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Effect of free-range rearing and α-tocopherol and copper supplementation on fatty acid profiles and susceptibility to lipid oxidation of fresh meat from Iberian pigs

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Abstract

The effects of the feeding system (free-range versus confinement) and the inclusion in concentrate feeds of α-tocopheryl acetate (α-TAc) (0.100 g kg^{-1}) and copper (II) sulphate (0.125 g kg^{-1}) or both on meat oxidative susceptibility, α-tocopherol, intramuscular lipid fraction fatty acid and volatile aldehyde contents were determined in *Biceps femoris* muscle from Iberian pigs. *m. Biceps femoris* from free-range-reared Iberian pigs showed a significantly higher oleic acid percentage in neutral lipids ($p < 0.001$) than muscles from pigs fed in confinement, whereas confinement-pig muscles yielded higher arachidonic acid percentage ($p < 0.05$) in intramuscular fat and linoleic acid percentage ($p < 0.05$) in polar lipids. Copper (II) sulphate supplementation did not show any significant effect on fatty acid composition of lipid fractions of the *B. femoris* muscle. α-TAc supplementation highly increased the α-tocopherol content of muscles ($p < 0.01$). Free-range-reared pigs had the highest muscle FF61-tocopherol concentrations ($p < 0.001$), reflecting the high content found in the pasture. Both free-range rearing and α-TAc supplementation reduced susceptibility to iron-ascorbate-induced peroxidation and reduced hexanal content ($p < 0.001$). A clear relationship between the susceptibility to iron-ascorbate-induced peroxidation and the ratio between the concentration of polyunsaturated fatty acids in polar lipids and α-tocopherol content was found. The present results suggest a beneficial effect on meat quality when rearing hogs outdoors. They further suggest potential benefits to dry-cured products elaborated from free-range pigs as a result of a lower lipid oxidative trend of the meat.

Keywords: Iberian pig; Fatty acid; Oxidation; Vitamin E; Copper (II) sulphate; Volatile aldehydes

1. Introduction

The Iberian pig is an autochthonous pig breed from the South-west of the Iberian Peninsula which is usually free-range-reared and fed on natural resources (acorn and pasture) to produce meat for dry-cured meat processing, mainly dry-cured hams. However, it is not always possible to feed all the pigs by this system, and so, it is becoming more usual to use formulated feeds for fattening pigs (Lopez-Bote, 1998). However, the replacement of free-range rearing and the substitution of acorn and pasture by concentrate diets in pigs produce a marked decrease in the sensory attributes of hams and consequently a lower acceptability of the products (García et al., 1996).

The lipid oxidative processes occurring during the ripening of the dry-cured hams promote the formation of numerous volatile compounds, mainly aldehydes (Buscailhon, Berdagué & Monin, 1993; García, Berdagué et al., 1991; López et al., 1992), that highly influence the flavour (Flores, Grimm, Toldrá & Spanier, 1997). López et al. found differences in the content and proportions of many volatile substances between hams from free-range-reared pigs fed on acorn and pasture and those fed concentrate feeds. These variations have been attributed to the different fatty acid compositions of the fat and intramuscular lipid fractions and the extension of the lipid oxidative processes (Cava et al., 1997; Ruiz et al., 1998).

The digestive characteristics of the gastrointestinal tract of pigs allow modification of the fatty acid composition of fat depots by modifications of the diet. In this sense, numerous experiments have been carried out using

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different dietary fats to modify pig lipid depots, and attempting to increase the proportion of oleic acid (Cava et al., 1997; Monahan, Buckley, Morrissey, Lynch & Gray, 1992; Ruiz et al., 1998; St John et al., 1987). Furthermore, Elliot and Bowland (1968) found that the supplementation of growing-finishing pigs with supranutritional copper diets produced an increase in the proportion of monounsaturated fatty acids and decreased the proportion of saturated fatty acids in the backfat.

Oxidation of lipids in meat has received much attention due to its relationship with off-flavour development (Igene & Pearson, 1979; Konopka, Guth & Grosch, 1995; López-Bote, Rey, Ruiz, Isabel & Sanz-Arias, 1997). The susceptibility of muscle tissue to lipid oxidation depends on a number of factors, the most important being the level of polyunsaturated fatty acids in the phospholipids present in cellular and subcellular membranes (mitochondria, microsomes) (Gray & Pearson, 1987). Because of their fatty acid composition, it is believed that membrane phospholipids are the sites for initial development of oxidised flavours in raw and cooked meat products during storage (Gray & Pearson; Igene & Pearson, 1979; Younathan, 1985). Moreover, the susceptibility of pig muscle to lipid oxidation is also influenced by the presence of antioxidants, as shown recently by the influence of dietary vitamin E (α-tocopherol) supplementation on the improvement of the oxidative stability of muscle from lean pigs (Buckley, Morrissey & Gray, 1995; Monahan et al., 1992).

Relatively little has been reported on the effects of rearing conditions and the use of antioxidant substances and fatty acid unsaturation promoters, such as copper, on meat characteristics or their contribution to meat oxidative stability. So, the purpose of this study was to determine the effect of a free-range rearing system and the enrichment of concentrate feeds with α-tocopheryl acetate and copper (II) sulphate, on the fatty acid composition, α-tocopherol level and oxidative status of meat from Iberian pigs.

2. Material and methods

2.1. Animals and diets

Twenty-five castrated male Iberian pigs weighing 103 ± 2.7 kg were randomly allotted into five groups (five pigs per group) according to raising conditions (confinement and free-range) and the type of feeding during the finish-fattening period (last 8 weeks prior to slaughter). One group (FRANG) was free-range-reared according to the traditional way in which hogs are fed on natural resources: acorns (*Quercus ilex* and *Q. rotundifolia*) and pasture. The other four groups were raised in confinement and were given free access to the appropriate diet: a control diet (CONT) containing 0.005 g kg^{-1} of α-tocopheryl acetate (α-TAc), and diets enriched with 0.125 g kg^{-1} of copper (II) sulphate (COPPER), 0.100 g kg^{-1} of α-TAc (VITE) and 0.125 g kg^{-11} of copper (II) sulphate + 0.100 mg kg^{-1} of α-TAc (CUVE). Dose of copper (II) sulphate used in the feeding of pigs was far below the maximum tolerable levels for pigs according to National Research Council criteria (NRC, 1980) and at the highest added levels fixed by the European Communities (Official Journal of the European Communities, 1998). Pigs were stunned and slaughtered at a local slaughterhouse after the fattening period at a live weight of 145 ± 8.2 kg.

Chemical analysis of acorns, pasture and concentrate feeds was carried out following the methods of the Association of Official Analytical Chemists (AOAC, 1984).

2.2. Sampling

Thighs were removed from the carcass and *m. Biceps femoris* was dissected and freed of visible fat. Muscles were vacuum-packed and frozen at −80°C until analysed. Analyses were carried out in duplicate within 3 weeks of slaughter.

2.3. Fatty acid profiles

Total intramuscular lipids from muscle were extracted according to the method described by Bligh and Dyer (1959). From the fat extracted, the neutral lipid (NL) and polar lipid (PL) fractions of muscle tissues were isolated using amino-propyl minicolums according to the method developed by Garcia-Regueiro, Gilbert and Díaz (1994). Fatty acid methyl esters (FAMEs) of total lipid extracts and neutral and polar lipid fractions were prepared by esterification in the presence of sulphuric acid (Cava et al., 1997). FAMEs were analysed using a Hewlett Packard (mod. HP-5890A) gas chromatograph, equipped with a flame ionisation detector (FID). FAMEs were separated on a Hewlett Packard HP FFAP-TPA fused-silica column (30 m length×0.53 mm i.d.×1.0 μm film thickness). The injector and detector were maintained at 230°C. Column oven temperature was maintained at 225°C. The carrier gas was nitrogen at a flow rate of 1.8 ml min^{-1}.

Identification of FAMEs was based on retention times of reference compounds (Sigma). Tridecanoic acid was used as internal standard. Standard curves for quantification were obtained for all fatty acids determined under the conditions described above.

2.4. Determination of α-tocopherol

Determination of α-tocopherol in muscle was carried out according to the method described by Rey, López-Bote, Soares and Isabel (1997). Representative samples were homogenised in a 0.054 mol l-1 dibasic sodium phosphate buffer adjusted to pH 7.0 with HCl. After mixing with absolute ethanol and hexane (1:1 vol/vol), the upper layer containing tocopherol was resolved and evaporated to dryness and resolved in ethanol prior to analysis by reverse phase HPLC on a Hewlett Packard mod. HP-1050, with a Hewlett Packard UV detector, mod. HPIB-10. Separation was made on a C-18 column (Hewlett Packard RP-18), the mobile phase was methanol:water (97:3 v/v) at a flow rate of 2ml min^{-1} and the detector was fixed at 292 nm.

2.5. Measurement of induced lipid oxidation

The liability of muscle tissue homogenates to iron-ascorbate-induced lipid oxidation was determined by the method of Kornbrust and Mavis (1980). Homogenates (approximately 1 mg protein ml^{-1} buffer) were incubated at 37°C in 40 nM tris-maleate buffer (pH 7.4) with 1 mM $FeSO_4$ in a total volume of 10 ml. At fixed time intervals, aliquots were removed for measurement of 2-thiobarbituric acid-reactive substances (TBARS). TBARS were expressed as nmols malonaldehyde (MDA)/ mg protein. Protein was measured by the procedure of Lowry, Rosebrough, Farr and Randall (1951).

2.6. Headspace volatile aldehydes

Volatile compounds of muscle samples were analysed by headspace gas chromatography according to the method described by López-Bote et al. (1997). After flaking pig muscles, 4 g, were transferred into a 10 ml headspace glass vial and 0.5 µl of 5-heptanone was added as internal standard and then sealed with teflon-faced silicone septums and aluminium caps. A Hewlett Packard mod. HP- 19395-A automated headspace sampler connected to a Hewlett Packard mod 5890 gas chromatograph equipped with FID was employed. Separation of volatile aldehydes was made on a HP5 (phenylmethyl silicone) fused capillary column (50 m length×0.32 mm i.d.×1.05 µm film thickness). Pressurisation and vent times were set at 10 and 20 s, respectively, and the injection time was 30 s. The carousel bath temperature was set to 90°C and the equilibrium time was 30 min. The manifold temperature was set to 95°C. Carrier gas (He) and auxiliary gas pressures were 0.3 and 2.3 bar, respectively. The detector was set at 240°C and the injector at 230°C. The initial oven temperature was 35°C and was increased to 200°C at 7°C min^{-1}. A split ratio of 24:1 was used. Some samples were analysed under the same headspace-gas chromatography as described above and peaks were identified by mass spectrometry.

2.7. Mass spectrometry

The transfer line to the mass spectrometer (MS) was maintained at 280°C. The mass spectra were obtained using a mass selective detector (Hewlett Packard mod. HP-5971 A) by electronic impact at 70 eV, a multiplier voltage of 1756 V and collecting data at a rate of 1 scan s^{-1} over the m/z range of 30 to 300. Compounds were tentatively identified by comparing their mass spectra with those from authentic standards and those contained in the NIST/EPA/NIH and Wiley libraries.

2.8. Statistical analysis

The effects of rearing conditions and α-TAc and copper (II) sulphate diet enrichment on fatty acid composition, lipid-induced oxidation, volatile aldehydes and tocopherol concentration were analysed using the General Linear Model of SAS (1997). An individual pig was the experimental unit for analysis of all data. Data were analysed as a completely randomised design. When a significant F was detected ($p<0.05$), the comparative analyses between means were conducted using orthogonal contrasts. Data were represented as the means of each group and pooled standard deviation together with the significance levels of the main effects and interactions. Correlations among parameters were determined using the CORR procedure of SAS.

3. Results and discussion

3.1. Analysis of diets

Data on chemical composition and fatty acid profiles of diets are given in Table 1. The chemical composition of acorns revealed a relatively high content of fat and a low content of protein. However, crude protein levels in concentrate feeds and pasture had three times higher levels than acorns.

Copper content of diets with the basal level of copper (II) sulphate, CONT and VITE diets, was 0.017 and 0.016 g kg^{-1} DM. The analysed copper content was 2.7 times higher in both COPPER (0.047 g kg^{-1} DM) and CUVE (0.042 g kg^{-1} DM) diets, after supplementation of diets with 0.125 g kg^{-1} copper (II) sulphate.

Regarding α-tocopherol, pasture showed the highest content of all the feeds analysed (0.171 g kg^{-1} DM), even higher than the supplemented diets (VITE and CUVE), while the content of acorns (0.020 g kg^{-1} DM) was similar to that of the non-supplemented feeds (CONT and COPPER). α-Tocopherol content of pasture has been described as higher than commonly recommended values for fattening pigs (NRC, 1988) and ranges from 0.070 to 0.200 g kg^{-1} (Lynch, 1991; Mutettika & Mahan, 1993).

Fatty acid composition of feeds revealed notable differences between the acorn, the pasture and the concentrate feeds. In contrast to concentrate feeds, acorns were higher in oleic acid (C18:1 n-9). Pasture was

Table 1
Chemical composition (g kg^{-1} DM) and fatty acid composition (g kg^{-1} fatty acids) of concentrate feeds, acorn and pasture

Diets[a]						
	Concentrate feeds				FRANG	
	CONT	VITE	COPPER	CUVE	Acorn	Pasture
Crude protein	136	136	122	124	47.1	137
Fat	44.7	47.5	48.4	42.4	63.4	62.6
Crude fibre	49.2	43.2	43.9	45.4	57.0	222
Ash	69.2	50.8	48.0	48.9	17.0	73.1
carbohydrate	701	723	738	729	816	505
α-Tocopherol (g kg^{-1} DM)	0.020	0.125	0.022	0.108	0.020	0.171
Copper (g kg^{-4} DM)	0.017	0.016	0.47	0.042	0.005	0.005
Fatty acid (FA) (g kg^{-1} FA)						
12:0	0.5	0.5	0.4	0.5	0.2	2.1
14:0	8.4	8.4	8.4	8.4	0.9	4.4
15:0	0.7	0.7	0.6	0.6	0.4	2.0
16:0	237	239	235	240	126	156
16:1 (n-7)	13.3	12.7	13.8	13.5	0.9	3.5
17:0	2.0	2.1	1.9	2.0	1.0	2.4
18:0	93.7	97.4	89.9	100	32.2	20.3
18:1 (n-9)	303	302	304	296	661	93.5
18:2 (n-6)	288	290	287	279	147	118
18:3 (n-3)	31.8	30.8	32.8	29.2	10.1	449

[a] The basal diet contained (g kg^{-1} DM): barley 475; wheat bran 400; soybean meal (44%) 80; lard 20; calcium carbonate 8; calcium phosphate 12; sodium chloride 3; mineral/vitamin mix 2. The diet had a calculated metabolisable energy content of 3190 kcal kg^{-1} DM.

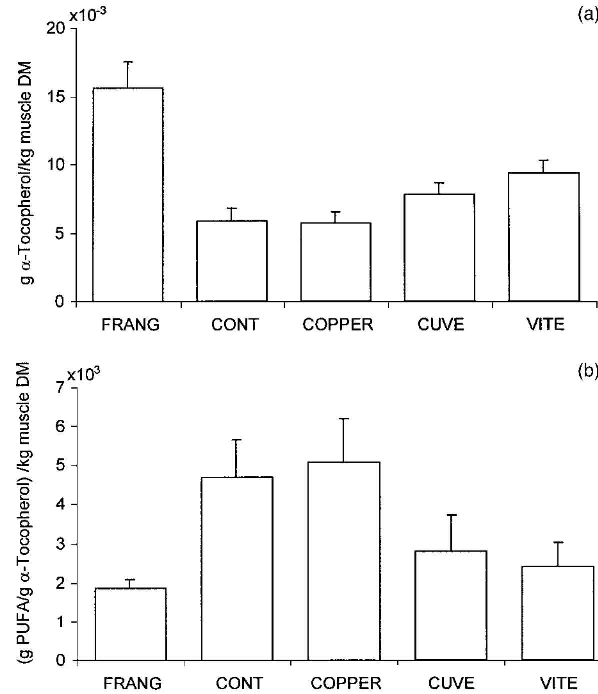

Fig. 1. Mean and standard deviation of α-tocopherol content (g kg^{-1} DM of meat) (A) and ratio of PUFA from PL/FF61-tocopherol (g PUFA/g α-tocopherol kg^{-1} muscle DM) (B) of *m. Biceps femoris* from free-range-reared pigs (FRANG), and pigs raised in confinement on concentrate feeds: basal diet (CONT), 0.125 g kg^{-1} of copper (II) sulphate supplementation (COPPER), 0.100 g kg^{-1} of α-TAc (VITE) and 0.125 g kg^{-1} of copper (II) sulphate + 0.100 g kg^{-1} of α-TAc (CUVE).

characterised by a relatively high proportion of linolenic acid (C18:3 n-3). Concentrate feeds had higher proportions of linoleic acid (C18:2 n-6), compared to acorns and pasture. Chemical and fatty acid composition of the diets are in agreement with data reported in previous papers involving the feeding of Iberian pigs (Cava et al., 1997; Rey, López-Bote & Sanz-Arias, 1997; Ruiz et al., 1998).

3.2. Concentrations of α-tocopherol in muscle

The α-tocopherol content in *m. Biceps femoris* was significantly influenced by diets (Fig. 1A). Muscles from pigs supplemented with 0.100 g kg^{-1} α-TAc contained 1.5 times more α-tocopherol than muscles from pigs fed basal level α-TAc diets (COPPER and CONT). Copper supplementation did not significantly affect the α-tocopherol concentrations in the muscle. These results are in accordance with those reported previously by several authors (Jensen et al., 1997; Monahan et al., 1992) who observed an increase in levels of α-tocopherol in muscle by α-TAc enriched diets.

Muscles from free-range pigs showed higher concentrations of α-tocopherol than muscles from pigs fed on basal or α-TAc-enriched diets ($p < 0.001$). α-Tocopherol content in muscles from FRANG pigs was 1.8 times higher than in muscles from α-TAc supplemented pigs and 2.7 times higher than in muscles from α-TAc basal level diets. This higher content of α-tocopherol of muscles from free-range pigs could be attributed to the high α-tocopherol content of pasture (0.171 g kg^{-1} DM). In this sense, Mutettika and Mahan (1993) reported higher concentrations of α-tocopherol in milk and serum of gilts feeding on pasture than in gilts given mixed diets supplemented with low levels of α-tocopherol. In spite of these results, free-range rearing is shown as a natural way of increasing the content of antioxidant substances in pigs.

3.3. Fatty acid profiles

Fatty acid composition of the neutral (NL) and polar lipid (PL) fractions are shown in Tables 2 and 3, respectively.

Copper supplementation at levels of 0.125 g kg^{-1} of feed did not produce differences in the percentages of monounsaturated fatty acids in NL and PL fractions. The absence of an effect on fatty acid profile of neutral lipids, due to supranutritional copper doses, could be attributed to the levels used in the formulated feed (100

Table 2
Effect of free-range rearing and α-TAc and copper (II) sulphate-enriched diets on fatty acid composition (%) of neutral lipids from *m. Biceps femoris*

	Rearing system [a]									
		Confinement					*p*-Value of contrast[b]			
	FRANG	CONT	VITE	COPPER	CUVE	SEM	1	2	3	4
14:0	1.54	1.55	1.47	1.51	1.60	0.03	ns	ns	ns	ns
16:0	23.0	25.2	24.7	24.9	25.2	0.17	0.011	ns	ns	ns
18:0	8.63	10.40	10.14	10.33	9.90	0.19	0.000	ns	ns	ns
Σ saturated	33.2	37.2	36.3	36.7	36.8	0.30	0.008	ns	ns	ns
16:1	4.63	4.27	4.02	4.14	4.30	0.10	ns	ns	ns	ns
18:1	55.1	51.9	52.4	52.1	52.0	0.31	0.002	ns	ns	ns
Σ Monousaturated	59.7	56.2	56.4	56.2	56.3	0.32	0.000	ns	ns	ns
18:2 (n-6)	5.66	5.02	5.52	5.16	5.11	0.20	ns	ns	ns	ns
18:3 (n-3)	0.68	0.64	0.63	0.71	0.64	0.03	ns	ns	ns	ns
20:4 (n-6)	0.76	1.00	1.09	1.19	1.18	0.07	ns	ns	ns	ns
Σ Polyunsaturated	7/11	6.66	7.26	7.05	6.94	0.29	ns	ns	ns	ns
Σ Unsaturated	66.8	62.8	63.7	63.3	63.3	0.30	0.000	ns	ns	ns
UI [c]0.75	0.75	0.72	0.74	0.73	0.73	0.01	ns	ns	ns	ns
ΣMonounsat/Σ sat	1.74	1.51	1.55	1.53	1.53	0.02	0.000	ns	ns	ns
Σ Unsat/Σ sat	1.94	1.69	1.75	1.73	1.72	0.02	0.000	ns	ns	ns
Σ Polyunsat/Σ sat	0.21	0.18	0.20	0.19	0.19	0.01	ns	ns	ns	ns

[a] Rearing system: Confinement: CONT, control diet; COPPER: control diet + 0.125g kg^{-1} copper (II) sulphate; VITE: control diet + 0.100 g kg^{-1} α-TAc; CUVE: control diet + 0.125 g kg^{-1} copper (II) sulphate + 0.100 g kg^{-1} α-TAc; Free-range: FRANG, natural resources (acorn and pasture).
[b] Contrasts were as follows: (1) free-range vs confinement; (2) copper supplementation vs no copper supplementation; (3) vitamin E supplementation vs no vitamin E supplementation; (4) interaction copper × vitamin E. ns, not significant ($p > 0.05$).
[c] UI (Unsaturation index), average number of double bonds per fatty acid residue.

Table 3
Effect of free-range rearing and α-TAc and copper (II) sulphate-enriched diets on fatty acid composition (%) of neutral lipids from *m. Biceps femoris*

	Rearing system [a]									
		Confinement					*p*-Value of contrast[b]			
	FRANG	CONT	VITE	COPPER	CUVE	SEM	1	2	3	4
14:0	1.41	1.55	0.88	1.14	1.14	0.06	ns	ns	0.004	0.004
16:0	11.8	10.9	10.3	10.30	9.85	0.30	0.048	ns	ns	ns
18:0	16.3	16.0	17.6	15.8	16.8	0.32	ns	ns	ns	ns
Σ saturated	29.6	28.4	28.7	27.2	27.8	0.30	0.036	ns	ns	ns
16:1	2.62	2.66	1,92	2.38	2.49	0.07	ns	ns	0.024	0.002
18:1	15.9	13,8	12.6	14.5	14.51	0.38	0.035	ns	ns	ns
Σ Monousaturated	18.47	16.5	14.5	16.9	17.00	0.42	0.036	ns	ns	ns
18:2 (n-6)	25.0	27.3	27.3	28.0	27.3	0.35	0.005	ns	ns	ns
18:3 (n-3)	5.23	5.75	3.63	4.66	4.87	0.19	ns	ns	0.004	0.000
20:4 (n-6)	21.7	22.0	25.9	23.7	23.0	0.50	ns	ns	ns	ns
Σ Polyunsaturated	52.7	55.2	56.8	55.9	55.2	0.55	0.005	ns	ns	ns
Σ Unsaturated	70.5	71.68	71.2	72.7	72.2	0.30	0.036	ns	ns	ns
UI [c]0.75	1.71	1.77	1.83	1.8	1.79	0.02	0.025	ns	ns	ns
ΣMonounsat/Σ sat	0.62	0.58	0.51	0.62	2.070.61	0.02	ns	0.034	ns	ns
Σ Unsat/Σ sat	2.38	2.53	2.48	2.70	2.60	0.04	0.040	ns	ns	ns
Σ Polyunsat/Σ sat	1.76	1.95	1.98	2.07	1.99	0.04	0.009	ns	ns	ns

[a] Rearing system: Confinement: CONT, control diet; COPPER: control diet + 0.125g kg^{-1} copper (II) sulphate; VITE: control diet + 0.100 g kg^{-1} α-TAc; CUVE: control diet + 0.125 g kg^{-1} copper (II) sulphate + 0.100 g kg^{-1} α-TAc; Free-range: FRANG, natural resources (acorn and pasture).
[b] Contrasts were as follows: (1) free-range vs confinement; (2) copper supplementation vs no copper supplementation; (3) vitamin E supplementation vs no vitamin E supplementation; (4) interaction copper × vitamin E. ns, not significant ($p > 0.05$).
[c] UI (Unsaturation index), average number of double bonds per fatty acid residue.

$mg\,kg^{-1}$) and the duration of feeding regime in contrast to copper (II) sulphate supplements of 250 $mg\,kg^{-1}$ used in previous studies (Elliot & Bowland, 1968). The mentioned authors described an increase in the proportion of palmitoleic (C16:1 n-7) and oleic (C18:1 n-9) fatty acids together with a decrease in the proportion of palmitic (C16:0) and stearic (C18:0) fatty acids in the backfat of growing-finishing pigs raised on copper-enriched diets.

With the exception of significant ($p < 0.05$) changes in minor fatty acids (myristic, palmitoleic and linolenic) in the PL fraction (Table 3), the fatty acid of NL (Table 2) and PL fractions were not substantially affected by dietary supplementation at supranutritional levels of α-TAc in feeds. These results are in agreement with those experiments reported in the literature for lean pigs (Buckley et al., 1995; Jensen et al., 1997; Monahan et al., 1992) in which α-TAc-enriched diets did not modify the fatty acid profiles of fat depots.

Fatty acid profiles of neutral and polar intramuscular lipid fractions were significantly affected by free-range rearing conditions of the pigs. The altering effect of acorn and pasture feeding was especially marked in the NL fraction (Table 2), while fatty acids from the PL fraction were slightly affected (Table 3). FRANG pigs showed significantly ($p < 0.0001$) higher percentages of monounsaturated fatty acids (primarily oleic acid C18:1 n-9), and significantly lower saturated fatty acid percentages ($p < 0.01$), i.e. palmitic (C16:0) ($p < 0.05$) and stearic (C18:0) ($p < 0.001$) acids, in the NL fraction, than pigs receiving concentrate feeds (Table 2), reflecting the fatty acid composition of acorn (Table 1). In the PL fraction, the percentages of palmitic and oleic acid were significantly higher ($p < 0.05$) and linoleic acid was significantly lower ($p < 0.01$) in free-range pigs, and the unsaturation index (UI) was lower ($p < 0.05$) also (Table 4). Similar findings were described by Cava et al. (1997) in *m. Masseter* from free-range Iberian pigs or fed in confinement on concentrate feeds.

3.4. Lipid oxidation in muscle

Fig. 2 shows the rates of iron-ascorbate-induced lipid peroxidation in *Biceps femoris* muscle homogenates of Iberian pigs fed the CONT, COPPER, VITE and CUVE diets and free-range Iberian pigs (FRANG). Muscle homogenates from Iberian pigs fed α-TAc-supplemented diets (VITE and CUVE) showed a significantly lower ($p < 0.001$) susceptibility to lipid oxidation than muscle homogenates from pigs fed

Table 4
Pearson correlation coefficient amoung TBARS values in iron-arcorbate-induced lipid peroxidation test at time 0, 50, 100 and 200 min and α-tocopherol content, PUFA content from PL and ratio PUFA/α-tocopherol content from *m. Biceps femoris*

	TBARS			
Time (min):	0	50	100	200
α-tocopherol	−0.004	−0.430	−0.667	−0.840
	ns[a]	*	***	***
PUFA from PL	0.498	0.410	0.306	0.275
	*	*	ns	ns
PUFA from PL/α-tocopherol	0.036	0.449	0.602	0.853
	ns	*	**	***

[a] ns: not significant; *$p < 0.05$;**$p < 0.01$; ***$p < 0.001$.

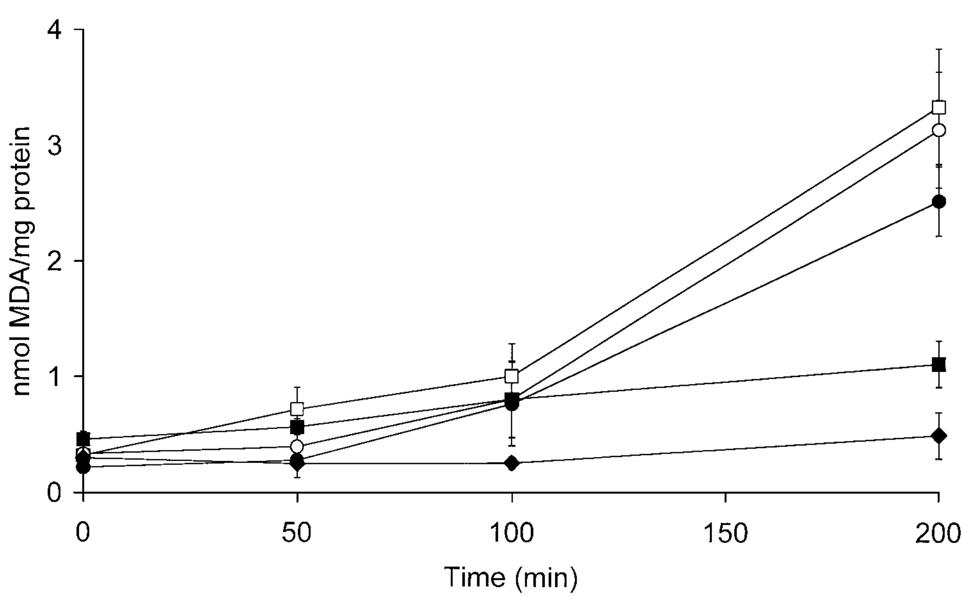

Fig. 2. Effect of free-range rearing and α-TAc and copper (II) sulphate enrichment in diets on the susceptibility of *m. Biceps femoris* to iron-ascorbate-induced lipid peroxidation. Pigs were free-range-reared on acorn and pasture (▲) or in confinement on a basal diet (□), or enriched with 0.125 $g\,kg^{-1}$ of copper (II) sulphate (○), 0.100 $g\,kg^{-1}$ of α-TAc +0.125 $g\,kg^{-1}$ of copper (II) sulphate (●) and 0.100 $g\,kg^{-1}$ of α-TAc.

α-TAc basal level diets (COPPER and CONT). Copper (II) sulphate administration in diets did not alter the susceptibility of muscle homogenates to lipid oxidation when compared to the control diet group. The protective effects of α-TAc supplementation against iron-ascorbate-induced lipid peroxidation of muscle homogenates have been widely reported in lean pigs (Monahan et al., 1992; Morrissey, Buckley, Sisk, Lynch & Sheehy, 1996).

In the case of muscles from FRANG group pigs, TBARS values during the experiment were significantly lower than in those from pigs raised on concentrate feeds.

Differences in the susceptibility of muscle homogenates to lipid oxidation, in pigs fed concentrate feeds, were clearly related to muscle levels of α-tocopherol. Meanwhile, the intensity of lipid oxidation in FRANG group muscle homogenates seemed to be associated with both fatty acid composition and unsaturation index of polar lipids (Table 3), and α-tocopherol content of muscles (Fig. 1A). The ratio between PUFA content from PL and α-tocopherol was significantly lower ($p<0.001$) in free-range pigs than in pigs fed concentrate feeds (Fig. 1B). In this sense, the TBARS values during the iron-ascorbate-induced lipid oxidation test were positively correlated with this ratio, being higher in the last test times than at the beginning (Table 4). Furthermore, a significant correlation was found at time 0 between PUFA content from PL and TBARS. This relationship was likely due to previous oxidation during sampling or thawing. It appears that, not only the PUFA content of PL and the α-tocopherol level in the muscle, but the ratio between both, highly influences susceptibility to lipid oxidation of muscle. As expected from the α-tocopherol content of the FRANG diet, pigs feeding on this diet have a high content of α-tocopherol in the muscle, and the muscle also has a high oxidative stability.

3.5. Volatile aldehydes of meat

The free-range rearing system and feeding regime significantly affected the headspace volatile aldehydes of pig muscles (Table 5).

Incorporation of α-tocopherol in Iberian pig diet produced significant differences in the levels of aldehydes of meat, significantly reducing pentanal, hexanal and heptanal. The protective effect of α-tocopherol against lipid oxidation, as consequence of the deposition of dietary α-tocopherol in muscle, resulted in a significant reduction in the volatile aldehyde amounts ($p<0.05$), basically hexanal content ($p<0.05$), in the headspace of meat from pigs fed α-TAc-enriched diets (Table 5). These results agree with findings from Pfalzgraf, Frigg, and Steinhart (1995) who found a significant reduction in hexanal content in meat from pigs fed 0.200 g kg^{-1} of α-TAc-enriched diets in contrast to meat from pigs fed α-TAc basal levels in diets.

The modification of muscle fatty acid composition, particularly of polyunsaturated fatty acids of PL and FF61-tocopherol content, through free-range rearing, not only reduced muscle homogenates lipid oxidation but also modified the amounts of volatile aldehydes in meat. Headspace of meat from FRANG group pigs showed significantly lower hexanal ($p<0.05$) and total volatile aldehyde concentration ($p<0.05$) than meat from pigs fed concentrate feeds, indicating a lower development of lipid oxidative reactions (Table 5). Larick, Turner, Schoenherr, Coffey, and Pilkington (1992) observed an increase in the aldehydes derived from oxidation of linoleic acid (hexanal, pentanal) in volatile compounds of meat from pigs fed increasing levels of linoleic acid. These authors suggested that an increase in the hexanal amounts, as a result of deposition in muscle of dietary linoleic acid, indicates that meat from pigs fed concentrate feeds are more prone to the development of oxidative processes which are related to rancid flavour

Table 5
Concentration (g kg^{-1} muscle DM) of headspace volatile aldehydes of *m. Biceps femoris* muscle from free-range pigs and pigs fed the experimental diets[a]

	Rearing system									
		Confinement					*p*-value of contrasts			
	FRANG	CONT	VITE	COPPER	CUVE	SEM	1	2	3	4
c4[b]	0.379	0.597	0.388	0.489	0.384	0.007	ns	ns	ns	ns
c5	0.077	0.415	0.074	0.299	0.121	0.001	ns	ns	0.022	ns
c6	1.21	2.58	1.601	2.72	1.91	0.039	0.034	ns	0.033	ns
c7	0.179	0.415	0.217	0.272	0.234	0.004	0.009	ns	0.002	0.025
c8	0.184	0.146	0.118	0.106	0.098	0.002	0.011	ns	ns	ns
c9	0.371	0.218	0.157	0.175	0.176	0.004	0.050	ns	ns	ns
sum	2.302	4.372	2.562	4.067	2.921	0.057	0.050	ns	0.015	ns

[a] Rearing system: Confinement: CONT, control diet; COOPER: control diet + 0.125g kg^{-1} copper (II) sulphate; VITE: control diet + 0.100 g kg^{-1} α-TAc; CUVE: control diet + 0.125 g kg^{-1} copper (II) sulphate + 0.100 g kg^{-1} α-TAc; Free-range: FRANG, natural resources (acorn and pasture).
[b] c4 = butanal, c5 = pentanal, c6 = hexanal,c7 = heptanal, c8 = octanal, c9 = nonanal.

in meat. Furthermore, free-range pig muscles showed a significantly higher content of volatile aldehydes derived from decomposition-oxidation of oleic acid, nonanal ($p < 0.05$) and octanal ($p < 0.05$), reflecting the oxidation of the main fatty acid in lipid fractions. In this sense, López-Bote et al. (1997), in an experiment in which rabbits were fed diets rich in oleic acid, described an increase in the headspace volatile concentration of nonanal and octanal. The increase in the amounts of oleic acid oxidation-derived products in meat from free-range pigs, in comparison to meat from pigs fed in confinement on concentrate feeds, is important from the point of view of the development of aromatic characteristics during the ripening of the dry-cured products elaborated from Iberian pig meat. These compounds have been identified in extracts of dry-cured Iberian hams (Antequera et al., 1992; García, Berdagué, et al., 1991) and have been associated with pleasant flavour (Flores et al., 1997; Grosch, 1987).

In view of the effect of rearing system and feeding regime on fatty acid composition and muscle oxidative susceptibility reported in this work, it is likely that these factors could be responsible for the different sensory quality of dry-cured hams elaborated from Iberian pig meat. This results from a distinct oxidative pattern during the ripening, as a consequence of both the α-tocopherol content and the fatty acid profiles. In this sense, other research is being conducted in our laboratories to confirm free-range rearing and α-TAc supplementation effectiveness on lipid stability during the maturation of hams and effects on the sensory quality of Iberian hams.

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