Quality of n-3 enriched Manchego lamb meat through refrigerated storage under modified atmospheres. Effect of supplementing antioxidants.

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Abstract- **The effect of supplementing either vitamin E (300 ppm/kg concentrate) or a grape extract rich in polyphenols (900 ppm/kg concentrate) on the quality of n-3 enriched lamb meat storaged at 4 ºC for 0, 6 and 12 days under a high-oxygen atmosphere was evaluated. A significant interaction between the dietary treatment and the time of storage was observed in both the TBARS values and the proportion of metmyoglobin. Regarding the TBARS, the meat from the lambs fed vitamin E showed stable levels over storage. However, the rest of the samples significantly rose at days 6 and 12. An increase in the proportion of metmyoglobin after 6 days of MAP storage was observed in all treatments. Nevertheless, from days 6 to 12, the samples supplemented vitamin E did not change, whereas the meat from the other dietary treatments doubled their values. The levels of PUFA tended to decrease in all treatments, the vitamin E group showing the lowest decrease after 12 days of MAP storage whereas the most pronounced decline was observed in the control samples. Among PUFA, the n-3 fatty acids underwent changes in a lesser extent.**

Keywords- **n-3 fatty acids; vitamin E; grape seed extract.**

I. INTRODUCTION

The fatty acid composition of ruminant meat has been widely studied, being characterized as rich in saturated fatty acids (SFA). For that reason, ruminant meat consumption might cause an increase in the risk of suffering cardiovascular diseases. There is, therefore, a growing interest in modifying the fatty acid profile of ruminant meat by reducing the levels of SFA while increasing those of polyunsaturated fatty acids (PUFA) and, especially, those belonging to the n-3 family [1].

Several studies have been performed in order to obtain n-3 enriched lamb meat by supplementing the animal´s diet with different components, such as extruded linseed, fish oil or algae [2,3]. However, higher PUFA levels in fat lead to an increase in lipid oxidation, since PUFA are the substrate in which the reaction begins. Oxidative phenomena leads to rancid flavours and to meat discoloration [4], these effects being more pronounced with longer storage periods.

In order to delay the aforementioned oxidative phenomena, natural antioxidants, such as vitamin E [5] or vegetal residues rich in polyphenols [6], have been used. The aim of the present study was to compare the effect of supplementing either vitamin E or a grape extract rich in polyphenols on the quality of n-3 enriched lamb meat.

II. MATERIALS AND METHODS

A. Animals, diets and experimental procedure

Thirty Manchego lambs with an initial live weight of 14.7 kg were randomly distributed into three groups and housed in individual pens. All diets consisted of an n-3 fatty acids enriched concentrated by using extrused linseed and fish oil as n-3 sources. A supplement of 300 ppm α-tocopherol (vitamin E)/kg concentrate was added to one of the 3 groups (VE), another supplement of 900 ppm grape (*vitis vinifera*) extract rich in polyphenols /kg concentrate was added to the second group (P) whereas the third one was kept as control (C).

All diets were offered *ad libitum*. The lambs were slaughtered at 26.5 kg in an industrial slaughterhouse. The day after, the lambs loins were chopped and the chops were packaged under a rich in oxygen (70 % O_2 and 30 % O_2) modified atmosphere (MAP) and the stored at 4 ºC for up to 12 days. Analyses were carried out at 0, 6 and 12 days of refrigerated MAP storage.

B. Analytical procedures

Lipid oxidation of fresh meat, expressed as mg malonaldehyde (MDA)/kg fresh meat, was measured by means of the Thiobarbituric acid reactive substances (TBARS) test [7]. The pigment composition was assessed by spectophotometry as suggested by [8]. Fatty acids were analysed following the method proposed by [9] in which both extraction and derivatization into methyl esters were carry out in one step. Fatty acid methyl esters were then analysed by gas chromatography coupled to a flame ionization detector.

C. Statistical analysis

Data were analyzed using the MIXED procedure by the Statistical Analysis System (SAS) package. A split model was used considering dietary treatment (D) as main plot effect and the time of storage (TS), a repeated measure, as subplot effect. Differences within treatments were studied by the Dunn-Šidak´s test.

III. RESULTS AND DISCUSSION

The TBARS values for the different dietary treatments tended to increase with the time of storage (Table 1), a more pronounced increase being observed in both C and P groups. Meat from lambs fed vitamin E showed the lowest TBARS values during all the storage period, thus indicating a lower extent of oxidation of these samples and confirming the antioxidant effect of vitamin E in meat.

Table 1 TBARS values (expressed as mg MDA/kg meat) and percentage of metmyoglobin (METMB) of muscle from n-3 enriched lambs fed on diets (D) containing different supplements: control (C), grape seed extract (P) and vitamin E (VE) during MAP storage (TS)

	TS	D			D	TS	D x TS
	(days)	C	P	VE			
TBARS	0	0.21 ± 0.04^{x}	0.16 ± 0.04^{x}	0.10 ± 0.04	***	***	***
	6	4.99 ± 0.27^{ay}	4.47 ± 0.26^{ay}	0.59 ± 0.27^{b}			
	12	8.52 ± 0.45^{az}	8.10 ± 0.43^{az}	1.24 ± 0.45^{b}			
METMB	0	9.65 ± 0.82^{x}	7.80 ± 0.77^{x}	7.47 ± 0.82^{x}	***	***	***
	6	30.82 ± 2.03^{y}	29.18 ± 1.92^{y}	32.85 ± 2.03^{y}			
	12	58.56 ± 2.96^{az}	62.97 ± 2.81^{az}	34.87 ± 2.96^{by}			

Significance: ns, P>0.05; *, P≤0.05; **, P≤0.01; ***, P<0.0001

[a.b.c.d] Different superscript letters within the same row indicate significant difference between treatments (P<0.05)

[x.y] Different superscript letters within the same column indicate significant difference with the time of storage (P<0.05)

These results are in agreement with previous studies, which report a delay in oxidation phenomena in lamb meat when doses of vitamin E above 287 mg/kg were supplemented [10].

The proportion of metmyoglobin increased with the time of storage (Table 1), non statistical differences among treatments being observed after 6 days of storage. In all cases, the percentage of metmyoglobin was below 40, in which meat shows a distinctly brown colour [11]. After 12 days of chilled storage the proportion of metmyoglobin showed differences within treatments, both C and P samples showing the highest levels. At the end of the study, the percentage of metmyoglobin was around 60 %, which usually entails the consumers´ rejection. On the other hand, the proportion of metmyoglobin hardly rose in the VE batch, this result pointing out to a better colour preservation when supplementing vitamin E.

SFA, MUFA and PUFA proportions showed a significant interaction ($P \leq 0.05$) between the studied factors (Table 2). Both SFA and MUFA increased during the time of storage in C and P samples while PUFA decreased. However, VE samples did not undergo any significant increase in the levels of those fatty acids during storage. Within SFA, it is worth highlighting the effect of the storage undertaken by both C14:0 ($P \leq 0.01$; data not shown) and C16:0 ($P \leq 0.001$) that leads to an increase in their proportions until day 12 for the three dietary treatments, in which the levels are the highest. Regarding with the PUFA levels, a decrease in both C and P samples was observed, showing the lowest levels after 12 days of chilled storage. However, VE samples did not undergo any significant change. Within the PUFA, the effect of the storage period on the levels of linoleic acid (C18:2 n-6) should be highlighted (Table 2), samples stored for 12 days showing significantly lower levels ($P \leq 0.001$). An interaction between both effects (D x TS) was observed for n-3 fatty acids, since samples from C and P groups underwent a significant reduction with longer storage periods whereas similar levels were observed during storage in VE samples. It should be highlighted that the decrease in n-3 fatty acids was less steep in P samples than in control. Hence, P diet seems to reduce n-3 PUFA oxidation, though is not as effective as the VE dietary treatment, since the latter seems to maintain the levels of oxidation during the

Table 2 Fatty acid composition (expressed as percentage) of muscle from n-3 enriched lambs fed on diets (D) containing different supplements: control (C), grape seed extract (P) and vitamin E (VE) during MAP storage (TS)

	TS (days)	D			D	TS	D x TS
		C	P	VE			
C16:0	0	22.94 ± 0.50	23.10 ± 0.46	23.78 ± 0.50	ns	***	ns
	6	23.87 ± 0.50	24.02 ± 0.46	24.34 ± 0.50			
	12	24.49 ± 0.50	24.69 ± 0.45	24.56 ± 0.51			
C18:2 n-6	0	8.93 ± 0.81	8.99 ± 0.74	9.36 ± 0.81	ns	**	ns
	6	8.84 ± 0.81	9.06 ± 0.74	9.15 ± 0.81			
	12	7.81 ± 0.81	7.86 ± 0.74	8.73 ± 0.82			
C18:3 n-3	0	1.21 ± 0.11^{a}	1.36 ± 0.11^{a}	1.10 ± 0.12^{a}	ns	***	**
	6	1.22 ± 0.11^{a}	1.44 ± 0.10^{b}	1.22 ± 0.11^{b}			
	12	1.06 ± 0.11^{b}	1.27 ± 0.10^{a}	1.21 ± 0.12^{ab}			
C20:5 n-3 (EPA)	0	2.86 ± 0.29^{a}	3.00 ± 0.26^{a}	3.12 ± 0.29	ns	***	**
	6	2.32 ± 0.30^{b}	2.58 ± 0.27^{b}	2.99 ± 0.29			
	12	1.60 ± 0.21^{cx}	1.76 ± 0.19^{cx}	2.74 ± 0.21^{y}			
C22:6 n-3 (DHA)	0	0.70 ± 0.06^{a}	0.82 ± 0.06^{a}	0.74 ± 0.06	ns	***	**
	6	0.55 ± 0.06^{b}	0.67 ± 0.06^{b}	0.68 ± 0.06			
	12	0.40 ± 0.06$^{c\ x}$	0.50 ± 0.05$^{c\ xy}$	0.68 ± 0.06^{y}			
SFA	0	38.78 ± 0.79^{a}	39.11 ± 0.73^{a}	40.63 ± 0.79	ns	***	*
	6	40.03 ± 0.79^{b}	40.28 ± 0.73^{b}	41.13 ± 0.79			
	12	41.24 ± 0.79^{b}	41.60 ± 0.72^{c}	41.48 ± 0.80			
MUFA	0	44.53 ± 0.98^{a}	43.18 ± 0.89^{a}	41.82 ± 0.98	ns	***	*
	6	44.58 ± 0.98^{a}	43.02 ± 0.89^{a}	41.74 ± 0.98			
	12	46.09 ± 0.98^{b}	44.80 ± 0.89^{b}	42.14 ± 0.99			
PUFA	0	15.52 ± 1.27^{a}	16.18 ± 1.16^{a}	16.26 ± 1.27	ns	***	*
	6	14.65 ± 1.27^{a}	15.62 ± 1.16^{a}	16.03 ± 1.27			
	12	12.20 ± 1.26^{b}	12.89 ± 1.15^{b}	15.31 ± 1.28			
n3	0	5.56 ± 0.48^{a}	6.25 ± 0.44^{a}	5.81 ± 0.48	ns	***	***
	6	4.71 ± 0.48^{b}	5.56 ± 0.44^{b}	5.72 ± 0.48			
	12	3.48 ± 0.39$^{c\ x}$	4.14 ± 0.35$^{c\ xy}$	5.42 ± 0.39^{y}			
n6	0	9.17 ± 0.83	9.25 ± 0.76	9.64 ± 0.83	ns	**	ns
	6	9.07 ± 0.83	9.31 ± 0.76	9.41 ± 0.83			
	12	7.99 ± 0.83	8.06 ± 0.75	8.98 ± 0.84			
n6/n3	0	1.63 ± 0.09^{a}	1.47 ± 0.08^{a}	1.68 ± 0.09	*	***	***
	6	1.91 ± 0.05$^{b\ x}$	1.68 ± 0.05$^{a\ y}$	1.66 ± 0.05$^{\ y}$			
	12	2.30 ± 0.09$^{c\ x}$	1.97 ± 0.08$^{b\ xy}$	1.66 ± 0.09^{y}			

n-3: C18:3 n-3+C20:5 n-3+C22:5 n-3+C22:6 n-3; n-6: C18:2 n-6+C20:4 n-6+C22:4 n-6; SFA: C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0; MUFA: C16:1 + C17:1 + C18:1; PUFA: C18:2 n-6 + C18:3 n-4 + C18:3 n-3 + cis-9-trans-11 C18:2 + C20:1 n-9 + C20:4 n-6 + C20:4 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3.

Significance: ns, P>0.05; *, P≤0.05; **; P≤0.01; ***, P<0.0001

$^{a.b.c}$ Different superscript letters within the same column indicate significant difference (P<0.05)

$^{x.y}$ Different superscript letters within the same row indicate significant difference (P<0.05)

whole storage period. Several studies have reported an enhancement of n-3 enriched lamb meat quality when supplementing polyphenols [12-13]. Nevertheless, the ability of vitamin E in delaying oxidation seems to be more effective [14], and even more if combined with other plant antioxidants [12].

Long-chain fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids gradually decreased only in C and P samples, whereas VE meat did not show any remarkable change during the experiment. Lambs supplemented VE showed higher levels of both EPA and DHA after 12 days of storage than the rest of the samples.

Supplementing grape extract showed a similar behaviour to that of vitamin E although it was less

pronounced.

The n-6/n-3 ratio increases with the time of chill storage when control and P dietary treatments were used, though it was lower for the P treatment. However, when using vitamin E as a supplement, the mentioned ratio did not significantly change. In addition, VE samples stored for 12 days showed the lowest n-6/n-3 ratios in comparison with the rest of the treatments. It is worth noting that the n-6/n-3 ratio was below 4 for all dietary treatments and hence within the dietary recommendations [15].

IV. CONCLUSIONS

Supplementing vitamin E prevents n-3 enriched lamb meat's lipid oxidation and discoloration in a higher extent than when using polyphenols from the grape. The grape extract reduces PUFA oxidation after 6 and 12 days of MAP storage, especially n-3 fatty acids thus improving the n-6/n-3 ratio in comparison with that of control. The aforementioned reduction is, nevertheless, more pronounced when vitamin E is used.

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REFERENCES

1. Wood JD, Enser M, Fisher AV et al. (2008) Fat deposition, fatty acid composition and meat quality: A review. Meat Sci 78: 343-358
2. Raes K, De Smet S, Demeyer D (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linolenic acid in lamb, beef and pork meat: a review. Animal Feed Sci and Tech 113: 199-221
3. Díaz MT, Cañeque V, Sánchez CI et al. (2011) Nutritional and sensory aspects of light lamb meat enriched in n-3 fatty acids during refrigerated storage. Food Chem 124: 147-155
4. Durand D, Scislowski V, Gruffat D et al. (2005) High-fat rations and lipid peroxidation in ruminants; consequences on the health of animals and quality of their products. EAPP Vol. 112, 55th Annual Meeting of the European Association for Animal Production, Bled, Slovenia, pp 137-150
5. Lauzurica S, De La Fuente J, Díaz MT et al. (2005) Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. Meat Sci 70: 639-646
6. O´Grady MN, Maher M, Troy DJ et al. (2006) An assessment of dietary supplementation with tea cathechins and rosemary extract on the quality of fresh beef. Meat Sci 73: 132-143
7. Maraschiello C, Sárraga C, García Regueiro JA (1999) Gluthatione peroxidise activity, TBARS and α-tocopherol in meat from chickens fed different diets. J Agric Food Chem 47: 867-872
8. Krzywicki K (1979) Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. Meat Sci 3: 1-10
9. Sukhija PS, Palmquist DL (1988) Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J Agric Food Chem 36: 1202-1206
10. Álvarez I, De La Fuente J, Cañeque, V et al. (2009) 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. J Agric Food Chem 57: 140-146
11. MacDougall DB (1982) Changes in the colour and opacity of meat. Food Chem 9: 75-88
12. Gobert M, Gruffat D, Habeanu M et al. (2010) Plants extracts combined with vitamin E in PUFA-rich diets of cull cows protect processed beef against lipid oxidation. Meat Sci 85: 676-683
13. Jacobsen C, Let MB, Nielsen NS et al. (2008) Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated lipids: A comparative study. Trends in Food Sci and Tech 19: 76-93
14. Juárez M, Durgan MER, Aalhus JL et al. (2011) Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. Meat Sci 88: 434-440
15. Scollan N, Hocwuete JF, Nuernberg K et al. (2006) Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci 74: 17-33