# Volatile compounds of omega-3 enriched Manchego lamb meat storaged under modified atmospheres. Effect of supplementing antioxidants.

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*Abstract*— **The effect of supplementing either vitamin E or a grape extract rich in polyphenols on the volatile profile of omega-3 enriched lamb meat stored under modified atmospheres (MAP) was assessed. The effect of antioxidants was moderate, vitamin E being more effective in limiting the extent of lipid oxidation than the grape extract. A high number of compounds showed lower levels after 6 days of MAP-packaging storage at 4 ºC. Several lipid-derived compounds rose during storage mainly in the control samples and, in a lesser extent, in the P group, showing a protecting effect of the grape seed extract against lipid oxidation. The samples from the VE samples showed minor changes in the course of storage, confirming the higher antioxidant potential.**

*Keywords*— **Omega-3 fatty acids; volatile compounds; lamb meat.**

## **I.** INTRODUCTION

The nutritional value of omega-3 polyunsaturated fatty acids (PUFA) in human heath is well documented [1]. Increasing the content of omega-3 PUFA in food is, therefore, of great importance, especially regarding ruminant meat, which is considered as a rich source of saturated fat. The fatty acid profile of ruminant tissue can be modified by including sources rich in omega-3 PUFA in the animal´s diet [2]. However, since PUFA are far more prone to oxidize than saturated fatty acids, omega-3 enriched meat is highly susceptible to undergo oxidative phenomena. Lipid oxidation not only affects the nutritional value of meat but also its organoleptic quality, especially when cooked, producing a large number of volatile compounds, such as aldehydes, ketones or carboxylic acids, besides other effects. Hence, the use of antioxidants might be of great interest to limit or delay the extent of oxidation phenomena. Concerns regarding the safety and toxicity of synthetic antioxidants prompted research into the use of antioxidants compounds from natural sources [3]. The antioxidants can be deposited on the surface of the meat before packaging or supplemented in the animal´s diet. In the latter case, the antioxidants can be incorporated within cell membranes, being more efficient in delaying lipid oxidation than when added post-mortem [4].

The aim of the present study was to evaluate the effect of supplementing either vitamin E or a grape extract rich in polyphenols on the volatile profile of lamb meat rich in omega-3 PUFA. The effect of refrigerated storage under a high-oxygen modified atmosphere packaging (MAP) was also assessed.

## **II.** MATERIALS AND METHODS

### *A. Animals, diets and experimental procedure*

Thirty Manchego male lambs with an initial live weight of 14.7 kg were randomly allocated on one of the three dietary treatments. All diets consisted of a concentrated rich in omega-3 fatty acids by using extrused linseed and fish oil as omega-3 sources. A supplement of 300 mg α-tocopherol (vitamin E) per kg concentrate was added to one of the 3 groups (VE group), another supplement composed of 900 mg grape (*vitis vinifera*) extract rich in polyphenols per 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 and chilled for 24 h. The day after slaughter, the lamb loins were cut into chops and those were packaged under MAP containing a high oxygen proportion (70 % $O_2$ and 30 % $CO_2$), afterwards the samples were stored at 4 ºC for up to 6 days. After refrigerated storage, the samples were vacuum-packaged in metallic pouches

and then frozen at -20 ºC until analysis. The volatile fraction was analyzed at 0 and 6 days of refrigerated MAP storage.

*B. Analysis of volatile compounds*

The volatile compounds were extracted by solid-phase microextraction (SPME) by means of a Divinylbenzene/Carboxen/Polydimethylsiloxane fibre (Supelco, Bellefonte, PA, USA) and subsequently analyzed by gas chromatography-mass spectrometry (GC-MS; Agilent 6850 GC-5975 C Triple-Axis Detector).

Before analysis, the samples were thawed overnight at 4 ºC, wrapped in aluminium foil and then cooked in an oven at 180 ºC to an inner temperature of 70 ºC. Five grams of *longissimus dorsi* were homogeneized in a mechanical grinder with 5 g of anhydrous sodium sulphate and 20 µL of an aqueous solution of 600 mg/L cyclohexanone as internal standard. An aliquot of the mixture (3.5 ±0.05 g) was placed in a sealed headspace vial and then in a thermoblock at 45 ºC, were both equilibration and extraction (1 h each) of the volatile compounds took place. After the extraction step, the fibre was inserted into the GC port for desorption (260 ºC/10 min in splitless mode). After each run, the fibre was cleaned up to avoid carry-over.

The chromatographic separation was carried out in a CP-Sil 8 CB column (60 m long; 0.25 mm i.d.; 0.25 µm film thickness; Chrompack, Middleburg, Holland) with 1 mL/min helium flow. The temperature program was as follows: 5 min at 45 ºC, ramp 5 ºC/min to 250 ºC and 11 min at 250 ºC.

Detection was performed with electron impact ionization, with 70 eV ionization energy operating in the full-scan mode (33-300 amu; 2.72 scans/s). Source, quadrupole and interface were 230, 150 and 280 ºC, respectively. Compound identification was carried out by injection of commercial standards, by spectra comparison using the NIST/EPA/NIH Mass Spactral Library (NIST 05) and/or by calculation of linear retention indexes (LRI) relative to a series of alkanes ($C_5$-$C_{20}$). The sums of abundances of up to four ions per compound were used for semi-quantitative determination.

*C. Statistical analysis*

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

A principal component analysis (PCA) was also performed on the 76 most correlated compounds with the SPSS Win 12.0 software.

## **III.** RESULTS AND DISCUSSION

A number of 102 compounds were identified in the volatile fraction of omega-3 enriched lamb meat. These compounds were clustered in the following chemical families: aldehydes (27 compounds), ketones (15), alcohols (14), alkanes (17), carboxylic acids (3), benzene compounds (11), heterocycles (4), esters (2), sulphur compounds (5) and terpenoids (2). The volatile profile was, as expected, mainly composed of compounds resulting from lipid oxidation, in agreement with other studies [5]. The fat enriched diet of animals, together with the conditions of the cooking procedure, seems to be the key factors for the obtained volatile profile.

Eighteen volatile compounds showed significant differences by the dietary treatment. Of these compounds, 13 showed significantly lower levels in the VE samples. As an example, the behaviour of 1-octen-3-ol, pentane, 2-heptanone and 1-penten-3-ol are depicted in Fig. 1A. A similar trend was observed for the following compounds: 3,5-octadien-2-one, 2-penten-1-ol, 2-decen-1-ol, 1,3,5-octatriene, benzene, 3-ethylbenzaldehyde, phenylacetaldehyde and trimethylamine.

The levels of other compounds, namely, 2,3-butanedione and 2,3-butanediol, were significantly lower in the controls, whereas the abundance of 2-propanone and dimethyl disulfide were significantly lower in the P samples (Fig. 1B). Supplementing antioxidants had an effect on the volatile profile of omega-3 enriched lamb. However, the effect was moderate and dependent on the antioxidant. Within the compounds significantly affected by the dietary treatment, most of the lipid-derived compounds

showed lower levels in the meat from the lambs supplemented vitamin E, thus confirming the higher ability of this substance to delay lipid oxidation. In animal tissues, vitamin E is located in the cell membrane, closely associated with the membranal phospholipids [6] where lipid oxidation is initiated. However, due to the different chemical nature of the polyphenols from grape in comparison with that of vitamin E, an uneven bioavailability, absorption or deposition could be expected. As an example, [7] suggested, when studying the effect of supplementing grape seed extract on the quality of pork meat, a lack of availability of condensed tannins present in grape due to complexion with the protein in the pig´s feed.

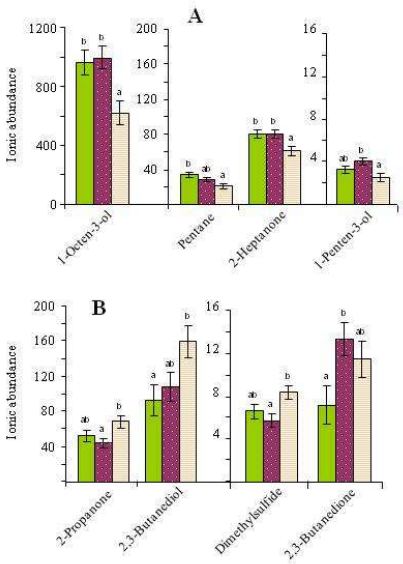


Fig.1. Levels of the volatile compounds significantly affected by the dietary treatment in omega-3 enriched lamb meat: Control; P group; VE group. [ab] Means with different letters for each compound differ significantly ($P<0.05$).

Fifty-five compounds were significantly affected by the time of storage, most of them showing lower levels after 6 days of MAP storage. The evolution over storage of propanal, pentanal, hexanal, 1-pentanol, 1-octen-3-ol and 2-pentylfuram is shown in Fig.2A. A similar behaviour was observed for 19 aldehydes (of which 8 were saturated and 10 were unsaturated), 9 ketones (3 of which were unsaturated), 4 alcohols, 1 unsaturated alkane, 2 esters, 2 benzene compounds, 2 heterocycles, a terpene and a sulphur compound.

Only 7 compounds significantly rose with the time of storage, as shown in Fig. 2B.

Previous studies have reported an increase in the levels of lipid-derived volatile compounds with longer storage periods. The rise in lipid oxidation with storage has been reported even after supplementing natural antioxidants, such as thyme leaves [8] or a rosemary extract [9], although it should be taken into account that both studies monitored lipid oxidation in stored meat that had previously been cooked.

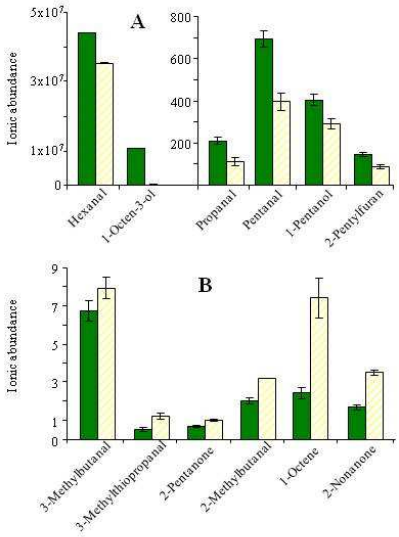


Fig.2. Levels of the volatile compounds significantly affected by the time of storage in omega-3 enriched lamb meat: 0 days; 6 days.

The levels of 11 compounds were affected by both effects, showing a significant interaction (data not shown). Most of these compounds, which were lipid-derived products, showed higher levels during storage in the controls and, in a lesser extent, in the P meat, while in the VE meat they scarcely changed. Again, the grape extract seems to be less efficient in protecting meat against lipid oxidation than vitamin E. Besides the aforementioned differences in the chemical structure of the polyphenols in comparison with that of vitamin E, the intake of polyphenols might somehow explain our results. It should be noted that the conditions of our study (meat rich in PUFA, high oxygen MAP, cooking) are highly oxidizing, therefore a greater concentration of the grape extract might be

necessary. In addition, the polyphenols can be degraded by the thermal treatment, thereby decreasing their antioxidant activity.

Seventy-six volatile compounds were used to build a PCA in order to identify correlated compounds. Ten principal components accounted for 89.4 % of the total variance. The principal components 1 and 2 (PC1 and PC2, respectively) were the functions that showed a meaningful distribution of the samples (Fig.3). The PC1, composed of dienals, enols and furans among others, seems to separate the samples according to the time of storage, whereas the PC2, mainly composed of alkanes and methylketones, could separate the samples from the VE group that had been stored for 6 days from the rest of the samples also stored for 6 days. Differences in the accumulation or degradation of volatile compounds can be observed, the volatile profile of the samples from both the C and P groups undergoing changes in the course of the storage, whereas the VE samples showing a volatile profile closer to that of fresh samples.

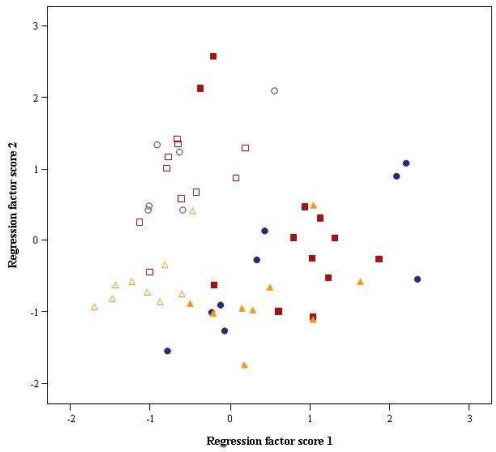


Fig.3. Loading plot of the factor scores of omega-3 enriched lamb meat samples supplemented antioxidants: ● Control; ■ P group; ▲ VE group. Full symbols correspond to non-stored samples (0 d), Open symbols correspond to samples stored for 6 days.

## IV. CONCLUSIONS

In the conditions of our study, the volatile profile of omega-3 enriched lamb meat mainly results from lipid degradation. Supplementing antioxidants has a protective effect against oxidative phenomena, however, vitamin E seems to be more effective than the polyphenols from the grape seed extract. Further research is necessary to determine the optimal conditions of supplementing the grape extract so that the polyphenols can be effective in protecting omega-3 enriched lamb meat against oxidative phenomena.

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