

# Improvement of Dry-cured Iberian Ham Quality Characteristics Through Modifications of Dietary Fat Composition and Supplementation with Vitamin E

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The effects of dietary fat composition and vitamin E supplementation on the quality characteristics of dry-cured Iberian hams ripened for two years were studied. Thirty Iberian × Duroc pigs were fed diets containing three levels of poly and monounsaturated fatty acids. Within each dietary fat treatment, one group was fed a basal level of vitamin E (20 mg  $\alpha$ -tocopheryl acetate/kg diet) and the other group received a supplemented level (200 mg  $\alpha$ -tocopheryl acetate/kg diet). Dietary fat composition significantly affected total saturated fatty acids content of neutral and polar lipids from dry-cured Iberian ham ( $p = 0.012$  and  $p = 0.003$ , respectively). However, diet fatty acids composition did not influence either total monounsaturated or total polyunsaturated fatty acids of neutral and polar lipids. Vitamin E supplementation significantly enhanced dry-cured Iberian ham  $\alpha$ -tocopherol content ( $p = 0.001$ ). This, in turn, led to significantly lower levels of TBARS on days 6 and 9 of storage in slices from dry-cured Iberian hams made of vitamin E supplemented pigs and also lower oxidation levels in an induced lipid oxidation test in samples from those pigs. Dietary fatty acid composition did not significantly affect either TBARS during slices storage or malonaldehyde content in the induced oxidation test. No effect of vitamin E supplementation was observed in ham volatile aldehyde profile, but dietary fat significantly affected hexanal ( $p = 0.02$ ), heptanal ( $p = 0.05$ ) and total aldehyde content ( $p = 0.02$ ), with those pigs fed a diet rich in PUFA showing higher values. Using diets supplemented in  $\alpha$ -tocopherol and rich in monounsaturated fatty acids seemed adequate dietary strategies for feeding Iberian hams reared indoors.

*Key Words:* dietary manipulation, Iberian pig, vitamin E, fatty acids, oxidation, dry-cured ham

## INTRODUCTION

Dry-cured hams from Iberian pigs achieve high consumer acceptance and prices due to their particular quality characteristics (Ruiz et al., 2002). These are partly based in the free-range system in which animals are fattened (García et al., 1996). As a direct consequence of the feeding sources in such a rearing system, mainly acorns and grass, muscles from Iberian pigs show high levels of oleic acid (C18:1 n-9) and  $\alpha$ -tocopherol (Ruiz et al., 1998; Rey and López-Bote, 2000) which in turn influence the extent and rate of

lipid oxidative phenomena during the processing (Isabel et al., 1999a).

Due to the increasing demand for dry-cured Iberian ham, a high proportion of Iberian pigs produced in Spain are currently being reared indoors and fed on concentrates. However, products from pigs fed this way do not reach the same quality as those from outdoors (García et al., 1996). Although several studies have dealt with the negative influence of feeding Iberian pigs indoors with concentrates on the quality characteristics of dry-cured meat products (Lopez et al., 1992; Ruiz et al., 1998; Carrapiso et al., 2002) there has been no attempt for improving the quality of dry-cured ham from Iberian pigs reared indoors through dietary manipulation.

Even though dietary strategies for improving pork quality characteristics are effective, their use in pig production is not widespread, since the extra feeding costs are difficult to recover in the commercial setting. However, the high added value of dry-cured Iberian ham could justify the use of dietary manipulation for

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reducing deterioration during storage, or for improving quality characteristics (Ruiz and López-Bote, 2002).

Commercialisation of sliced and packaged dry-cured Iberian ham is becoming more and more important. Quality characteristics of dry-cured meat products easily deteriorate after slicing, due to discoloration of the surface, moisture loss and rancid flavour development (Kemp et al., 1988; Ventanas et al., 2001), all these phenomena being related with lipid oxidation. Low deterioration of  $\alpha$ -tocopherol during the processing of dry-cured hams has been reported (Isabel et al., 1999b). This suggests that enhancing  $\alpha$ -tocopherol in the raw material by dietary means could be a useful approach for controlling deterioration of sliced dry-cured Iberian ham.

The present experiment was designed to provide information about the effect of dietary vitamin E supplementation in diets with different concentration of mono- and polyunsaturated fatty acids on the quality characteristics of dry-cured Iberian hams and on discoloration, lipid oxidation and moisture loss of sliced dry-cured Iberian ham during storage.

## MATERIAL AND METHODS

### Animals and Diets

Thirty Iberian  $\times$  Duroc gilts were randomly distributed and located in individual cages and fed a conventional pig diet until they reached approximately 100 kg live weight. At this time six experimental diets were randomly assigned to groups of five pigs. All pigs were fed ad libitum with the appropriate diet, which were formulated to contain three levels of poly- (mainly linoleic acid, C18:2 n-6) and monounsaturated fatty acids (mainly oleic acid, C18:1 n-9), but maintaining a constant concentration of saturated fatty acids. Within each dietary fat treatment, one group was fed a basal level of vitamin E (20 mg  $\alpha$ -tocopheryl acetate/kg diet) (Hoffman La Roche, Switzerland) and the other group received a supplemented level (200 mg  $\alpha$ -tocopheryl acetate/kg diet). Chemical composition and fatty acids of the experimental diets are shown in Table 1. Pigs were slaughtered at an average weight of 150 kg by electrical stunning and exsanguination at a local slaughterhouse. Feeders were emptied 24 h prior to slaughter.

### Sample Collection and Chemical Analysis

Proximate composition of diets was carried out according to the following AOAC procedures (AOAC, 1990): nitrogen content by the Kjeldahl method (976.05), crude protein (954.01), crude fat (920.39) and crude fibre (962.09). Ash content was analysed as described in ISO – method 1442 – (ISO, 1973).

$\alpha$ -Tocopherol was extracted from feed as previously described by Rey et al. (2001). Analyses were carried out by HPLC (Hewlett Packard 1050, with a UWD, HPIB 10 detector and a RP-18 end-capped column) (Waldbronn, Germany). The eluting solvent was methanol:water (97:3) at a flow rate of 2 mL/min. Dietary fatty acids were extracted and quantified following the one-step procedure described by Sukhija and Palmquist (1988) in lyophilised samples. Pentadecanoic acid (C15:0) (sigma, Alcobendas, Madrid) was used as internal standard. Fatty acid methyl esters (FAMES) were prepared by acidic-trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) as described elsewhere (López-Bote et al., 1997). FAMES were identified by gas chromatography using a 6890 Hewlett Packard gas chromatograph and a 30 m  $\times$  0.32 mm  $\times$  0.25  $\mu$ m cross-linked polyethylene glycol capillary column. A temperature program of 170 to 245 °C was used. The injector and detector were maintained at 250 °C. The carrier gas (helium) flow rate was 3 mL/min.

The right thigh from each pig was obtained at cutting (24 h after slaughter) and processed in a traditional manner for approximately 22 months to produce a dry-cured Iberian ham as previously described (Ruiz et al., 1999). Briefly, hams were salted in piles for 10 days at 2 °C and 80–85% relative humidity (salting), subsequently salt was brushed off and hams were held at 2–5 °C and 85–75% relative humidity for 80 days (postsalting phase). Temperature was thereafter increased from 5 to 28 °C over 90 days, while relative humidity was progressively reduced to 65% (drying stage). Finally, hams were kept in a cellar (cellar phase) at 15–18 °C and 65–75% relative humidity for 16 additional months.

Samples of *Biceps femoris* were taken from each ham and analysed as follows: neutral and polar lipids from 2.5 g ham samples were obtained using the method of Marmer and Maxwell (1981). FAMES of neutral and polar lipid fractions were prepared and analysed as explained above for dietary samples.

For the determination of  $\alpha$ -tocopherol, 1 g muscle was homogenised in 10 mL 0.054 M dibasic sodium phosphate buffer adjusted to pH 7.0 with HCl. After mixing with absolute ethanol and hexane, the upper layer containing tocopherol was evaporated and dissolved in ethanol prior to analysis by reverse phase HPLC (HP 1050, with a UVD, HPIB 10 detector) (Hewlett Packard, Waldbronn, Germany). Separation was made on a Lichrocart PR 18 endcapped column (250  $\times$  4 mm i.d., 5  $\mu$ m particle size) (Merck Darmstadt, Germany), the mobile phase was methanol:water (97:3 v/v) at a flow rate of 2 mL min<sup>-1</sup>, and peaks were registered at 292 nm (Rey et al., 1996).

The liability of dry-cured ham homogenates to iron-induced lipid oxidation was determined as described by Konbrust and Mavis (1980). To prepare homogenates

**Table 1.** Chemical composition of experimental diets.

Ingredients (g/kg of diet)	Diets <sup>1</sup>					
	Mono		Medium		Poly	
	Basal	Suppl	Basal	Suppl	Basal	Suppl
Barley	500	500	500	500	500	500
Wheat	381	381	381	381	381	381
Soybean meal	41.4	41.4	41.4	41.4	41.4	41.4
Lard	15.4	15.4	18.7	18.7	22.0	22.0
Olive oil	24.6	24.6	12.3	12.3	–	–
Sunflower oil	–	–	9	9	18.0	18.0
Sodium chloride	3	3	3	3	3	3
Calcium carbonate	9.9	9.9	9.9	9.9	9.9	9.9
Vitamin and mineral premix	1	1	1	1	1	1
$\alpha$ -tocopheryl acetate (50%) <sup>2</sup>	–	0.4	–	0.4	–	0.4
Proximate analysis (per kg d.m.)						
Dry matter (g/kg feed d.m.)	888.0	891.0	892.0	895.2	888.6	890.6
Crude protein (g/kg d.m.)	135.5	133.0	136.6	136.9	133.7	135.3
Crude fat (g/kg d.m.)	68.8	71.3	71.9	68.4	68.1	68.0
Crude fibre (g/kg d.m.)	50.8	49.0	49.0	43.0	48.0	47.0
Ash (g/kg d.m.)	56.6	60.0	59.0	63.0	62.0	59.0
Nitrogen free extractives (g/kg d.m.)	688.3	686.7	683.5	688.7	688.2	690.7
Lysine (g/kg d.m.)	8.1	8.0	7.8	8.0	8.0	8.1
$\alpha$ -tocopherol (mg/kg d.m.)	39.8	255.0	60.0	256.3	66.7	260.4
Calculated ME (MJ/kg feed)	13.1	13.1	13.2	13.2	13.4	13.4
Fatty acids (g/kg d.m.)						
C14:0	0.4	0.4	0.5	0.4	0.5	0.6
C16:0	10.1	9.7	10.2	10.1	10.1	10.2
C16:1 (n-7)	0.5	0.5	0.1	0.1	0.1	0.1
C17:0	0.2	0.2	0.2	0.2	0.2	0.2
C17:1	0.1	0.1	0.1	0.1	0.1	0.1
C18:0	3.1	2.8	3.0	3.0	3.0	3.1
C18:1 (n-9)	20.9	21.8	16.7	16.0	14.0	14.2
C18:1 (n-7)	0.9	1.0	0.9	0.8	0.8	0.9
C18:2 (n-6)	9.9	9.9	14.9	15.4	18.9	18.7
C18:3 (n-3)	1.4	1.4	1.4	1.2	1.3	1.3
C20:0	0.2	0.2	0.2	0.2	0.2	0.3
C20:1 (n-9)	0.7	0.8	0.7	0.7	0.7	0.6
C20:3 (n-9)	0.2	0.2	0.1	0.3	0.1	0.2
C20:5 (n-3)	0.1	0.2	0.2	0.3	0.3	0.3
C22:5 (n-3)	0.2	0.2	0.2	0.3	0.4	0.4
C22:6 (n-3)	0.2	0.2	0.2	0.3	0.4	0.4
$\Sigma$ Monounsaturated fatty acids (MUFA)	23.1	24.3	18.4	17.6	16.0	16.4
$\Sigma$ Polyunsaturated fatty acids (PUFA)	12.2	12.2	17.2	17.8	21.4	21.3
$\Sigma$ Saturated fatty acids (SFA)	13.8	13.3	14.0	13.8	14.0	14.3

<sup>1</sup>Mono: diet containing a high concentration of monounsaturated fatty acids (23.1–24.3g/kg d.m.); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3–21.4g/kg); Medium: diet with an intermediate concentration (16.7–18.4g monounsaturated fat and 13.8–14.0g polyunsaturated/kg d.m.), in each case with a basal or supplemented (Suppl) level of  $\alpha$ -tocopheryl acetate (200mg/kg feed).

<sup>2</sup> $\alpha$ -Tocopheryl acetate supplement = 50%  $\alpha$ -tocopheryl acetate + 50% carrier.

3g ground *Biceps femoris* from dry-cured hams were homogenised with 27mL 0.15MKCl for 45s. During homogenisation tubes were kept in ice to avoid heating. Protein content was measured in 1mL homogenate following the Lowry procedure (Lowry et al., 1951). One mL of muscle homogenate was incubated at 37°C in 40mM tris-maleate buffer (pH 7.4) with 1mM FeSO<sub>4</sub> and 2mM ascorbic acid in a total volume of 10mL. At fixed intervals (0, 30, 60, 90 and 120min) aliquots were removed for measurement of TBARS by the method of Buege and Aust (1978).

TBARS were expressed as nmoles of malonaldehyde (MDA) per mg protein.

Slices of processed dry-cured hams (1.5mm thick) were obtained and placed on polystyrene trays, wrapped in an oxygen-permeable PVC (permeability 0.65 L/m<sup>2</sup>·h at 23°C) and stored at 4°C under fluorescent light. Lipid oxidation was assessed by the 2-thio-barbituric acid method (Salih et al., 1987) on days 0, 3, 6 and 9 of storage.

Volatile aldehydes of stored samples were analysed by headspace gas chromatography. After flaking

dry-cured ham slices, 4g were transferred to 10ml headspace glass vials (Hewlett Packard). 4-Heptanone (408.5ng) was added to the vials as an internal standard, and the vials were sealed with a Teflon-faced silicone septum and aluminium caps. A Hewlett Packard 19395A automated headspace sampler connected to a Hewlett Packard 5890 gas chromatograph equipped with FID was employed. Separation was performed on a HP-5 (phenylmethyl silicone) fused capillary column (50ml  $\times$  0.32mm i.d.  $\times$  1.05  $\mu$ m film thickness) (Hewlett Packard, Avondale, PA, USA). Pressurisation and vent times were 10 and 20s, respectively, and the injection time was 30s. The carousel bath manifold temperatures were 90°C and 95°C, respectively and the equilibrium time 30min. Carrier gas (He) and auxiliary gas pressures were 0.3 and 2.3 bar, respectively. The detector temperature was 240°C and that of the injector 230°C. The initial oven temperature was 35°C and was ramped to 200°C at 7°C/min. A split ratio of 24:1 was used. Identification of aldehydes was performed by comparing their retention times with those of authentic standards (Sigma, St Louis, MO, USA). Standard curves were obtained for all aldehydes determined and 4-heptanone under the conditions described above. Some samples were analysed under the same headspace gas chromatographic conditions and peaks were identified by mass spectrometry. For mass spectrometry identification the transfer line to the mass spectrometer was maintained at 280°C. The mass spectra were obtained using a mass selective detector (Hewlett-Packard HP-5971A) by electronic impact at 70eV, a multiplier voltage of 1756V and collecting data at a rate of 1 scan/s over the m/z range of 30 to 300. Compounds were tentatively identified by comparing their mass spectra with those of authentic standards and those contained in the NIST/EPA/NIH and Willey libraries.

For the determination of weight loss during storage, approximately 1cm<sup>3</sup> samples were taken in duplicate from the *Biceps femoris* muscle in the final product. Immediately after cutting, samples were weighed and stored in a laboratory cabinet (Kowel, Model CC-3-1, Barrioplano, Navarra, Spain) at a controlled temperature (20  $\pm$  0.1°C) and relative humidity (75  $\pm$  2%). Samples were weighed again at day 2, 3, 4, 5, 6 and 7 of storage.

### Statistical Analysis

An individual pig was the experimental unit for analysis of all data. Response data were evaluated using the General Linear Model of SAS version 8 (SAS, 1999). The effect of dietary fat and  $\alpha$ -tocopherol supplementation was tested using the following model:

$$Y_{ij} = \mu + \alpha_1 + \beta_j + \alpha\beta_{ij} + e_{ijk}$$

where  $Y_{ij}$  is the dependent variable,  $\mu$  is the overall mean,  $\alpha_1$  refers to the effect of dietary fat composition,  $\beta_j$  refers to the effect of  $\alpha$ -tocopherol supplementation,  $\alpha\beta_{ij}$  refers to the interaction between dietary fat composition and  $\alpha$ -tocopherol supplementation, and  $e_{ijk}$  refers to the experimental error. A repeated measurement test was used to compare differences in oxidation rates between groups.

## RESULTS AND DISCUSSION

Dietary fat treatment markedly influenced fatty acid composition of both, neutral (Table 2) and polar lipids (Table 3), while  $\alpha$ -tocopherol supplementation only affected linoleic acid (C18:2 n-6) content in neutral lipids. However, dietary fat composition did not affect oleic (C18:1 n-9) nor linoleic (C18:2 n-6) acid content of neutral and polar lipids, while these fatty acids showed marked differences between diets (Table 1). Consequently, total MUFA and PUFA levels of neutral and polar lipids were also unaffected. On the other hand, dietary fat treatment significantly affected saturated fatty acid (SFA) content in dry-cured ham neutral and polar lipids ( $p = 0.012$  and  $p = 0.003$ , respectively), samples from animals fed the diet high in MUFA showing lower values. This lack of differences in MUFA and PUFA content between dry-cured Iberian hams from pigs fed different dietary fat could be a result of lipolysis and lipid oxidation phenomena that takes place during the ripening (Antequera et al., 1992). As a consequence of those processes, there happened a reduction in phospholipid content and a decrease in the proportion of polyunsaturated fatty acids of phospholipids occurs (Martín et al., 1999). In fact, the fatty acid composition of fresh *longissimus dorsi* muscle from the same pigs showed higher values for linoleic (C18:2 n-6), arachidonic (C20:4 n-6) and total PUFA in polar lipids, and a significant effect of dietary fat on total MUFA and PUFA of polar lipids (data not shown).

Dietary fat treatment did not significantly influence  $\alpha$ -tocopherol concentration in dry-cured hams (Table 4), whereas  $\alpha$ -tocopherol supplementation increased the amount of vitamin E in dry-cured Iberian ham. This is in agreement with previous research about fresh meat, in which the deposition of  $\alpha$ -tocopherol in pig muscle was dependent upon the concentration of vitamin E in the feed (Monahan et al., 1992; Morrissey et al., 1996). This meant that, after almost two years of processing, differences in muscle  $\alpha$ -tocopherol concentration between non-supplemented and supplemented pigs still remained significant (8.08 vs 16.00).

Dry-cured Iberian ham slices from  $\alpha$ -tocopherol supplemented pigs showed lower levels of TBARS on days 6 and 9 of storage ( $p = 0.03$  and  $p = 0.05$ , respectively, Table 4). A significant effect of dietary

**Table 2.** Fatty acid composition of intramuscular neutral lipids in *Biceps femoris* samples of dry-cured hams from Iberian pigs fed different diets.

Fatty acid (g/100g of total FAMES)	Fat <sup>1</sup>			Vit E		Pooled SD	p-value		
	Mono	Medium	Poly	Basal	Suppl		Fat	Vit E	Fat* Vit E
C14:0	1.34	1.29	1.31	1.35	1.27	0.25	0.919	0.499	0.703
C16:0	21.41 <sup>b</sup>	24.65 <sup>a</sup>	23.91 <sup>a</sup>	22.86	23.79	2.16	0.044	0.413	0.846
C16:1 (n-9)	4.28	3.81	3.84	4.12	3.83	0.89	0.548	0.436	0.280
C18:0	8.80 <sup>b</sup>	9.80 <sup>b</sup>	12.10 <sup>a</sup>	9.98	10.48	1.19	0.002	0.330	0.314
C18:1 (n-9)	48.44	47.14	45.54	47.07	47.01	2.99	0.227	0.914	0.618
C18:1 (n-7)	1.62	2.16	3.57	2.14	2.76	1.51	0.057	0.341	0.357
C18:2 (n-6)	5.77	5.54	4.79	5.88	4.85	1.10	0.197	0.035	0.086
C18:3 (n-3)	0.73	0.49	0.44	0.63	0.47	0.24	0.066	0.131	0.566
C20:1 (n-9)	1.50	1.11	0.58	1.10	1.03	0.62	0.059	0.675	0.173
C20:4 (n-6)	1.10	1.13	0.65	0.96	0.96	0.44	0.082	0.991	0.836
C22:4 (n-6)	0.57	0.33	0.56	0.48	0.49	0.46	0.469	0.802	0.576
C22:5 (n-3)	1.22 <sup>a</sup>	0.69 <sup>b</sup>	0.40 <sup>b</sup>	0.87	0.67	0.40	0.005	0.239	0.203
C22:6 (n-3)	0.78	0.72	0.98	0.84	0.81	0.64	0.800	0.531	0.999
Σ SFA	31.95 <sup>b</sup>	36.03 <sup>a</sup>	37.53 <sup>a</sup>	34.47	35.87	3.13	0.012	0.323	0.642
Σ MUFA	56.40	54.30	53.75	54.79	54.84	2.99	0.272	0.944	0.490
Σ PUFA	11.65	9.67	8.72	10.73	9.29	2.27	0.065	0.208	0.673

<sup>1</sup>Mono: diet containing a high concentration of monounsaturated fatty acids (23.1–24.3g/kg d.m.); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3–21.4g/kg); Medium: diet with an intermediate concentration (16.7–18.4g monounsaturated fat and 13.8–14.0g polyunsaturated/kg d.m.), in each case with a basal or supplemented (Suppl) level of  $\alpha$ -tocopheryl acetate (200mg/kg feed).

Means with different superscript within a row shows statistical differences ( $p < 0.05$ ) between dietary fat treatments.

**Table 3.** Fatty acid composition of intramuscular polar lipids in *Biceps femoris* samples of dry-cured hams from Iberian pigs fed different diets.

Fatty acid (g/100g of total FAMES)	Fat <sup>1</sup>			Vit E		Pooled SD	P-value		
	Mono	Medium	Poly	Base	Suppl		Fat	Vit E	Fat* Vit E
C14:0	0.72	0.72	0.86	0.66	0.87	0.319	0.657	0.178	0.222
C16:0	15.49 <sup>b</sup>	17.85 <sup>a</sup>	18.66 <sup>a</sup>	16.87	17.80	2.308	0.030	0.257	0.051
C16:1 (n-9)	2.21	1.92	2.12	2.32	1.85	0.513	0.579	0.047	0.138
C18:0	9.87 <sup>b</sup>	11.47 <sup>a</sup>	11.72 <sup>a</sup>	10.97	11.07	0.930	0.003	0.798	0.252
C18:1 (n-9)	23.60	21.06	25.73	23.45	23.48	6.299	0.385	0.993	0.799
C18:1 (n-7)	4.53	4.18	4.61	4.46	4.42	0.483	0.230	0.845	0.543
C18:2 (n-6)	16.99	20.80	20.23	18.81	19.87	3.848	0.169	0.530	0.244
C18:3 (n-3)	2.77	2.98	1.91	2.82	2.29	1.300	0.266	0.355	0.387
C20:1 (n-9)	1.89	0.73	0.95	1.27	1.10	1.220	0.259	0.803	0.913
C20:4 (n-6)	5.98	6.13	5.05	5.88	5.56	1.436	0.314	0.606	0.340
C22:4 (n-6)	2.50	1.74	1.50	1.84	1.98	0.986	0.166	0.749	0.460
C22:5 (n-3)	2.06 <sup>a</sup>	1.84 <sup>b</sup>	1.22 <sup>b</sup>	1.83	1.58	0.559	0.026	0.328	0.021
C22:6 (n-3)	2.76 <sup>a</sup>	1.40 <sup>b</sup>	1.17 <sup>b</sup>	1.72	1.83	1.128	0.039	0.822	0.506
Σ SAT	27.49 <sup>b</sup>	31.78 <sup>a</sup>	31.67 <sup>a</sup>	29.73	30.89	2.213	0.003	0.186	0.181
Σ MUFA	34.46	28.32	33.74	32.30	32.05	5.866	0.153	0.742	0.571
Σ PUFA	38.04	39.90	34.59	37.96	37.06	6.973	0.330	0.877	0.455

<sup>1</sup>Mono: diet containing a high concentration of monounsaturated fatty acids (23.1–24.3g/kg d.m.); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3–21.4g/kg); Medium: diet with an intermediate concentration (16.7–18.4g monounsaturated fat and 13.8–14.0g polyunsaturated/kg d.m.), in each case with a basal or supplemented (Suppl) level of  $\alpha$ -tocopheryl acetate (200mg/kg feed).

Means with different superscript within a row shows statistical differences ( $p < 0.05$ ) between dietary fat treatments.

$\alpha$ -tocopherol supplementation was also observed for induced oxidation of dry-cured Iberian ham samples after 30, 60, 90 and 120 min of incubation ( $p = 0.001$ ,  $p = 0.011$ ,  $p = 0.001$  and  $p < 0.0001$ , respectively) samples of hams from supplemented pigs showing lower values in all cases.

Reduction of TBARS in fresh, frozen and cooked meats from pigs fed supplemented vitamin E levels are

well documented (Ashgar et al., 1989; Monahan et al., 1992; López-Bote et al., 1997). Lower levels of induced lipid oxidation have also been reported for meat from pigs fed supranutritional levels of vitamin E (Rey et al., 2001). The little degradation of  $\alpha$ -tocopherol during the ripening of dry-cured Iberian ham after 20 months, despite lipid oxidation occurring during processing (Antequera et al., 1992), explained the lower levels of

**Table 4.**  $\alpha$ -Tocopherol concentration, thiobarbituric acid reactive substances (TBARS) during refrigerated storage, iron-induced oxidation along incubation in *Biceps femoris* samples of dry-cured hams from Iberian pigs fed different diets.

	Fat <sup>1</sup>			Vit E		Pooled SD	P-value		
	Mono	Medium	Poly	Base	Suppl		Fat	Vit E	Fat* Vit E
$\alpha$ -tocopherol (mg/kg d.m.)	12.15	13.17	10.80	8.08	16.00	4.464	0.220	0.001	0.150
TBARS (mg MDA/kg)									
Day 0	1.19	1.24	1.11	1.16	1.21	0.10	0.06	0.23	0.48
Day 3	1.45	1.36	1.48	1.43	1.42	0.09	0.31	0.40	0.24
Day 6	1.73	1.80	1.68	1.78	1.69	0.15	0.31	0.03	0.47
Day 9	1.81	1.83	1.77	1.86	1.73	0.19	0.59	0.05	0.92
Induced oxidation (nmol MDA/mg protein)									
0 min	4.74	4.56	4.13	4.75	4.20	0.86	0.29	0.09	0.75
30 min	8.35	8.03	7.61	8.69	7.31	0.98	0.25	0.001	0.88
60 min	10.21	9.70	9.69	10.27	9.46	0.80	0.28	0.011	0.66
90 min	10.94	10.97	11.01	11.50	10.44	0.72	0.98	0.001	0.67
120 min	11.43	10.85	11.50	11.84	10.68	0.65	0.07	0.0001	0.98

<sup>1</sup>Mono: diet containing a high concentration of monounsaturated fatty acids (23.1–24.3 g/kg d.m.); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3–21.4 g/kg); Medium: diet with an intermediate concentration (16.7–18.4 g monounsaturated fat and 13.8–14.0 g polyunsaturated/kg d.m.) in each case with a basal or supplemented (Suppl) level of  $\alpha$ -tocopherol acetate (200 mg/kg feed).

Means with different superscript within a row shows statistical differences ( $p < 0.05$ ) between dietary fat treatments.

lipid oxidation in dry-cured Iberian ham slices during storage and in dry-cured Iberian ham samples in an induced oxidation test. The antioxidant effect of  $\alpha$ -tocopherol in other meat products, even after cooking, has been reported (Monahan et al., 1992; López-Bote et al., 1998b) which suggested low degradation even under pro-oxidant conditions. Direct exposition of dry-cured ham slices to air leads to rapid development of rancidity (Ruiz and López-Bote, 2002). Therefore, feeding supplemented levels of  $\alpha$ -tocopherol to Iberian pigs, due to its low degradation throughout processing, appears to be an interesting strategy for maintaining quality during storage of sliced dry-cured Iberian ham.

Neither TBARS during storage of dry-cured Iberian ham slices, nor levels of malonaldehyde (MDA) in an induced oxidation test showed any effect for dietary fat composition (Table 4). Previous studies reported a marked effect of dietary fat source on fresh meat lipid oxidation (Monahan et al., 1992; Lin et al., 1989). In those reports muscle fatty acid composition was markedly affected by dietary treatment, samples showing higher amount of polyunsaturated fatty acids developing faster and more intense lipid oxidation. However, in our work neither total MUFA nor total PUFA levels of neutral and polar lipids were significantly affected by dietary treatment. Thus, lipid oxidative changes in slices and induced oxidation samples were consistent with the dry-cured ham fatty acid composition found.

No significant effect of  $\alpha$ -tocopherol supplementation was observed in volatile aldehyde profile (Table 5). Conversely, dietary fat composition significantly affected hexanal, heptanal and total aldehyde content, dry-cured hams from Iberian pigs fed a diet high in PUFA showing higher values. This behaviour is consis-

tent with previous research on meat and meat products, in which products from animals fed higher amounts of linoleic acid (C18:2 n-6) showed higher levels of hexanal (Gandemer, 2002), the major compound from linoleic acid oxidation. Higher levels of hexanal have been linked to off flavour development in cooked meat (Skibsted et al., 1998) and also dry-cured ham (Ruiz et al., 1999). Therefore, feeding Iberian pigs with diets high in MUFA rather than in PUFA could be an interesting approach for obtaining dry-cured Iberian hams with lower rancid notes.

The effect of dietary treatment on the rate of weight loss in dry-cured Iberian ham samples placed in a controlled environment of reduced relative humidity was also investigated. Ham samples from Iberian pigs fed higher levels of vitamin E showed a tendency to lower weight loss than those from pigs fed a basal level of vitamin E (Table 6). Dietary fat showed a significant effect on weight loss on days 4, 5, 6 and 7 of storage, samples from Iberian pigs fed a diet enriched in monounsaturated fat showing lower weight losses in all cases.

Vitamin E dietary supplementation has been reported to markedly affect drip loss of fresh and thawed pork (Monahan et al., 1992). Ashgar et al. (1991) reported that frozen pork chops from pigs fed different dietary levels exhibited different rates of drip loss upon thawing and during refrigerated storage for 10 days. Several authors (Monahan et al., 1994; Ashgar et al., 1991) have suggested that  $\alpha$ -tocopherol could preserve the integrity of muscle cell membranes by preventing the oxidation of membrane phospholipids during storage, and this could inhibit the passage of sarcoplasmic fluid through the muscle cell membrane. Biological membranes function as important barriers

**Table 5.** Volatile aldehyde concentration in *Biceps femoris* of dry-cured hams from Iberian pigs fed different diets.

Aldehyde (mg/kg)	Fat <sup>1</sup>			Vit E		Pooled SD	P-value		
	Mono	Medium	Poly	Basal	Suppl		Fat	Vit E	Fat* Vit E
Butanal	1077.6	1129.1	1444.4	1107.7	1326.4	478	0.07	0.51	0.69
Pentanal	326.1	176.3	419.7	287.3	327.4	347	0.09	0.62	0.74
Hexanal	3360.1 <sup>b</sup>	2638.9 <sup>b</sup>	5574.5 <sup>a</sup>	2897.6	4818.1	2984	0.02	0.46	0.67
Heptanal	302.5 <sup>b</sup>	224.1 <sup>b</sup>	454.9 <sup>a</sup>	224.0	430.4	228	0.05	0.95	0.31
Octanal	115.0	109.3	272.5	133.0	198.2	115	0.08	0.40	0.47
Nonanal	252.4	273.7	393.7	274.2	339.0	114	0.91	0.26	0.18
Sum	5433.7 <sup>b</sup>	4551.5 <sup>b</sup>	8559.7 <sup>a</sup>	4923.8	7439.5	3865	0.02	0.45	0.64

<sup>1</sup>Mono: diet containing a high concentration of monounsaturated fatty acids (23.1–24.3g/kg d.m.); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3–21.4g/kg); Medium: diet with an intermediate concentration (16.7–18.4g monounsaturated fat and 13.8–14.0g polyunsaturated/kg d.m.), in each case with a basal or supplemented (Suppl) level of  $\alpha$ -tocopheryl acetate (200mg/kg feed).

Means with different superscript within a row shows statistical differences ( $p < 0.05$ ) between dietary fat treatments.

**Table 6.** *Biceps femoris* dry matter and weight loss of dry-cured ham samples during storage (stored at 20°C with a relative humidity of 75%) from Iberian pigs fed different diets.

	Fat <sup>1</sup>			Vit E		Pooled SD	P-value		
	Mono	Medium	Poly	Base	Suppl		Fat	Vit E	Fat* Vit E
Dry matter % loss initial weight	48.62	48.64	48.92	49.45	48.00	2.398	0.967	0.187	0.440
Weight loss									
Day 2	1.15	1.89	2.34	2.38	1.21	1.16	0.38	0.10	0.45
Day 3	1.49	2.03	3.58	3.12	1.62	2.16	0.18	0.12	0.61
Day 4	2.00 <sup>b</sup>	2.39 <sup>b</sup>	5.41 <sup>a</sup>	4.12	2.41	2.68	0.04	0.15	0.61
Day 5	2.56 <sup>b</sup>	2.59 <sup>b</sup>	6.07 <sup>a</sup>	4.52	2.96	2.63	0.03	0.18	0.47
Day 6	3.39 <sup>b</sup>	3.46 <sup>b</sup>	6.98 <sup>a</sup>	5.41	3.81	2.86	0.04	0.20	0.43
Day 7	4.39 <sup>b</sup>	4.23 <sup>b</sup>	8.02 <sup>a</sup>	6.36	4.73	3.16	0.05	0.24	0.50

<sup>1</sup>Mono: diet containing a high concentration of monounsaturated fatty acids (23.1–24.3g/kg d.m.); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3–21.4g/kg); Medium: diet with an intermediate concentration (16.7–18.4g monounsaturated fat and 13.8–14.0g polyunsaturated/kg d.m.), in each case with a basal or supplemented (Suppl) level of  $\alpha$ -tocopheryl acetate (200mg/kg feed).

Means with different superscript within a row shows statistical differences ( $p < 0.05$ ) between dietary fat treatments.

to changes that can affect the quality of foods of animal origin (Stanley, 1991). Nevertheless, less loss in weight caused by vitamin E supplementation could also be related to lower levels of protein insolubilisation, polymerisation and cross-linking due to interaction with oxidised lipids (Khayat and Schwall, 1983; Nakhost and Karel, 1984). Excessive drying of the surface negatively affects the appearance and acceptability of hams, with negative commercial implications (Ventanas et al., 2001). Therefore, supplementation with supranutritional levels of vitamin E could be a useful way of improving sliced dry-cured meat products for retailing.

Dietary fat unsaturation affected water migration in dry-cured meat products (Girard et al., 1989; López-Bote et al., 1998a). Excessive accumulation of SFA led to a high rate of water loss during ripening. On the other hand, the levels of PUFA in fatty tissues of pigs used for dry-cured ham production are critical since an excessive accumulation in tissues would need the use of a prolonged drying period in dry-cured products (Girard et al., 1989). Our results did not support these hypotheses. An explanation for the higher weight losses in samples from pigs fed the diet rich in PUFA

could be that, at least in part, those losses would be liquid fat, since these samples showed a softer and more fluid fat.

In conclusion, dietary fat source and  $\alpha$ -tocopheryl acetate supplementation influences several quality characteristics of dry-cured Iberian ham, including the concentration of  $\alpha$ -tocopherol, susceptibility to oxidation of sliced samples, induced lipid oxidation and volatile aldehyde content. Diets supplemented in  $\alpha$ -tocopherol and rich in monounsaturated fatty acids, rather than polyunsaturated ones, appeared as adequate dietary strategies for feeding Iberian hams reared indoors. However, further studies including sensory analysis and in-depth analysis of volatile profiles are required to optimise diet composition for Iberian pigs.

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## REFERENCES

- Antequera T., López-Bote C.J., Córdoba J.J., García C., Asensio M.A. and Ventanas J. (1992). Lipid oxidative changes in the processing of the Iberian pig hams. *Food Chemistry* **45**: 105–110.
- AOAC (1990). In: Williams S., *Official Methods of Analysis*. Arlington, VA: Association of Official Analytical Chemists.
- Ashgar A., Gray J.I., Booren A.M., Gomaa E.A., Abouzieed M.M., Miller E.R. and Buckley D.J. (1991). Effect of supranutritional dietary vitamin E levels on subcellular deposition of  $\alpha$ -tocopherol in the muscle and on pork quality. *Journal of the Science of Food and Agriculture* **57**: 31–41.
- Ashgar A., Lin C.F., Gray J.I., Buckley D.J., Booren A.M., Crackel R.L. and Flegal C.J. (1989). The influence of oxidized dietary oil and antioxidant supplementation on membrane-bound lipid stability in broiler meat. *British Poultry Science* **30**: 817–825.
- Buege J.A. and Aust S.D. (1978). Microsomal lipid peroxidation. In: Fleischer S. and Parker L. (eds), *Methods in Enzymology* Vol. 52. New York: Academic Press, pp. 302–314.
- Carrapiso A.I., Jurado A., Timón M.L. and García C. (2002). Odor-active compounds of Iberian hams with different aroma characteristics. *Journal of Agricultural and Food Chemistry* **50**: 1996–2000.
- Gandemer G. (2002). Lipids in muscles and adipose tissues, change during processing and sensory properties of meat products. *Meat Science* **62**: 309–321.
- García C., Ventanas J., Antequera T., Ruiz J., Cava R. and Alvarez P. (1996). Measuring sensorial quality of Iberian Ham by Rasch model. *Journal of Food Quality* **19**: 397–412.
- Girard J.P., Bucharles C., Berdague J.L. and Ramihone M. (1989). The influence of unsaturated fats on drying and fermentation processes in dry sausages. *Fleischwirtschaft* **69**: 255–260.
- Isabel B., Timón M.L., Cava R., García C., Ruiz J., Carmona J.M. and López-Bote C.J. (1999a). Dietary  $\alpha$ -tocopherol acetate supplementation modifies volatile aldehyde and sensory properties of dry-cured ham. *Irish Journal of Agricultural and Food Research* **38**: 137–142.
- Isabel B., López-Bote C.J., Rey A.I. and Sanz R. (1999b). Influence of dietary  $\alpha$ -tocopherol acetate supplementation on oxidative deterioration and weight loss in sliced dry-cured ham. *Meat Science* **51**: 227–232.
- ISO (1973). *Meat and meat products – determination of ash and moisture content*. Geneva, Switzerland: International Standard Organization.
- Kemp J.D., Langlois B.E., Akers K., Means W.J. and Aaron D.K. (1988). Effect of storage-temperature and time on the quality of vacuum packaged dry-cured ham slices. *Journal of Food Science* **53**: 402–406.
- Khayat A. and Schwall D. (1983). Lipid oxidation in seafood. *Food Technology* **7**: 130–140.
- Konbrust D.J. and Mavis R.D. (1980). Relative susceptibility of microsomes from lung, heart, kidney, brain and testes to lipid peroxidation: correlation with vitamin E content. *Lipids* **15**: 315–322.
- Lin C.F., Gray J.I., Ashgar A., Buckley D.J., Booren A.M. and Flegal C.J. (1989). Effect of dietary oils and  $\alpha$ -tocopherol supplementation on lipid peroxidation in broiler meat. *Journal of Food Science* **54**: 1457–1460.
- López M.O., de la Hoz L., Cambero M.I., Gallardo E., Reglero G. and Ordóñez J.A. (1992). Volatile compounds of dry hams from Iberian pigs. *Meat Science* **31**: 267–277.
- López-Bote C.J., Gomaa E., Gray J.I. and Flegal C.J. (1998a). Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. *British Poultry Science* **39**: 235–240.
- López-Bote C.J., Isabel B. and Rey A.I. (1998b). Alimentación del cerdo Iberico y calidad de la producción carnica. *Anaporc* **177**: 52–73.
- López-Bote C.J., Rey A., Sanz M., Gray J.I. and Buckley J.D. (1997). Dietary vegetable oils and  $\alpha$ -tocopherol reduce lipid oxidation in rabbit muscle. *Journal of Nutrition* **127**: 1176–1182.
- Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with the Folin-phenol reagent. *Journal of Biological Chemistry* **193**: 265–275.
- Marmer W.N. and Maxwell R.J. (1981). Dry column method for the quantitative extraction and simultaneous class separation of lipids from muscle-tissue. *Lipids* **16**: 365–371.
- Martín L., Córdoba J.J., Ventanas J. and Antequera T. (1999). Changes in intramuscular lipids during ripening of Iberian dry-cured ham. *Meat Science* **51**: 129–134.
- Monahan F.J., Buckley D.J., Morrissey P.A., Lynch P.B. and Gray J.I. (1992). Influence of dietary fat and  $\alpha$ -tocopherol supplementation on lipid oxidation in pork. *Meat Science* **31**: 229–241.
- Monahan F.J., Gray J.I., Ashgar A., Haug A., Strasburg G.M., Buckley D.J. and Morrissey P.A. (1994). Influence of diet on lipid oxidation and membrane structure in porcine muscle microsomes. *Journal of Agricultural and Food Chemistry* **42**: 59–63.
- Morrissey P.A., Buckley D.J., Sisk H., Lynch P.B. and Sheehy P.J.A. (1996). Uptake of  $\alpha$ -tocopherol in porcine plasma and tissues. *Meat Science* **44**: 275–283.
- Nakhost Z. and Karel M. (1984). Measurement of oxidation-related changes in proteins of freeze-dried meats. *Journal of Food Science* **49**: 1171–1173.
- Rey A., López-Bote C., Soares M. and Isabel B. (1996). Determination of  $\alpha$ -tocopherol in pork with high intramuscular fat content. *Grasas Aceites* **47**: 331–334.
- Rey A.I. and López-Bote C.J. (2000). Effect of dietary copper and vitamin E supplementation, and extensive feeding with acorn and grass on longissimus muscle composition and susceptibility to oxidation in Iberian pigs. *Journal of Animal Physiology and Animal Nutrition* **85**: 281–292.
- Rey A.I., Kerry J.P., Lynch P.B., López-Bote C.J., Buckley D.J., Morrissey P.A. (2001). Effect of dietary oils and  $\alpha$ -tocopherol acetate supplementation on lipid

- (TBARS) and cholesterol oxidation in cooked pork. *Journal of Animal Science* **79**: 1201–1208.
- Ruiz J. and López-Bote C. (2002). Improvement of dry-cured ham quality by lipid modification through dietary means. In: Toldrá F. (ed.), *Research Advances in the Quality of Meat and Meat Products*. Research Signpost, India: Trivandrum, pp. 255–271.
- Ruiz J., Cava R., Antequera T., Martín L., Ventanas J. and López-Bote C.J. (1998). Prediction of the feeding background of Iberian pigs using the fatty acid profile of subcutaneous, muscle and hepatic fat. *Meat Science* **49**: 155–165.
- Ruiz J., García C., Muriel E., Andrés A.I. and Ventanas J. (2002). Influence of sensory characteristics on the acceptability of dry-cured ham. *Meat Science* **61**: 347–354.
- Ruiz J., Ventanas J., Cava R., Andrés A.I. and García C. (1999). Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. *Meat Science* **52**: 19–27.
- Salih A.M., Smith D.M., Price J.F. and Dawson L.E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Science* **66**: 1483–1488.
- SAS (1999). SAS Institute, Inc. SAS User's guide: Statistics. Cary, NC: SAS Institute Inc.
- Skibsted L.H., Mikkelsen A. and Bertelsen G. (1998). Lipid derived off-flavours in meat. In: Shahidi F. (ed.), *Flavor of Meat, Meat Products and Seafood*, London: Blackie Academic and Professional, pp. 217–256.
- Stanley D.W. (1991). Biological membrane deterioration and associated quality losses in food tissues. *Critical Reviews in Food Science and Nutrition* **30**: 487–553.
- Sukhija P.S. and Palmquist D.L. (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and faeces. *Journal of Agricultural and Food Chemistry* **36**: 1202–1206.
- Ventanas J., Ruiz J. and Córdoba J.J. (2001). El jamon curado de cerdo Iberico: descripcion del proceso tradicional de elaboracion. In: Ventanas J. (ed.), *Tecnología del Jamon Ibérico*. Madrid: Mundi-Prensa, pp. 45–72.