140 J. Agric. Food Chem. 2009, 57, 140–146

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Changes in the Fatty Acid Composition of *M. longissimus dorsi* of Lamb during Storage in a High-Oxygen Modified Atmosphere at Different Levels of Dietary Vitamin E Supplementation

Inma Álvarez,[†] Jesús De La Fuente,[‡] Vicente Cañeque,[†] Sara Lauzurica,[‡] Concha Pérez,[§] and María Teresa Díaz*[,†]

Departamento de Tecnología de los Alimentos, INIA, Ctra. De la Coruña km. 7.5, 28040, Madrid, Spain, and Departamento de Producción Animal and Departamento de Fisiología (Fisiología Animal), Facultad de Veterinaria (UCM), Avda. Puerta de Hierro, s/n, 28040, Madrid, Spain

The effect of vitamin E supplementation on fatty acid composition during storage of lamb meat packed in a high-oxygen modified atmosphere was studied. Lambs were fed with diets supplemented with four levels of vitamin E (0, 250, 500, and 1000 mg acetate-α-tocopherol/kg feed). Slices of *M. longissimus dorsi* were packed in a high-oxygen modified atmosphere (70% O_2:30% CO_2) and stored at 2 ± 1 °C in the dark for 14, 21, and 28 days. The nonsupplemented group (E0) showed an increase in saturated fatty acids (SFA) and monounsaturated fatty acids and a reduction in polyunsaturated fatty acids (PUFA) in total fatty acids. The proportion of SFA and PUFA was unchanged in the supplemented groups (E250, E500, and E1000). The supplementation with 250 mg acetate-α-tocopherol/kg feed prevented the oxidation of PUFA in the total lipids and in the polar lipids fraction, while 500 mg acetate-α-tocopherol/kg feed was necessary to prevent the oxidation of PUFA in free fatty acids. Supplementing vitamin E not only inhibited lipid oxidation but also maintained nutritional value [essential fatty acids, linoleic acid (C18:2*n*-6) and linolenic acid (C18:3*n*-3), and beneficial fatty acids, eicosapentaenoic acid (C20:5*n*-3) and docosahexaenoic acid (C22:6*n*-3)] throughout the storage period.

KEYWORDS: Lamb meat; vitamin E; fatty acids; lipid fractions; modified atmosphere packaging

INTRODUCTION

Fresh lamb meat is generally sold chilled to a temperature of 4 °C. The shelf life of refrigerated lamb meat does not exceed 10 days (*1*), after which spoilage can be evident. To provide longer storage times, meat is stored in modified atmosphere packaging. Modified atmosphere retail packaging with a high level of oxygen (70−80%) preserves the bright red color of fresh meat (*2*). In addition, high-oxygen modified atmosphere packaging prevents microbial growth (*3*). However, high concentrations of oxygen may increase levels of lipid oxidation in meat, giving rise to oxidative rancidity and discoloration. As a result, lipid oxidation is one of the major causes of quality deterioration in meat packed at a high-oxygen concentration. Lipid oxidation can alter flavor, color, and texture (*4*) and reduce nutritional value and safety of meat.

* To whom correspondence should be addressed. Tel: +34 913474038. Fax: +34 913572293. E-mail: diaz.teresa@inia.es.

[†] Departamento de Tecnología de los Alimentos, INIA.

[‡] Departamento de Producción Animal, Facultad de Veterinaria (UCM).

[§] Departamento de Fisiología (Fisiología Animal), Facultad de Veterinaria (UCM).

Some authors (*5, 6*) have demonstrated that the fatty acid profile of meat could be modified during storage by hydrolytic and oxidative processes. The oxidative stability of lipid fractions is associated with their fatty acid composition. Polar lipids have a high content of unsaturated fatty acids and are therefore more susceptible to oxidation than triglycerides, rich in saturated fatty acids (SFA) (*7*). Because the susceptibility of unsaturated fatty acids to oxidation is related to the degree and the content of unsaturation, polyunsaturated fatty acids (PUFA) are more prone to be oxidized than monounsaturated fatty acids (MUFA) (*8*). In this way, lipid oxidation could reduce PUFA essential fatty acids (C18:2*n*-6 and C18:3*n*-3) and long-chain PUFA (C20:5*n*-3 and C22:6*n*-3). Long-chain PUFA play an important role in the development of cerebral and retinal tissues and in the prevention of heart diseases and some cancers (*9*).

Furthermore, the process of lipid oxidation leads to vitamin losses, such as vitamin A, carotenoids, vitamin C, and vitamin E (VE), due to the free radicals generated by the process that co-oxidizes these vitamins (*10*). On the other hand, oxidation of muscle lipids results in the production of oxidation products, such as aldehydes and oxysterols, which could have an adverse effect on human health (*11*).

10.1021/jf801940c CCC: $40.75 © 2009 American Chemical Society
Published on Web 12/18/2008

Deterioration caused by lipid oxidation may be reduced by incorporating natural antioxidants. Antioxidants delay or inhibit the process of oxidation, even at low concentrations. VE, a natural antioxidant, functions as a lipid-soluble antioxidant in cell membranes. Many studies have shown that VE supplementation improves the stability of lipids and color in beef (*12*), pork (*13*), and lamb (*14*). The concentration of muscle VE via dietary supplementation depends on the level of supplementation, the muscle evaluated, and the active form of the VE supplemented (*12*).

The aforementioned studies of lamb meat have described the oxidation changes through the evaluation of the thiobarbituric acid reactive substances (TBARS), but until now, no studies have appeared on fatty acid composition during storage and the possible effect of VE.

Part of this research was published in a previous paper by Lauzurica et al. (*15*). That paper (*15*) described the effect of dietary VE supplementation on α-tocopherol deposition and TBARS, color, and bacterial load of lamb meat packed under a modified atmosphere. The objective of the present study was to report the modifications in fatty acid composition of lamb meat during storage in high-oxygen modified atmosphere packaging, in relation to different levels of dietary VE supplementation. To carry out more precise examination on unsaturated fatty acids, which are mainly concentrated in the polar lipid fraction, the fatty acid compositions of the neutral and polar lipids and the free fatty acid fractions were evaluated.

MATERIALS AND METHODS

Feeding Experiments. Thirty-six weaned male Manchego breed lambs were assigned to four treatment groups of nine animals each. Four levels of VE (0, 250, 500, and 1000 mg acetate-α-tocopherol/kg feed) were added to a basal diet containing 20 mg VE/kg feed. Thus, four experimental diets were obtained as follows: E0, E250, E500, and E1000. The basal diet contained (g/kg feed) the following: barley, 281.9; corn meal, 200; soybean meal, 171.5; corn (whole shelled), 127.7; wheat, 120; oats, 50; fat, 23; limestone, 15.9; salt, 6; sodium bicarbonate, 2; and mineral and vitamin premix, 2. Feed, water, and barley straw were offered ad libitum throughout the fattening period. The lambs were individually penned (1 m^2). The fattening period lasted an average of 37 ± 1.5 days, from an initial weight of 13.2 ± 0.5 kg to a slaughter weight of 26.2 ± 0.3 kg, a usual commercial slaughter weight in Spain. Lambs were slaughtered in a commercial abattoir in accordance with commercial practices.

Samples. After the carcasses were chilled for 24 h at 4 °C, *M. longissimus dorsi* from the right half of the carcasses was dissected and cut into four slices randomly assigned to one of four storage periods (SPs): 0, 14, 21, and 28 days. Muscle slices for 0 days of storage were vacuum-packed in metallic pouches and then frozen at −20 °C for subsequent analyses. Muscle slices for 14, 21, and 28 days were packed in a modified atmosphere (MAP) using BB41 pouches (150 μm, polyamide/polyethylene, 50/100, Cryovac) with low gas permeability (7 cc/m^2/24 h O_2 at 4 °C and 80% relative humidity, 150 cc/m^2/24 h O_2 at 23 °C and 75% relative humidity) and low water vapor transmission rate (1.5 g/m^2/24 h at 38 °C and 100% relative humidity) and flushed with 70% O_2 and 30% CO_2 (EAP 20, Carburos Metálicos, S.A.) with a 2:1 gas volume to meat ratio in each pack. Samples were kept in the darkness at 2 ± 1 °C. After the SP, slices were vacuum-packed in metallic pouches and frozen at −20 °C for subsequent fatty acid composition analyses.

Lipid Extraction. Intramuscular fat was extracted from the muscle samples according to Hanson and Olley (*16*). Total lipids extracts were dried in a rotatory evaporator. The fat obtained was used for fatty acid composition of total lipids and for lipid fractionation.

Lipid Extracts Fractionation. For further fatty acid composition characterization, total lipid extracts were fractionated into neutral lipids, polar lipids, and free fatty acids (*17*). An aliquot of the total lipid extract was applied to amino-propylsilica minicolumn (Isolute NH_2, 500 mg, 10 mL) to separate neutral lipids (10 mL of chloroform:2-propanol 2:1), free fatty acids (10 mL of 2% acetic acid in diethyl ether), and polar lipids (10 mL of 2% HCL−methanol in 0.5 N methanol).

Fatty Acid Composition. Fatty acid methyl esters (FAMEs) of the total lipids and lipid fractions were formed in accordance with the Morrison and Smith method (*18*), using 14% boric trifluoride in methanol. Chromatographic analysis of FAMEs was performed using a Perkin-Elmer gas chromatograph (Perkin-Elmer, United States) equipped with a split-splitless injector and a flame ionization detector (FID) and a fused silica capillary column (0.32 mm internal diameter and 30 m long). The mobile phase consisted of helium C-50 at a flow of 9 psig. Fatty acids were identified using reference standards. Data regarding fatty acid composition were expressed in percentages by weight of the total of FAMEs identified.

Statistical Analysis. All data were analyzed using the Statistical Analysis System (SAS) package (*19*). A split-plot design was used in which dietary VE supplementation level was examined as a main plot effect, and SP, a repeated measurement, was examined as a subplot effect. The lowest Bayesian Information Criterion was used to choose the matrix of the error structure among the following structures: compound symmetry, first-order autoregressive, first-order heterogeneous autoregressive, and unstructured. When main effects (VE or SP) or interaction were significant, planned comparisons of means were performed using the Dunn-Šidák method (*20*) (comparing dietary supplementation levels for each SP and SPs for each dietary supplementation level, $p \leq 0.05$).

RESULTS AND DISCUSSION

The concentration of α-tocopherol in meat increased as VE supplementation in diet increased (*15*). Thus, the α-tocopherol concentration in E0 was 0.95 mg/kg meat, whereas in E250 and E500, it was over two times greater (2.17 and 2.67 mg/kg meat, respectively). In E1000 samples, the α-tocopherol concentration was more than 3.75 times greater (3.57 mg/kg meat) E0 (*15*).

Total Fatty Acids. Table 1 shows the fatty acid proportions of intramuscular fat expressed as a percentage of the total of FAMEs identified.

Initially (day 0), the profile of fatty acids in intramuscular fat was similar in the four experimental groups, with mean proportions of 41.55% for SFA, 41.11% for MUFA, and 17.34% for PUFA. The meat fatty acid composition is mainly affected by the fatty acid composition of animal diet (*21*). The experimental diets in the present study were carried out with the same raw materials, and composition only varied in the content of VE [data shown in Lauzurica et al.(*15*)]. Salvatori et al.(*22*) studied the effect of the VE supplementation on the fatty acid composition of lamb meat and found no differences between the supplementation groups.

A significant interaction was observed between the level of supplementation and SP for SFA proportion ($p = 0.003$) and the major SFA, palmitic acid (C16:0)($p = 0.019$); MUFA proportion ($p = 0.007$) and the major MUFA, oleic acid (C18:1) ($p = 0.014$); and PUFA proportion ($p = 0.0002$) and all PUFA individually, except for docosapentaenoic acid (C22:5*n*-3). The fatty acid proportions changed in different ways during the SP in the experimental groups. Thus, in the nonsupplemented group (E0), the SFA proportion increased from 0 to 28 days, mainly due to the increase in C16:0. The MUFA proportion also increased from 0 to 14 days, mainly due to the increase in C18:1. After 14 days of storage, a reduction of the PUFA proportion was observed in E0 due to the reduction of all PUFA individually, except C22:5*n*-3. Because of PUFA reduction, SFA and MUFA increased since they are expressed as proportions. The reduction in the proportion of PUFA could be related to oxidative reactions. Monin et al.(*6*) obtained an increase in SFA

Table 1. Effect of VE Supplementation on Fatty Acid Composition (Grams Per 100 g of FAMEs) during the SP of Meat Packed in MAP (LS Means ± Standard Errors)[a]

		VE				p		
		n = 9						
fatty acid	SP (days)	E0	E250	E500	E1000	VE	SP	VE × SP
SFA	0	41.7 ± 0.71 x	42.2 ± 0.86	41.4 ± 0.68	40.9 ± 0.68	0.0017	0.0011	0.0029
	14	42.6 ± 0.75 x	43.3 ± 0.86	39.9 ± 0.74	39.9 ± 0.70			
	21	43.5 ± 0.78 a,xy	43.3 ± 0.81 ab	39.6 ± 0.77 b	40.7 ± 0.68 ab			
	28	46.0 ± 0.83 a,y	42.4 ± 0.86 ab	41.6 ± 0.70 b	42.0 ± 0.70 b			
C14:0	0	2.69 ± 0.15	2.81 ± 0.17	2.75 ± 0.14	2.71 ± 0.14	0.7380	0.2268	0.8479
	14	2.60 ± 0.15	2.71 ± 0.17	2.52 ± 0.15	2.55 ± 0.14			
	21	2.66 ± 0.16	2.79 ± 0.17	2.47 ± 0.15	2.56 ± 0.14			
	28	2.82 ± 0.17	2.70 ± 0.17	2.59 ± 0.14	2.63 ± 0.14			
C16:0	0	23.6 ± 0.45 x	23.9 ± 0.53	23.5 ± 0.43	22.7 ± 0.43	0.0095	0.0001	0.0192
	14	24.5 ± 0.46 xy	24.4 ± 0.53	23.3 ± 0.45	22.8 ± 0.44			
	21	25.1 ± 0.48 yz	24.6 ± 0.51	23.2 ± 0.47	23.1 ± 0.43			
	28	26.2 ± 0.50 a,z	24.5 ± 0.53 ab	23.9 ± 0.44 b	23.9 ± 0.44 b			
C18:0	0	11.8 ± 0.42	11.9 ± 0.49	11.7 ± 0.40	11.9 ± 0.40	0.2714	0.0001	0.1772
	14	12.9 ± 0.43	13.1 ± 0.51	11.8 ± 0.42	12.1 ± 0.41			
	21	13.1 ± 0.44	13.4 ± 0.47	11.7 ± 0.43	12.5 ± 0.41			
	28	13.8 ± 0.48	13.1 ± 0.49	12.6 ± 0.41	12.8 ± 0.41			
MUFA	0	40.6 ± 0.84 x	41.4 ± 0.97 x	40.4 ± 0.79 x	42.1 ± 0.79 x	0.4300	0.0001	0.0074
	14	43.8 ± 0.85 y	43.8 ± 0.97 y	41.9 ± 0.82 xy	43.9 ± 0.81 y			
	21	44.6 ± 0.87 y	42.6 ± 0.95 xy	42.3 ± 0.83 y	43.5 ± 0.79 xy			
	28	43.4 ± 0.89 y	43.2 ± 0.97 xy	41.5 ± 0.81 xy	42.5 ± 0.81 xy			
C16:1	0	1.65 ± 0.07	1.70 ± 0.08	1.56 ± 0.07	1.60 ± 0.07	0.4137	0.9308	0.8489
	14	1.65 ± 0.07	1.71 ± 0.08	1.52 ± 0.07	1.59 ± 0.07			
	21	1.70 ± 0.07	1.63 ± 0.08	1.54 ± 0.07	1.59 ± 0.07			
	28	1.65 ± 0.08	1.70 ± 0.08	1.55 ± 0.07	1.55 ± 0.07			
C18:1	0	38.0 ± 0.78 x	38.9 ± 0.90 x	38.0 ± 0.74 x	39.7 ± 0.74 x	0.4520	0.0001	0.0141
	14	41.2 ± 0.79 y	41.1 ± 0.89 y	39.6 ± 0.75 y	41.3 ± 0.74 y			
	21	42.0 ± 0.80 y	40.1 ± 0.88 xy	39.9 ± 0.76 y	41.1 ± 0.74 xy			
	28	41.0 ± 0.84 y	40.5 ± 0.90 xy	39.2 ± 0.75 xy	40.0 ± 0.75 xy			
PUFA	0	17.7 ± 1.05 x	16.5 ± 1.23	18.2 ± 0.99	17.0 ± 0.99	0.0085	0.0001	0.0002
	14	13.7 ± 1.08 y	13.0 ± 1.23	18.3 ± 1.05	16.3 ± 1.02			
	21	11.9 ± 1.12 a,y	14.1 ± 1.19 ab	18.1 ± 1.09 b	15.8 ± 0.99 ab			
	28	10.6 ± 1.17 a,y	14.3 ± 1.23 ab	16.9 ± 1.02 b	15.5 ± 1.02 ab			
C18:2*n*-6	0	9.60 ± 0.61 x	8.87 ± 0.71 x	10.40 ± 0.58	8.42 ± 0.58	0.0119	0.0001	0.0005
	14	8.10 ± 0.62 ab,y	7.23 ± 0.7 a,y	10.53 ± 0.60 b	8.10 ± 0.59 ab			
	21	7.34 ± 0.63 a,y	7.83 ± 0.70 ab,xy	10.44 ± 0.61 b	8.04 ± 0.58 ab			
	28	6.91 ± 0.65 a,y	8.00 ± 0.71 ab,xy	9.88 ± 0.59 b	8.06 ± 0.59 ab			
C18:3*n*-3	0	0.50 ± 0.05 x	0.48 ± 0.05	0.45 ± 0.04 x	0.43 ± 0.04	0.6983	0.0030	0.0001
	14	0.47 ± 0.04 x	0.47 ± 0.05	0.54 ± 0.04 y	0.48 ± 0.04			
	21	0.46 ± 0.06 x	0.48 ± 0.06	0.51 ± 0.04 xy	0.47 ± 0.05			
	28	0.35 ± 0.05 y	0.49 ± 0.05	0.51 ± 0.04 xy	0.41 ± 0.04			
C20:3*n*-6	0	0.55 ± 0.04 x	0.38 ± 0.05	0.44 ± 0.04	0.48 ± 0.04	0.0175	0.0004	0.0036
	14	0.36 ± 0.04 y	0.32 ± 0.05	0.42 ± 0.04	0.49 ± 0.04			
	21	0.30 ± 0.04 y	0.32 ± 0.05	0.42 ± 0.04	0.46 ± 0.04			
	28	0.25 ± 0.05 y	0.31 ± 0.05	0.46 ± 0.04	0.36 ± 0.04			
C20:4*n*-6	0	4.77 ± 0.37 x	4.94 ± 0.42 x	5.17 ± 0.34	5.47 ± 0.34	0.0001	0.0001	0.0012
	14	3.19 ± 0.37 a,y	3.41 ± 0.42 ab,y	5.00 ± 0.36 b	5.19 ± 0.35 b			
	21	2.56 ± 0.39 a,y	3.78 ± 0.40 ab,xy	5.00 ± 0.38 b	4.92 ± 0.34 b			
	28	1.92 ± 0.41 a,y	3.96 ± 0.42 b,xy	4.43 ± 0.35 b	4.74 ± 0.35 b			
C20:5*n*-3	0	0.61 ± 0.11 x	0.64 ± 0.10 x	0.41 ± 0.08	0.44 ± 0.09	0.6542	0.0001	0.0156
	14	0.31 ± 0.05 xy	0.28 ± 0.06 y	0.35 ± 0.05	0.42 ± 0.05			
	21	0.24 ± 0.05 y	0.31 ± 0.06 y	0.35 ± 0.05	0.38 ± 0.05			
	28	0.20 ± 0.07 y	0.35 ± 0.07 xy	0.32 ± 0.05	0.45 ± 0.05			
C22:5*n*-3	0	0.47 ± 0.14	0.59 ± 0.16	0.75 ± 0.13	0.64 ± 0.13	0.0036	0.0368	0.1571
	14	0.63 ± 0.07	0.63 ± 0.08	0.83 ± 0.06	0.85 ± 0.06			
	21	0.51 ± 0.08	0.70 ± 0.09	0.79 ± 0.08	0.82 ± 0.08			
	28	0.36 ± 0.08	0.70 ± 0.09	0.76 ± 0.07	0.78 ± 0.07			
C22:6*n*-3	0	0.29 ± 0.03 x	0.22 ± 0.04	0.23 ± 0.03	0.26 ± 0.03	0.1390	0.0638	0.0002
	14	0.21 ± 0.03 y	0.20 ± 0.03	0.27 ± 0.03	0.31 ± 0.03			
	21	0.15 ± 0.03 y	0.22 ± 0.03	0.27 ± 0.03	0.26 ± 0.03			
	28	0.11 ± 0.03 y	0.25 ± 0.03	0.23 ± 0.03	0.23 ± 0.03			
PUFA/SFA	0	0.43 ± 0.03 x	0.40 ± 0.04	0.44 ± 0.03	0.42 ± 0.03	0.0034	0.0001	0.0011
	14	0.32 ± 0.03 ab,y	0.30 ± 0.04 a	0.46 ± 0.03 b	0.41 ± 0.03 ab			
	21	0.27 ± 0.03 a,y	0.33 ± 0.03 ab	0.46 ± 0.03 b	0.39 ± 0.03 ab			
	28	0.23 ± 0.03 a,y	0.34 ± 0.04 ab	0.41 ± 0.03 b	0.37 ± 0.03 ab			

[a] a,b: Different letters within the same row indicate significant difference ($p \leq 0.05$). x,y,z: Different letters within the same column indicate significant difference ($p \leq 0.05$).

and MUFA proportions and a reduction in the PUFA proportion in pork meat stored under aerobic conditions for 9 days, and they suggested that this decrease in PUFA was the result of lipid oxidation.

Table 2. Effect of VE Supplementation on Fatty Acids (Grams Per 100 g of Total FAMEs) in the Lipid Fractions during the SP of Meat Packed in MAP (LS Means ± Standard Errors)

		VE				*p*		
		$n = 9$						
fatty acids in	SP (days)	E0	E250	E500	E1000	VE	SP	VE × SP
neutral lipids	0	63.3 ± 2.32	62.7 ± 2.88	61.4 ± 2.07	60.7 ± 2.19	0.0369	0.6847	0.7531
	14	64.6 ± 2.45	64.0 ± 3.16	60.5 ± 2.57	57.7 ± 2.29			
	21	66.7 ± 3.11	66.4 ± 2.89	59.1 ± 2.78	58.7 ± 2.29			
	28	69.3 ± 3.11	64.0 ± 3.17	61.1 ± 2.28	59.8 ± 2.58			
polar lipids	0	35.4 ± 2.23	36.1 ± 2.77	37.3 ± 2.00	37.9 ± 2.10	0.0449	0.0863	0.8060
	14	32.7 ± 2.36	31.0 ± 3.05	36.7 ± 2.48	39.2 ± 2.21			
	21	30.2 ± 3.00	30.5 ± 2.78	37.5 ± 2.69	37.7 ± 2.21			
	28	27.8 ± 2.30	32.9 ± 3.06	35.1 ± 2.19	36.1 ± 2.49			
free fatty acids	0	1.33 ± 0.13	1.26 ± 0.17	1.23 ± 0.12	1.37 ± 0.13	0.0662	0.0001	0.4074
	14	2.64 ± 0.17	3.04 ± 0.23	2.78 ± 0.19	3.08 ± 0.16			
	21	3.10 ± 0.43	3.05 ± 0.39	3.46 ± 0.38	3.56 ± 0.31			
	28	3.05 ± 0.37	3.07 ± 0.37	3.81 ± 0.26	4.13 ± 0.30			

The reduction (around 60%) in long chain PUFA (C20:4*n*-6, C20:5*n*-3, and C22:6*n*-3) was higher than the reduction (around 30%) in medium chain PUFA with a lower degree of unsaturation (C18:2*n*-6 and C18:3*n*-3). As expected, the fatty acids most prone to be oxidized were those with longer chains and with a higher degree of unsaturation (*23*).

Meat is refrigerated to increase its shelf life and reduce microbial spoilage. However, even when meat is stored at low temperatures, biochemical changes, lipolysis, and lipid oxidation develop after slaughtering in muscle lipids, limiting meat shelf life (*24*). In our study, the use of packaging at a high-oxygen concentration could have accelerated the lipid oxidation of the intramuscular fat during storage (*25*), particularly when the concentration of antioxidant, VE, in meat was low (group E0).

In the supplemented groups (E250, E500, and E1000), the proportion of SFA and PUFA did not change during the SP, whereas the proportion of MUFA showed a slight increase from 0 to 14 days in E250 and E1000 and from 0 to 21 days in E500. Thus, the increase in the concentration of α-tocopherol in meat via dietary supplementation protected against the oxidation of PUFA, starting at a level of supplementation of 250 mg/kg feed. Previous papers (*15, 26*) have shown that VE is effective in the reduction of the oxidation processes measured by TBARS values. Meat and meat products from animals supplemented with VE had lower TBARS values than nonsupplemented animals. Nevertheless, these papers have not evaluated changes in fatty acid composition.

Within the PUFA, C18:2*n*-6 and C18:3*n*-3 are considered essential fatty acids for humans (*27*), and C20:5*n*-3 and C22:6*n*-3 fatty acids have beneficial effects on health (*9*). Thus, a reduction in the proportion of these fatty acids could be considered a reduction in the nutritional value of the meat. According to our results, supplementation with 500 and 1000 mg acetate-α-tocopherol/kg feed kept the nutritional value of meat stable throughout the SP, while without supplementation, the nutritional value of the meat decreased.

In addition, the changes occurring in the fatty acid profile produced a modification in PUFA/SFA ratio (**Table 1**) during storage. Thus, the PUFA/SFA ratio decreased in the nonsupplemented group from an initial value of 0.43 to 0.23 after 28 days of storage. The PUFA/SFA ratio is an indicator of the nutritional quality of the lipids in a food (*28, 29*). The PUFA/SFA ratio must be near 0.45 (*28*), and lower values indicated less nutritional quality of the lipids. Therefore, the meat from nonsupplemented lambs was less healthy after a period of storage than the meat at the beginning of the storage.

Fatty Acids Proportion in the Lipid Fractions. Table 2 shows the changes of fatty acid proportions of neutral lipids, polar lipids, and free fatty acids fractions.

Initially, 62% of the total fatty acids of intramuscular fat was in the neutral lipids fraction. The neutral lipids were 90% triglycerides, and the rest were cholesterol esters, cholesterol, monoglycerides, and diglycerides (*17*). On the other hand, 36% of the total fatty acids was in the polar lipids fraction. This fraction is mainly composed of phospholipids (*30*). Less than 1.5% of the total fatty acids was free fatty acids at the beginning of storage, since fatty acids are predominantly part of more complex lipid structures and are hardly ever free (*31*).

VE supplementation showed a significant effect on the proportion of fatty acids in the neutral ($p = 0.037$) and polar lipids ($p = 0.045$) fractions (**Table 2**). The proportion of fatty acids in neutral lipids decreased as the level of VE supplementation increased from 66.0 ± 1.83% for E0 to 59.3 ± 1.63% for E1000 (data not shown in the table), whereas the proportion of fatty acids in polar lipids increased as supplementation increased (from 31.5 ± 1.74% for E0 to 37.7 ± 1.55% for E1000, data not shown in the table).

The SP (**Table 2**) did not significantly affect the proportion of fatty acids in neutral and polar lipids. However, the proportion of free fatty acids increased during storage ($p = 0.0001$), from 1.30 ± 0.07% at day 0 to 2.88 ± 0.10% at 14 days and to 3.51 ± 0.16% at 28 days (data not shown in the table). Previous studies showed an increase in free fatty acids during refrigerated storage of fresh pork (*6*), rabbit (*32*), and chicken (*33*) meat and during the ham curing process (*34*). Some specific enzymes of the adipose tissue and muscle fibers (lipases, sterases, phospholipases, and lysophospholipases) are involved in the hydrolytic processes of muscle lipids during refrigeration (*35*) that release free fatty acids from both neutral and polar lipids (*32, 33*). Thus, the increase in free fatty acids in meat shows the degree of lipolysis during storage (*31*).

In our study, the contribution of neutral and polar lipids to the increase in free fatty acids during storage is not clear. Alasnier et al.(*33*), in studies on refrigerated fresh chicken meat, found that neutral and polar lipids contributed to the increase in free fatty acids, although the contribution of phospholipids, the main components of polar lipids, was higher than triglycerides, the main components of neutral lipids. Buscailhon et al.(*34*) in dry-cured hams observed that phospholipids varied to a great extent during processing, whereas neutral lipids experienced little change throughout the curing process.

Table 3. Effect of VE Supplementation on the Proportion of SFA, MUFA, and PUFA (g Per 100 g of FAMEs in Neutral Lipids) of the Neutral Lipids Fraction during the SP of Meat Packed in MAP (LS Means ± Standard Errors)[a]

		VE $n = 9$				p		
fatty acids	SP (days)	E0	E250	E500	E1000	VE	SP	VE × SP
SFA	0	46.9 ± 0.80	48.0 ± 0.94	46.5 ± 0.76	45.8 ± 0.76	0.0517	0.0096	0.6000
	14	47.2 ± 0.83	48.8 ± 0.94	45.8 ± 0.78	45.8 ± 0.76			
	21	47.4 ± 0.82	49.6 ± 0.90	45.9 ± 0.81	46.6 ± 0.76			
	28	48.6 ± 0.86	49.0 ± 0.90	47.7 ± 0.78	46.7 ± 0.78			
MUFA	0	47.0 ± 0.63	46.1 ± 0.75	47.2 ± 0.60	47.7 ± 0.60	0.3813	0.0006	0.6619
	14	48.1 ± 0.65	46.6 ± 0.75	48.1 ± 0.62	48.4 ± 0.60			
	21	47.9 ± 0.65	46.4 ± 0.75	47.8 ± 0.67	48.0 ± 0.60			
	28	45.9 ± 0.68	46.5 ± 0.72	46.7 ± 0.62	46.9 ± 0.64			
PUFA	0	5.56 ± 0.34 x	5.70 ± 0.39 x	5.56 ± 0.32	5.62 ± 0.32	0.0689	0.0001	0.0292
	14	4.46 ± 0.34 xy	4.30 ± 0.39 y	5.68 ± 0.33	4.94 ± 0.31			
	21	4.32 ± 0.34 y	4.26 ± 0.37 y	5.61 ± 0.33	4.82 ± 0.32			
	28	4.47 ± 0.37 xy	4.42 ± 0.37 y	5.38 ± 0.32	5.55 ± 0.32			

[a] x,y: Different letters within the same column indicate significant difference ($p \leq 0.05$).

Table 4. Effect of VE Supplementation on the Proportion of SFA, MUFA, and PUFA (g Per 100 g of FAMEs in Polar Lipids) of the Polar Lipids Fraction during the SP of Meat Packed in MAP (LS Means ± Standard Errors)[a]

		VE $n = 9$				p		
fatty acids	SP (days)	E0	E250	E500	E1000	VE	SP	VE × SP
SFA	0	29.7 ± 0.68 x	29.8 ± 0.75	30.4 ± 0.59	29.5 ± 0.61	0.0001	0.0001	0.0006
	14	32.5 ± 0.35 a,y	30.8 ± 0.40 ab	30.6 ± 0.32 b	30.4 ± 0.32 b			
	21	34.1 ± 0.47 a,z	31.0 ± 0.52 b	30.7 ± 0.46 b	30.8 ± 0.43 b			
	28	35.7 ± 0.57 a,z	30.8 ± 0.50 b	30.9 ± 0.47 b	31.2 ± 0.49 b			
MUFA	0	30.8 ± 1.45 x	33.9 ± 1.62	30.4 ± 1.35	33.5 ± 1.35 x	0.1532	0.0001	0.0001
	14	35.8 ± 1.43 y	35.7 ± 1.62	31.8 ± 1.35	36.1 ± 1.35 y			
	21	37.2 ± 1.42 y	36.3 ± 1.61	32.2 ± 1.36	36.3 ± 1.35 y			
	28	37.2 ± 1.46 y	36.9 ± 1.61	32.4 ± 1.35	34.6 ± 1.35 xy			
PUFA	0	38.3 ± 1.60 x	36.0 ± 1.75	38.9 ± 1.46	35.2 ± 1.46	0.0329	0.0001	0.0001
	14	31.7 ± 1.54 y	33.3 ± 1.75	37.6 ± 1.45	33.3 ± 1.45			
	21	28.7 ± 1.54 a,z	32.7 ± 1.73 ab	37.1 ± 1.47 b	32.9 ± 1.45 ab			
	28	26.8 ± 1.60 a,z	32.2 ± 1.73 ab	36.3 ± 1.45 b	33.7 ± 1.46 ab			

[a] a,b: Different letters within the same row indicate significant difference ($p \leq 0.05$). x,y,z: Different letters within the same column indicate significant difference ($p \leq 0.05$).

Table 5. Effect of VE Supplementation on the Proportion of SFA, MUFA, and PUFA (g Per 100 g of FAMEs in Free Fatty Acids) of the Free Fatty Acids during the SP of Meat Packed in MAP (LS Means ± Standard Errors)[a]

		VE $n = 9$				p		
fatty acids	SP (days)	E0	E250	E500	E1000	VE	SP	VE × SP
SFA	0	44.0 ± 1.23 xy	44.8 ± 1.73	44.7 ± 1.23	45.9 ± 1.80 x	0.0035	0.0016	0.0013
	14	43.3 ± 0.72 x	43.8 ± 1.06	40.5 ± 0.75	40.3 ± 0.72 y			
	21	43.8 ± 0.80 x	42.4 ± 0.90	41.4 ± 0.71	41.1 ± 0.71 xy			
	28	47.6 ± 0.87 a,y	41.4 ± 1.16 b	40.2 ± 0.86 b	41.8 ± 0.87 b,xy			
MUFA	0	38.3 ± 0.97	40.8 ± 1.34	42.5 ± 0.96	41.0 ± 1.01	0.9223	0.8306	0.0250
	14	41.3 ± 1.00	41.2 ± 1.45	40.4 ± 1.04	40.7 ± 1.01			
	21	41.1 ± 1.16	40.1 ± 1.34	39.6 ± 1.04	40.7 ± 1.05			
	28	40.1 ± 1.00	41.9 ± 1.34	40.3 ± 1.00	39.3 ± 1.01			
PUFA	0	15.2 ± 1.08	13.6 ± 1.49	12.1 ± 1.08 x	12.9 ± 1.14 x	0.0920	0.0001	0.0001
	14	14.9 ± 1.10	14.4 ± 1.54	18.9 ± 1.13 y	17.9 ± 1.12 y			
	21	14.9 ± 1.21	17.4 ± 1.46	18.7 ± 1.14 y	17.8 ± 1.15 y			
	28	12.4 ± 1.14 a	16.3 ± 1.50 ab	18.6 ± 1.13 b,y	19.2 ± 1.14 b,y			

[a] a,b: Different letters within the same row indicate significant difference ($p \leq 0.05$). x,y: Different letters within the same column indicate significant difference ($p \leq 0.05$).

Fatty Acid Composition of Neutral Lipid, Polar Lipid and Free Fatty Acids. Tables 3−5 show the proportion of SFA, MUFA, and PUFA of neutral lipids, polar lipids, and free fatty acids fractions during storage. The fatty acids of neutral lipids were mainly short chain fatty acids, saturated (47%) and monounsaturated (47%), and only 6% were polyunsaturated. The polar lipids fraction was initially 37% PUFA, 30% SFA, and 31% MUFA, with no differences between the experimental groups. Aurousseau et al.(*36*) found a lipid profile in the triglycerides similar to that of our study in concentrate-fed lambs weighing 35 kg at slaughter. The lipid profile of the free fatty acids fraction was initially 45% SFA, 40% MUFA, and 14% PUFA, without significant differences between the experimental groups. As commented above, this fraction came from the

lipolysis of neutral and polar lipids; therefore, its fatty acid composition was intermediate between the neutral and the polar lipid fractions.

SP had a significant effect on the proportion of SFA and MUFA ($p = 0.010$ and $p = 0.0006$, respectively) in the neutral lipid fraction. Thus, SFA increased throughout the SP (from 46.79 ± 0.41% at 0 days to 48.02 ± 0.42% at 28 days, data not shown in the tables), while MUFA decreased slightly (47.01 ± 0.32% at 0 days and 46.51 ± 0.33% at 28 days, data not shown in the tables). There was a significant interaction between VE supplementation and SP (VE × SP) for PUFA proportion ($p = 0.029$) in the neutral lipid fraction (**Table 3**). The PUFA proportion decreased from 0 to 21 days in E0 and from 0 to 14 days in E250, while in E500 and E1000, it remained unchanged during the SP. The fatty acid composition of neutral lipids showed little change throughout the SP. Buscailhon et al. (*34*) observed that the neutral lipids fraction showed great stability throughout the process of curing in hams.

The interaction between VE supplementation and SP was significant for the proportion of SFA ($p = 0.0006$), MUFA ($p = 0.0001$), and PUFA ($p = 0.0001$) in the polar lipid fraction (**Table 4**). The composition in fatty acids of the polar lipids fraction in the nonsupplemented group (E0) showed substantial changes during the SP. Thus, SFA and MUFA proportions increased and PUFA decreased throughout the SP. However, in the supplemented groups, SFA and PUFA proportions remained unchanged during the SP. The reduction in the PUFA proportion in the nonsupplemented group (E0) could indicate that the degradation of polar lipids during storage was also a consequence of oxidation changes. In this sense, Lauzurica et al. (*15*) found the TBARS values in non-VE-supplemented lamb meat increased significantly during SP. The highest susceptibility of polar lipids to oxidation as compared with neutral lipids has been demonstrated previously (*37*) and is mainly due to their high content in PUFA, which are more susceptible to oxidation, and due to their location in cell membranes, close to heme pigments and other prooxidant systems (*38*). VE supplementation (E250, E500, and E1000) prevented to a large extent the oxidation processes of polar lipids. When VE was supplemented in the animal diets, it was deposited within muscle cell membranes adjacent to polar lipids (*39*), protecting them from oxidation. According to Mitsumoto et al. (*40*), the α-tocopherol incorporated into muscle via dietary supplementation (endogenous origin) improved pigment and lipid stability much more effectively than α-tocopherol added as a postmortem supplement (exogenous origin).

In free fatty acids (**Table 5**), the interaction between VE supplementation and SP was significant for SFA ($p = 0.0013$), MUFA ($p = 0.0250$), and PUFA ($p = 0.0001$) proportions. Thus, in E0, the SFA proportion increased, while MUFA and PUFA proportions were unchanged during storage. In the E250 group, no significant changes occurred in SFA, MUFA, and PUFA proportions. In the E500 group, the proportions of SFA and MUFA were unchanged, and the proportion of PUFA increased from 0 to 14 days. However, in the group that received the highest supplementation (E1000), a reduction of the SFA proportion from 0 to 14 days and an increase in the PUFA proportion during the first 14 days of storage were observed. The increase in PUFA proportion in the groups E500 and E1000 indicated that the antioxidant effect of VE prevented oxidation of PUFA released by the lipolysis of phospholipids. According to Nawar (*41*), the fatty acids released by lipolysis are more susceptible to lipid oxidation; therefore, they require higher levels of VE supplementation (minimum of 500 mg acetate-α-tocopherol/kg feed) to prevent oxidation.

In summary, supplementation with 250 mg acetate-α-tocopherol/kg feed prevented the oxidation of PUFA of the total lipids and the polar lipids fraction of packaged meat conserved for 28 days at a high-oxygen modified atmosphere. However, 500 mg acetate-α-tocopherol/kg feed was necessary to prevent the oxidation of the PUFA of free fatty acids. Polar lipids and free fatty acids were the lipid fractions that showed the greatest changes during storage.

LITERATURE CITED

(1) Williams, G. W. Assessment of marketing strategies to enhance returns to lamb procedures. Texas Agricultural Market Research Center Commodity Market Research Report No. CM-1-91; Texas A&M University: College Station, 1991.

(2) Penney, N.; Bell, R. G. Effect of residual oxygen on the color, odor and taste of carbon-dioxide-packaged beef, lamb and pork during short-term storage at chill temperatures. *Meat Sci.* **1993**, *33*, 245–252.

(3) Okayama, T.; Muguruma, M.; Murakami, S.; Yamada, H. Studies on modified atmosphere packaging of thin sliced beef. 1. Effect of 2 modified atmosphere packaging systems on ph value, microbial-growth, metmyoglobin formation and lipid oxidation of thin sliced beef. *J. Jpn. Soc. Food Sci. Technol.* **1995**, *42*, 498–504.

(4) Lynch, M. P.; Kerry, J. P.; Buckley, D. J.; Faustman, C.; Morrissey, P. A. Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef. *Meat Sci.* **1999**, *52*, 95–99.

(5) Morcuende, D.; Estevez, M.; Ruiz, J.; Cava, R. Oxidative and lipoytic deterioration of different muscles from free-range reared Iberian pigs under refrigerated storage. *Meat Sci.* **2003**, *65*, 1157–1164.

(6) Monin, G.; Hortós, M.; Díaz, I.; Rock, E.; García-Regueiro, J. A. Lipolysis and lipid oxidation during chilled storage of meat from large White and Pietrain pigs. *Meat Sci.* **2003**, *64*, 7–12.

(7) Pickul, J.; Leszczynski, D. E.; Kummerow, F. A. Relative role of phospholipids, triacylglycerols, and cholesterol esters on malonaldehyde formation in fat extracted from chicken meat. *J. Food Sci.* **1984**, *49*, 704–708.

(8) Mottram, D. S. Flavour formation in meat and meat products: A review. *Food Chem.* **1998**, *62*, 415–424.

(9) Simopoulos, A. P. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* **2002**, *56*, 365–379.

(10) Kanner, J. Oxidative processes in meat and meat products: quality implications. *Meat Sci.* **1994**, *36*, 169–189.

(11) Dobarganes, C.; Marquez-Ruiz, G. Oxidized fats in foods. *Curr. Opin. Clin. Nutr. Metab. Care* **2003**, *6*, 157–163.

(12) Liu, Q.; Lanari, M. C.; Schaefer, D. M. A review of dietary vitamin E supplementation for improvement of beef quality. *J. Anim. Sci.* **1995**, *73*, 3131–3140.

(13) Hasty, J. L.; Van Heugten, E.; See, M. T.; Larick, D. K. Effect of vitamin E on improving fresh pork quality in Berkshire- and Hampshire-sired pigs. *J. Anim. Sci.* **2002**, *80*, 3230–3237.

(14) De la Fuente, J.; Diaz, M. T.; Alvarez, I.; Lauzurica, S.; Cañeque, V.; Perez, C. Effect of dietary supplementation with vitamin E on characteristics of vacuum-packed lamb. *J. Sci. Food Agric.* **2007**, *87*, 651–659.

(15) Lauzurica, S.; de la Fuente, J.; Diaz, M. T.; Alvarez, I.; Perez, C.; Cañeque, V. Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. *Meat Sci.* **2005**, *70*, 639–646.

(16) Hanson, S. W. F.; Olley, J. Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. *Biochem. J.* **1963**, *89*, 101–102.

(17) García-Regueiro, J. A.; Gibert, J.; Diaz, I. Determination of neutral lipids from subcutaneous fat of cured ham by capillary gas-

chromatography and liquid-chromatography. *J. Chromatogr. A* **1994**, *667*, 225–233.

(18) Morrison, W. R.; Smith, L. M. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron fluoride-methanol. *J. Lipids Res.* **1964**, *5*, 600–608.

(19) SAS/STAT. *SAS User's Guide*, Version 8.2; SAS Institute: Cary, NC, 1996.

(20) Sokal, R. R.; Rohlf, F. J. Biometry. In *The Principles and Practice of Statistics in Biological Research*, 3rd ed.; W. H. Freeman & Co.: New York, 1995; pp 207−271.

(21) Raes, K.; De Smet, S.; Demeyer, D. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. *Anim. Feed Sci. Technol.* **2004**, *113*, 199–221.

(22) Salvatori, G.; Pantaleo, L.; Di Cesare, C.; Maiorano, G.; Filetti, F.; Oriani, G. Fatty acid composition and cholesterol content of muscles as related to genotype and vitamin E treatment in crossbred lambs. *Meat Sci.* **2004**, *67*, 45–55.

(23) Cosgrove, J. P.; Church, D. F.; Pryor, W. A. The kinetics of the autoxidation of polyunsaturated fatty-acids. *Lipids* **1987**, *22*, 299–304.

(24) Morrissey, P. A.; Sheehy, P. J. A.; Galvin, K.; Kerry, J. P.; Buckley, D. J. Lipid stability in meat and meat products. *Meat Sci.* **1998**, *49*, S73–S86.

(25) Jeremiah, L. E. Packaging alternatives to deliver fresh meats using short- or long- term distribution. *Food Res. Int.* **2001**, *34*, 749–772.

(26) Formanek, Z.; Kerry, J. P.; Higgins, F. M.; Buckley, D. J.; Morrisey, P. A.; Farkas, J. Addition of synthetic and natural antioxidants to α-tocopheryl acetate supplemented beef patties: Effects of antioxidants and packaging on lipid oxidation. *Meat Sci.* **2001**, *58*, 337–341.

(27) Beare-Rogers, J. Nutritional attributes of fatty acids. *JAOCS* **1988**, *65*, 91–95.

(28) Nutritional Aspects of Cardiovascular Disease; Report on Health and Social Subjects, 46; HMSO: London, United Kingdom, 1994.

(29) Wood, J. D.; Enser, H. Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *Br. J. Nutr.* **1997**, *78*, 549–560.

(30) Gandemer, G. Phospholipids in muscle—Composition and alteration during technological treatments. *Rev. Fr. Corps Gras.* **1990**, *37*, 75–81.

(31) García-Regueiro, J. A.; Hortós, M.; Díaz, I. Ácidos grasos libres en carne de pollo. *Eurocarne* **2003**, *114*, 1–5.

(32) Alasnier, C.; David-Briand, E.; Gandemer, G. Lipolysis in muscles during refrigerated storage as related to the metabolic type of the fibres in the rabbit. *Meat Sci.* **2000**, *54*, 127–134.

(33) Alasnier, C.; Meynier, A.; Viau, M.; Gandemer, G. Hydrolytic and oxidative changes in the lipids of chicken breast and thigh muscles during refrigerated storage. *Food Chem. Toxicol.* **2000**, *65*, 9–14.

(34) Buscailhon, S.; Gandemer, G.; Monin, G. Time-related changes in intramuscular lipids of French dry-cured ham. *Meat Sci.* **1994**, *37*, 245–255.

(35) Alasnier, C.; Gandemer, G. Activities of phospholipases A and lysophospholipases in glycolytic and oxidative skeletal muscles in the rabbit. *J. Sci. Food Agric.* **2000**, *80*, 698–704.

(36) Aurousseau, B.; Bauchart, D.; Galot, A. L.; Prache, S.; Micol, D.; Priolo, A. Indoor fattening of lambs raised on pasture: 2. Influence of stall finishing duration on triglyceride and phospholipid fatty acids in the *longissimus thoracis* muscle. *Meat Sci.* **2007**, *76*, 417–427.

(37) Igene, J. O.; Pearson, A. M.; Gray, J. I. Effect of length of frozen storage cooking and holding temperatures upon components phospholipids and the fatty acid composition of meat triglycerides and phospholipids. *Food Chem.* **1981**, *7*, 289–303.

(38) Asghar, A.; Gray, A.; Buckley, D. J.; Pearson, A. M.; Booren, A. D. Perspectives on warmed-over flavor. *Food Technol.* **1988**, *42*, 102–108.

(39) Halliwell, B. Oxidants and human-disease some new concepts. *FASEB J.* **1987**, *1*, 358–364.

(40) Mitsumoto, M.; Arnold, R. N.; Schaefer, D. M.; Cassens, R. G. Dietary versus postmortem supplementation of vitamin E on pigment and lipid stability in ground beef. *J. Anim. Sci.* **1993**, *71*, 1812–1816.

(41) Nawar, W. W. Lipids. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Marcel Dekker: New York, 1996; pp 139−244.

Received for review April 11, 2008. Revised manuscript received November 14, 2008. Accepted November 18, 2008. This study was supported by the Ministerio de Ciencia y Tecnología of Spain (Project CAL02-033-C2-2).

JF801940C