363	2.224
Total Polyunsaturated	14.60	16.54	14.72	0.523	0.338	2.546
Total n-6	13.53	12.49	11.33	0.257	0.470	2.183
Total n-3	1.07	4.05	3.39	0.000	0.088	0.484
n-6/n-3 fatty acid ratio	12.64	3.09	3.37	0.000	0.622	0.780
Iodine Value[(c)]	63.72	62.98	61.79	0.410	0.520	2.511

[(a)]C, control (Extruded linseed, 0 g kg^{-1}); LE, (Extruded linseed, 50 g kg^{-1}; vitamin E (α-tocopheryl acetate), 200 mg and Se 0.21 mg kg^{-1}); LGSE, (Extruded linseed, 50 g kg^{-1}; red grape skin extract 3 g kg^{-1}).

[(b)]Root Mean Square Error.

[(c)]IV=85.703 + [C14:0] x 2.740 - [C16:0] × 1.085 - [C18:0] × 0.710 + [C18:2n-6] × 0.986

On the whole, these trends led to a significant ($P<0.01$) reduction in the n-6/n-3 PUFA ratio, as was previously observed also by MUSELLA *et al*. (2009) and RILEY *et al*. (2000). In our study, the n-6/n-3 PUFA ratio dropped from 12.6 in control pigs to less than 3.5 in linseed fed pigs. Thus, the inclusion of 5% extruded linseed in the finishing diet enabled to bring this ratio far below the threshold indicated by SIMOPOULOS (2008) to avoid adverse health consequences, without impairing technological parameters of subcutaneous adipose tissue. In fact, most Authors (e.g. LEBRET and MOUROT, 1998; LO FIEGO *et al*., 2005) indicate, as a guarantee of good preservation aptitude, contents of stearic acid (C18:0) and linoleic acid (C18:2n-6) higher than 12 and lower than 15%, respectively and an Iodine value minor than 70.
As showed in Table 3, and as expected, no difference was found between the linseed fed groups (LE *vs* LGSE) for any of the parameters taken into account.
Table 4 shows the fatty acid composition in LTL muscle. In general, the variations observed in FA compositions resembled what already seen in the backfat tissue. In fact, total SFA, MUFA and PUFA percentages did not differ among dietary treatments ($P>0.05$). Compared to C, linseed groups showed a higher content of lauric acid (C12:0; $P<0.01$) and lower in vaccenic acid (C18:1n-7; $P<0.05$). No hypothesis could be put forward to explain these variations that, though trivial, were statistically significant.
Total n-6 PUFA content, as in the backfat depot, although is tendentially higher in C group, was not affected by the dietary treatment and the only significant variation was shown by the docosatetraenoic acid (C22:4n-6; $P<0.01$), which resulted higher in the C group. The same trend was observed by RILEY *et al*. (2000). The total n-3 PUFA proportion, as seen in the backfat, was significantly higher ($P<0.01$) in the linseed groups. In detail, α-linolenic, eicosatrienoic, and eicosapentaenoic acids ($P<0.01$) more than tripled. Thus, EPA that did not change with diet in adipose tissue, increased in LTL muscle. This agrees with the results of CORINO *et al*. (2008), who observed that EPA is preferentially stored in the muscle rather than in the adipose tissue, and RILEY *et al*. (2000), who inferred that α-linolenic acid intake elicits eicosapentaenoic acid increments more in muscle than in adipose tissue. Hence, also in the muscle, the n-6/n-3 PUFA ratio was significantly reduced ($P<0.01$) to one-third in linseed fed pigs.
Not even in this tissue, except for the proportion of lauric acid, any variation was found between the two different dietary antioxidants, for any of the parameters taken into account.

3.4. Quality characteristics of meat stored in MAP

The analysis of the inner gaseous atmosphere composition of the experimental samples showed that the relative percentages of the gases have not changed throughout the storage time (data not shown). This result is not unexpected, because of the refrigerated (3±1°C) and short storage time length (8 days), the high barrier materials used, which strongly limits the gas transfers in and out the packages, and the bacteriostatic activity of CO_2 that, slowing the microbial growth, avoids oxygen consumption and as a consequence its decrease. In these conditions, all the modifications registered on the meat samples can be attributed only to the presence or absence of oxygen in the atmosphere surrounding the product and to meat composition.
The effects of dietary treatments and of gaseous mixtures in packaging during the 8 days of refrigerated storage on various physico-chemical characteristics of LTL muscle are shown in Table 5. The dietary treatments influenced most of the parameters studied.

Linseed groups exhibited lower weight losses; however, the reduction was significant (P<0.05) only on days 4 and 6.

Table 4. Fatty acid profile (% of total fatty acids) of *Longissimus thoracis et lumborum* muscle from pigs fed with the experimental diets.

Items	Dietary treatment[(a)]			P-value		R-MSE[(b)]
	C (n=4)	LE (n=4)	LGSE (n=4)	C *vs* LE+LGSE/2	LE *vs* LGSE	
C 10:0 (capric)	0.11	0.29	0.30	0.355	0.993	0.306
C 12:0 (lauric)	0.05	0.16	0.11	0.000	0.019	0.024
C 14:0 (myristic)	1.07	1.18	1.13	0.343	0.646	0.140
C 16:0 (palmitic)	23.11	23.85	23.48	0.442	0.656	1.117
C 17:0 (heptadecanoic)	0.12	0.14	0.21	0.303	0.185	0.076
C 18:0 (stearic)	13.41	13.50	13.10	0.780	0.381	0.616
C 20:0 (eicosanoic)	0.12	0.12	0.13	0.871	0.822	0.020
C 16:1 (palmitoleic)	2.81	2.99	2.73	0.801	0.261	0.304
C 17:1 (heptadecenoic)	0.07	0.15	0.18	0.062	0.560	0.075
C 18:1n-7 (vaccenic)	3.30	2.84	2.74	0.038	0.686	0.345
C 18:1n-9 (oleic)	39.39	39.79	40.68	0.612	0.646	2.639
C 20:1 (eicosenoic)	0.59	0.52	0.55	0.249	0.618	0.071
C 18:2n-6 (linoleic)	10.52	9.07	9.03	0.276	0.976	2.069
C 18:3n-3 (α-linolenic)	0.40	1.27	1.37	0.000	0.540	0.218
C 18:3n-6 (γ-linolenic)	0.08	0.09	0.13	0.350	0.352	0.057
C 20:2n-6 (eicosadienoic)	0.21	0.19	0.19	0.380	0.971	0.040
C 20:3n-3 (eicosatrienoic)	0.05	0.16	0.18	0.001	0.481	0.043
C 20:4n-6 (arachidonic)	3.53	2.49	2.50	0.093	0.991	0.897
C 20:5n-3 (eicosapentaenoic)	0.07	0.35	0.36	0.004	0.954	0.120
C 22:2n-6 (docosadienoic)	0.01	0.01	0.01	0.167	0.600	0.004
C 22:4n-6 (docosatetraenoic)	0.51	0.19	0.26	0.006	0.497	0.132
C 22:5n-3 (docosapentaenoic)	0.39	0.52	0.60	0.227	0.599	0.214
C 22:6n-3 (docosahexaenoic)	0.08	0.12	0.04	0.992	0.255	0.095
Total Saturated	38.00	39.23	38.46	0.364	0.469	1.452
Total Monounsaturated	46.16	46.29	46.88	0.811	0.776	2.824
Total Polyunsaturated	15.85	14.47	14.67	0.574	0.941	3.574
Total n-6	14.85	12.04	12.11	0.173	0.977	3.064
Total n-3	1.00	2.43	2.56	0.002	0.755	0.551
n-6/n-3 fatty acid ratio	15.33	4.99	4.76	0.000	0.827	1.438

[(a)]C, control (Extruded linseed, 0 g kg^{-1}); LE, (Extruded linseed, 50 g kg^{-1}; vitamin E (α-tocopheryl acetate), 200 mg and Se 0.21 mg kg^{-1}); LGSE, (Extruded linseed, 50 g kg^{-1}; red grape skin extract 3 g kg^{-1}).
[(b)]Root Mean Square Error.

The comparison between the antioxidants added to the linseed diets did not reveal significant differences (P>0.05) in this parameter.
In any time-lapse interval considered, the weight loss was unaffected by the packaging atmosphere.
The pH of LTL muscle ranged from 5.46 to 5.55 during the 8 days storage. These values are quite common in medium-heavy pigs. Dietary linseed inclusion did not affect the pH values, whereas grape skin extract addition yielded higher pH values than synthetic

antioxidant at day 6 (P< 0.01). However, in absence of a definite trend, this difference can be attributed to inter-animal variation. Our results conflict with the findings of LORENZO *et al*. (2014), who found that the addition of natural antioxidants, derived from grape seed extract, lowered the value of pH throughout storage. This difference is likely to be ascribable to the different origin of the grape extracts utilized (seed or skin) and, especially, to the fact that in the present work the extract was added to the diet of the pigs and not into the meat.

Table 5. Effect of the diet and packaging on the weight loss (%), pH, TBARS values and color variation (ΔE), measured on *Longissimus thoracis et lumborum* muscle, refrigerated 8 days (3 ± 1°C).

Items	Dietary treatment[(a)]			Packaging		P-value	P-value		R-MSE[(b)]
	C (n=24)	LE (n=24)	LGSE (n=24)	O_2 (n=36)	N_2 (n=36)	O_2 *vs* N_2	C *vs* (LE+ LGSE/2)	LE *vs* LGSE	
Day 4									
Weight loss %	3.67	2.70	2.84	2.86	3.28	0.233	0.021	0.737	0.834
pH	5.52	5.54	5.54	5.55	5.51	0.003	0.116	0.641	0.031
TBARS[(c)]	0.138	0.143	0.131	0.159	0.115	<0.001	0.932	0.186	0.016
ΔE0_4[(d)]	3.58	3.83	3.94	5.30	2.27	0.001	0.724	0.911	1.978
Day 6									
Weight loss %	4.57	3.07	3.87	3.39	4.29	0.076	0.043	0.190	1.178
pH	5.46	5.46	5.50	5.47	5.49	0.059	0.083	0.008	0.026
TBARS[(c)]	0.078	0.032	0.044	0.103	0.001	<0.001	0.015	0.497	0.035
ΔE0_6[(d)]	3.81	4.23	3.30	5.12	2.44	<0.001	0.953	0.271	1.644
Day 8									
Weight loss %	5.13	4.22	4.78	4.44	4.98	0.314	0.272	0.396	1.286
pH	5.51	5.51	5.53	5.53	5.50	0.007	0.309	0.230	0.026
TBARS[(c)]	0.157	0.091	0.058	0.177	0.027	0.021	0.208	0.656	0.147
ΔE0_8[(d)]	3.88	4.17	3.75	5.33	2.53	0.001	0.922	0.641	1.761

[(a)]C, control (Extruded linseed, 0 g kg^{-1}); LE, (Extruded linseed, 50 g kg^{-1}; vitamin E (α-tocopheryl acetate), 200 mg and Se 0.21 mg kg^{-1}); LGSE, (Extruded linseed, 50 g kg^{-1}; red grape skin extract 3 g kg^{-1}).

[(b)]Root Mean Square Error;

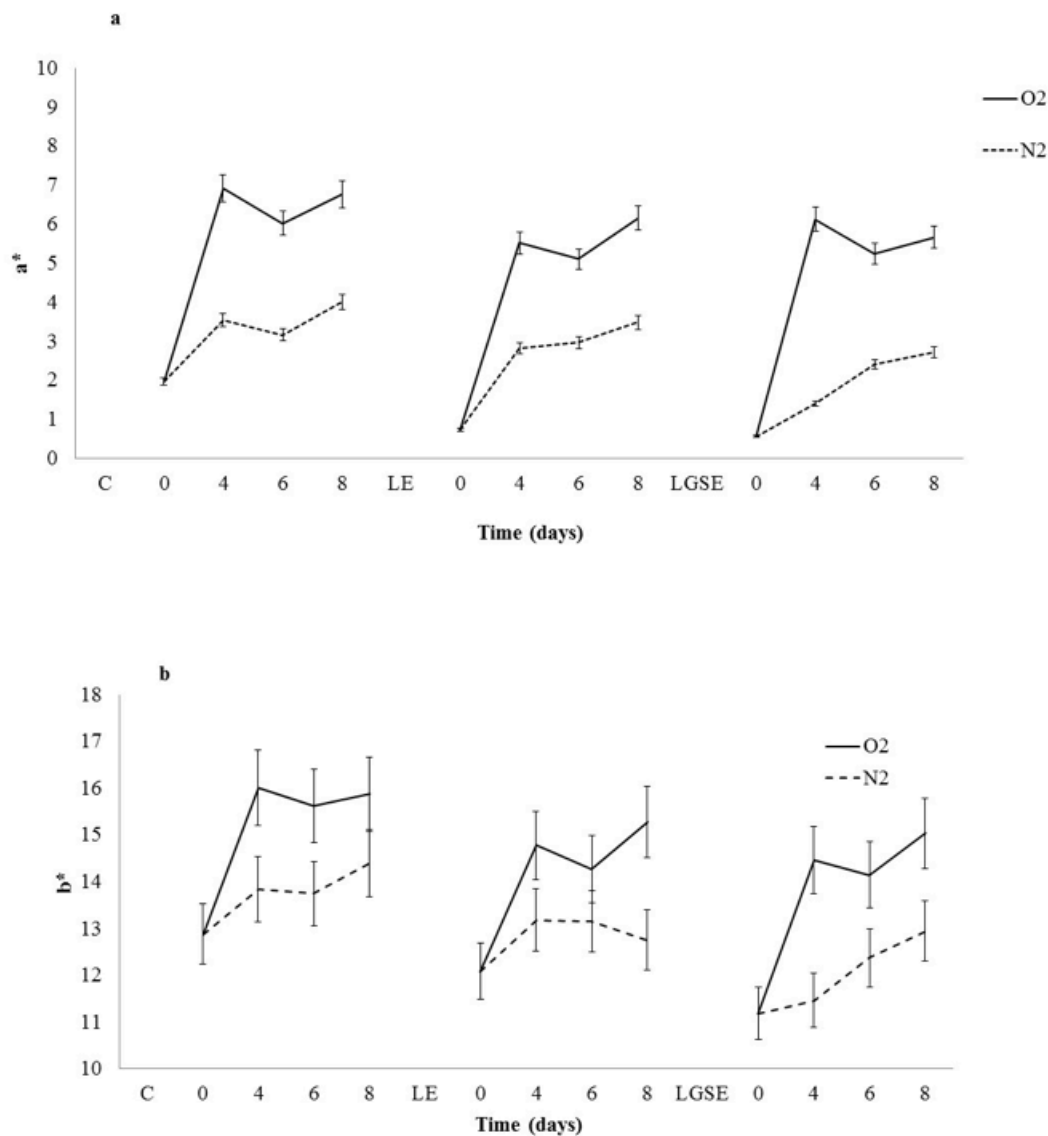
[(c)]TBARS (Thiobarbituric acid reactive substances) expressed in mg of malondialdehyde per kilogram of muscle.

[(d)]$\Delta Ei_j = \sqrt{(Lj - Li)^2 + (aj - ai)^2 + (bj - bi)^2}$

In 0_2 packaged samples, significant, though negligible, increases (P<0.01) in pH values were recorded at 4 and 8 days of storage. This agrees with MUHLISIN *et al*. (2014), who showed that, at increasing oxygen content in the gas mixture, pH of pork was higher than in samples stored at higher levels of nitrogen. However, VIANA *et al*. (2005) found that MAP did not exert a strong effect on pH of fresh pork loin.
The color evolution, expressed by ΔE, during refrigerated storage time, did not differ among dietary treatments. The ΔE values were all above the value of 2, which is considered the threshold to appreciate visual changes of the color (LORENZO *et al*., 2014). With regard to the gas mixtures, the ΔE in the samples stored in oxygen were significantly higher (P<0.01) in any time-lapse interval considered. Over storage, the ΔE values remained almost constant in the two gas mixtures, higher than 5 in O_2 and roughly half that in N_2.

Fig. 1 reports LTL color parameters evolution. In detail, as shown in Fig. 1a, the a* value, which is an index of redness, was consistently higher in high oxygen MAP over storage, regardless of the dietary treatment. The values observed in N_2 MAP are perceived as grey color, as the values ranged from 3.2 and 4.6 (DE SANTOS *et al*., 2007). The same pattern was observed for the b* value (Fig. 1b).

Figure 1. *Longissimus thoracis et lumborum* muscle color parameters evolution: redness "a*" values (**a**) and yellowness "b*" values (**b**) in relation to storage time in MAP: oxygen (O_2) or nitrogen (N_2).
C, control group (Extruded linseed, 0 g kg^{-1}); LE group, (Extruded linseed, 50 g kg^{-1}; vitamin E (α-tocopheryl acetate), 200 mg and Se 0.21 mg kg^{-1}); LGSE group, (Extruded linseed, 50 g kg^{-1}; red grape skin extract 3.0 g kg^{-1})



As concerns the oxidative stability, in the time-lapses considered, TBARS values were unaffected by the dietary treatment on day 4 and 8 (Table 5). Only on day 6, meat from the control showed a greater oxidation ($P<0.05$) than in antioxidants groups. No difference was found between LE *vs* LGSE.

The different modified atmospheres affected the oxidative stability of the muscle, that was always lower in samples packaged in high O_2 MAP, which yielded to a significantly higher TBARS values on day 4 and 6 ($P<0.01$), and on day 8 ($P<0.05$) of storage. When oxygen is readily available, a substrate such as meat is more prone to oxidation (SMIDDY *et al*., 2002) and, in agreement with our results, SPANOS *et al*. (2016) observed that samples of LTL muscle stored in MAP oxygen concentration of 50% or higher showed a significantly lower oxidative stability compared to samples stored under 0% oxygen.

However, even the highest determined value of TBARS was lower than 0.18 mg MDA/kg meat, far below the threshold value of 1.0 mg MDA/kg muscle for organoleptic detection of rancidity as suggested by O'GRADY *et al*. (2008).

Considering that TBARS values up to 0.6 mg of MDA/kg of fresh meat are considered fresh (TARLADGIS *et al*., 1960), all our samples, regardless of the different MAPs, could be classified as fresh meat, through the 8 days of storage.

4. CONCLUSIONS

Our results confirm that 5% of dietary extruded linseed included in the pig finishing diet is a suitable means to increase n-3 PUFA content and reduce the n-6/n-3 PUFA ratio in pig tissues without affecting on live and slaughter performance and impairing technological characteristics of adipose depots. In general, under the point of view of human nutrition, it ameliorates the fatty acid profiles in both backfat and LTL muscle.

Also, qualitative characteristics and chemical composition of muscle are not affected by dietary linseed inclusion associated with either synthetic or natural antioxidants.

In this research linseed feeding, supplemented with supranutritional doses of antioxidants, does not impair oxidative stability compared to a standard diet and reduces weight losses during chilled storage.

As expected, high concentration of oxygen in MAP brings about an increase in oxidative products and yields redder meat, irrespective of the dietary treatment.

In linseed fed pigs, dietary red grape skin extract is as effective as synthetic antioxidant in maintaining quality characteristics of pork during storage, even in high oxygen MAP.

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