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Effect of dietary supplementation with either red wine extract or vitamin E on the volatile profile of lamb meat fed with omega-3 sources

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ABSTRACT

The effect of dietary supplementation with either vitamin E (300 ppm) or a red wine extract rich in polyphenols (900 ppm) in an omega-3 enriched concentrate on the volatile fraction of lamb meat was assessed. The effect of refrigerated storage (0 and 6 days) under high-oxygen atmospheres (70% O_2/30% CO_2) was also studied. Extraction and analysis of the volatile compounds was carried out by headspace solid-phase microextraction (HS-SPME) and GC–MS, respectively. Vitamin E supplementation led to lower levels of lipo-oxidation compounds, such as 2-heptanone and 1-penten-3-ol. The red wine extract was less efficient against lipid oxidation than vitamin E but more efficient than the control (no added antioxidants). The levels of numerous lipid-derived compounds were found to be lower after 6 days of storage which could be due to further interactions with protein-related compounds.

1. Introduction

There is an increasing awareness of the relationship between food consumption and human health. In this respect, meat consumption is gradually becoming to be regarded by consumers as one of the main causes of an increased risk of chronic diseases such as obesity, cardiovascular disease or cancer (Weiss, Gibis, Schuh, & Salminen, 2010). To prevent these detrimental heath effects, the World Health Organization (WHO) has established minimum daily intakes of n-3 polyunsaturated fatty acids (PUFA). As a result of its highly saturated fatty acid profile, ruminant meat has a poor image in terms of nutritional quality. However, one of the main factors affecting the proportion of fatty acids of nutritional importance in lamb meat is the fatty acid profile of the animal's diet (Díaz et al., 2005), thus meaning that the fatty acid profile of ruminant meat can be modified by means of diet, as reported previously (Raes, De Smet, & Demeyer, 2004).

The higher susceptibility of PUFA to undergo oxidation than saturated fatty acids (SFA) means that PUFA-enriched meats are more prone to lipid oxidation, thus resulting in adverse effects on the quality, healthiness and acceptability of meat. Meat aroma, which is one of the most important quality attributes of muscle foods, can also be affected by oxidation, thereby resulting in changes in its volatile profile. Thus, lipid oxidation leads to the formation of aldehydes, which are responsible for the development of rancid flavours (Guillén-Sans & Guzmán-Chozas, 1998), and a large number of small molecular weight degradation products that are often volatile (Weiss et al., 2010). For this reason, the study of the volatile compounds of meat after any treatment can help to determine those changes that may affect its flavour. As far as the effect of supplementing omega-3 fatty acids on the volatile profile of meat is concerned, previous studies have found an increase in the levels of compounds resulting from lipid oxidation, such as unsaturated aldehydes and hydrocarbons, amongst others, when fish oil was used (Elmore, Mottram, Enser, & Wood, 2000). However, to the best of our knowledge, no studies have dealt with the volatile profile of omega-3 enriched meat after storage.

The use of natural ingredients to prevent or delay oxidation processes in meat, while maintaining the label "preservative-free", appears to be an interesting nutritional strategy. Natural antioxidants are primarily phenolic compounds found in all parts of plants, such as fruits, vegetables, or seeds (Jamilah, Mohamed, Abbas, Abdul Rahman, & Karim, 2009). Tocopherols, which are also synthesized by photosynthetic organisms, are also known for their potent antioxidant properties. Indeed, plant extracts have been used to delay lipid oxidation in meats or meat products (Tang et al., 2006). However, natural antioxidants are usually added *post-mortem* and their incorporation by dietary supplementation is much less common.

The aim of the present study was to investigate the effect of dietary supplementation of either α-tocopherol (vitamin E) or a red wine extract rich in polyphenols in an omega-3 enriched diet on the volatile profile of lamb meat. Although various articles have focused

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on the antioxidant effect of grape extract, to the best of our knowledge the effect of red wine extract has not been studied. The effect of refrigerated storage (4 °C) under high-oxygen modified atmospheres was also assessed.

2. Materials and methods

2.1. Animals, diets and experimental procedure

Thirty Manchego weaned male lambs, with an average initial live weight of 14.3 kg (SD ± 1.3), were randomly allocated to one of three dietary treatments. All animals were fed a basal diet rich in omega-3 fatty acids composed of 19.4% corn meal, 34% barley, 18.2% wheat, 14.6% soya-bean meal, 3.8% sunflower meal, 2.2% limestone (calcium carbonate plus sodium bicarbonate), 0.5% salt, 0.3% lamb additives (a mixture of vitamins and minerals comprising 1650 UI retinol, 330 UI cholecalciferol, 5 mg dL-α-tocopherol, 0.6 mg riboflavin, 2 mg ascorbic acid, 4 mg niacin, 0.002 mg cyanocobalamin, 0.18 mg vitamin K, 12.6 mg Mn, 22 mg Zn, 1 mg Cu, 0.12 mg I, 4.2 mg Fe, 0.2 mg Co, 0.03 mg Se, 50 mg Mg, and 69 mg S per g of product), as well as 6.0% extruded linseed and 1.5% deodorized fish oil as omega-3 sources. The dietary treatments differed in terms of the antioxidant used as supplement. Thus, one contained 900 mg of red wine extract rich in polyphenols (Provinols™, Seppic, Paris, France) per kg of concentrate (RW group), another contained 300 mg of DL-α-tocopheryl acetate (BASF, Limburgerhof, Germany) per kg of concentrate (VE group), and the final control treatment (C) involved no additional supplementation. All diets were pelleted. According to the manufacturer, the red wine extract mainly consists of epicatechin, catechin, proanthocyanidoles B1–B4, chlorogenic acid, B2-3O gallate, p-coumaric acid, resveratrol, epicatechin 3Ogallate and gallic acid.

The lambs were housed in individual pens (1 m^2) and fed *ad libitum* until they reached a live weight of 26.6 kg (SD ± 1.5). They were then transported to, and slaughtered in, a commercial abattoir following standard commercial practices. After a 24-h chilling period (4 °C), the carcasses were jointed and the loins were cut into 2-cm thick chops; these from between the fifth and sixth rib were used to analyse the volatile compounds. The chops from the right side were directly vacuum-packaged in metallic pouches (EV-15-1CD-SC, Tecnotrip, S.A., Barcelona, Spain) and then frozen at −20 °C until analysis (0 d lot), whereas the chops from the left side were stored under high-oxygen (70% O_2/30% CO_2, EAP 20, Carburos Metálicos, S.A.) modified atmospheres packaging (MAP) for 6 days in the dark at 4 °C (6 d lot). The BB41 pouches (Cryovac) used for MAP storage showed the following characteristics: 150 μm polyamide/polyethylene, 50/100, 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 vapour transmission rate (1.5 g/m^2/24 h O_2 at 38 °C and 100% relative humidity). The chops were subsequently vacuum-packaged and frozen as described above until analysis.

2.2. Analysis of volatile compounds

Before analysis, samples were thawed overnight at 4 °C, wrapped in aluminium foil and then cooked at 180 °C in a convection oven (TV29U, Memmert GmbH & Co., Schwabach, Germany) to an inner temperature of 70 °C, as monitored using a thermocouple probe (K-type thermocouple) inserted in the *longissimus dorsi*.

Volatile compounds were extracted by solid-phase microextraction (SPME) and analysed by gas chromatography–mass spectrometry (GC–MS). Five grams of cooked *longissimus dorsi* (trimmed of fat and visible connective tissue) were homogenised in a mechanical grinder (IKA Labortechnik, Staufen, Germany) with 5 g of Na_2SO_4 and 20 μL of an aqueous solution of 600 mg/L cyclohexanone as internal standard (IS). The IS was used to rule out any problems during the sample preparation and injection steps. An aliquot of the mixture (3.5 ± 0.05 g) was placed in a 15 mL headspace glass vial sealed with a polytetrafluoroethylene (PTFE)-faced silicone septum (Supelco, Bellefonte, PA, U.S.A.). Vials were placed in a block heater (SHT100D, Bibby Steriling Ltd., Staffordshire, UK) at 45 °C for both equilibration and extraction (1 h each). A 2 cm × 50/30 μm Stable-Flex divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)-coated fiber (Supelco, Bellefonte, PA, U.S.A.) was used for the extraction, after which it was inserted into the GC injection port (Agilent 6850 GC-5975C Triple-Axis Detector) for desorption (260 °C/10 min in splitless mode, provided with an SPME liner). Before use, the fiber was conditioned in the injection port of the GC at 270 °C for 1 h, as recommended by the manufacturer. After each run, the fiber was cleaned to avoid carry-over problems and, the fiber's sensitivity was tested, periodically, with a dilution of our IS. All analyses were run using the same fiber unit.

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 and the following temperature program: 5 min at 45 °C, ramp at 5 °C/min to 250 °C and 5 min at 250 °C.

Detection was performed by electron impact ionization (70 eV), operating in the full-scan mode from 33 to 300 amu at 2.72 scans/s. The interface, source and quadrupole temperatures were 280, 230 and 150 °C, respectively. Compound identification was carried out by injection of commercial standards, by spectral comparison using the NIST/EPA/NIH Mass Spectral Library (NIST 05) and/or by calculation of linear retention indexes (LRI) relative to a series of n-alkanes (C_5-C_{20}). The sums of abundances of up to four characteristic ions per compound were used for semi-quantitative determination. To ease comprehension, data shown throughout the manuscript were multiplied by 10^{-5}.

2.3. Statistical analysis

Data were analysed using the 9.1.2 Statistical Analysis System (SAS) package (SAS Institute, Cary, U.S.A.). Volatile compounds were analysed using the MIXED procedure. A split-plot design considering the dietary treatment as main plot effect and the time of storage, a repeated measure, as subplot effect was used. Each animal was considered to be one experimental unit. The analysis of repeated measures data firstly requires modelling the covariance structure of data, which refers to variances at individual times and to correlation between measures at different times on the same animal, and, secondly, the choice of the most appropriate covariance structure for each variable, as explained by Littell, Henry, and Ammerman (1998). To meet the first requirement, the compound symmetry (CS), first-order autoregressive (AR), first-order heterogeneous autoregressive (HAR) and unstructured (UN) were used for each volatile compound. The lowest Bayesian Information Criterion (BIC) was used to choose the matrix of error structure amongst the cited structures, the matrix showing the lowest BIC being chosen for each volatile compound. When significant differences were observed among treatments a Dunn–Šidak test was used for mean comparison ($P \leq 0.05$). If the interaction between both effects (interaction × storage period) was significant, planned comparisons among means were performed using Dunn–Šidak test ($P \leq 0.05$).

A Principal Component Analysis (PCA) was also performed on the most correlated compounds.

3. Results

A total of 103 volatile compounds were identified in the headspace of omega-3 enriched lamb meat. Table 1 lists these compounds ordered by chemical family together with their chromatographic indices, the ions used for semi-quantification and the method of identification. The significance of the effects, dietary treatment (DT), time of storage (TS) and the interaction between these two factors