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Original Article

Fatty acid composition, cholesterol and α-tocopherol of Barrosã-PDO veal produced in farms located in lowlands, ridges and mountains

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ABSTRACT

Forty-five Barrosã calves produced according to Protected Denomination of Origin (PDO) guidelines were used to characterise intramuscular fat composition (neutral and polar lipid fractions), cholesterol and α-tocopherol contents in Barrosã-PDO meat obtained from lowlands (<400 m), ridges (400–700 m) and mountains (>700 m). In addition, meat fatty acids were used as chemical markers to differentiate veal from farms located at distinct grazing altitudes through a canonical discriminant analysis. Although the altitude of farm location seems to slightly affect the fat composition of Barrosã-PDO veal, still it depends on the muscle considered. The fatty acid composition of the polar fraction from biceps femoris muscle was the most influenced by the altitude of farm location (14:0, 16:1*c*, 18:1*c9*, 18:2n-6, 20:4n-6, odd chain saturated fatty acids, branched chain fatty acids, cis-monounsaturated fatty acids, n-6 and total polyunsaturated fatty acids; $P < 0.05$), followed by the polar fraction of supraspinatus muscle (18:0, conjugated linoleic acids, 20:4n-6 and even-chain saturated fatty acids; $P < 0.05$). Furthermore, the results herein suggest that intramuscular fatty acid profile has little relevance to discriminate Barrosã-PDO meat from lowlands, ridges and mountains.

1. Introduction

Meat is an essential dietary component, providing a major proportion of consumer requirements for amino acids, fatty acids, some vitamins and minerals. Fatty acid (FA) composition in meat has been implicated in some of the most prevalent chronic diseases in Western countries such as cardiovascular diseases, cancer and obesity, mainly due to its high contents of saturated fatty acids and cholesterol (Wood et al., 2003; Biesalski, 2005). Moreover, the cholesterol oxidation products of meat exhibit mutagenic, carcinogenic and cytotoxic properties (Homma et al., 2004) and their content in food is strongly dependent on cholesterol and α-tocopherol concentrations. In fact, it is well known that α-tocopherol is the primary lipid-soluble antioxidant in biological systems (Kerry et al., 2000). Thus, the determination of FA composition, cholesterol and α-tocopherol contents could provide valuable information regarding meat nutritional quality. Meat consumption is affected by several factors of which, origin, safety and nutritional properties are among the most important ones.

Recent EU policies—with the objective of benefitting sustainable agriculture systems—have given meat obtained from regional cattle breeds with the opportunity of being commercialised with a "certification of origin", that is known as Protected Denomination of Origin (PDO). This is a consumer's guarantee that the meat was produced in a specific, predetermined geographic region and according to defined rules (Council Regulation no. 2081/92 of 14/7, EEC for Barrosã-PDO meat). Despite its higher price, consumer demand for legally protected PDO bovine meat has increased in Portugal in the past few years. However, frequent quality deviation of this meat (lack of homogeneity) is one of the main reasons for consumers for not repeating the purchase, and this thus poses a threat for their assertion in the Portuguese market. Barrosã-PDO meat is one of the most important commercial Portuguese PDO veals (249 carcass tons in 2005, which represent 10% of all the PDO meat produced in Portugal (Oliveira, 2007)), and it is sold mainly in supermarkets with no information concerning production.

According to PDO guidelines, calves are kept in barns, suckling their mothers until weaning (6–7 months of age) and are then supplemented with farm products (hay, turnips, straw, ryegrass, oat forages and maize, depending on the farm location) and

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concentrated. However, the climatic environment differs with farm altitude and can shorten the availability of grass and forage, so that it becomes necessary to increase the concentrated level to finish the calves. The dams graze on permanent grasslands including shrubs and forest, and are supplemented with the same forages. Their milk composition could be influenced by grazing conditions (botanic composition, altitude and distance to pasture) (Bugaud et al., 2001) and feed supplementation. Both milk fatty acid composition and the length of the suckling period, could be influenced by farm altitude, and may be responsible for differences in meat nutritional and sensory traits (Velasco et al., 2004; Moreno et al., 2006).

The Barrosã Breeders Association has requested studies that could help implementat suitable strategies to effectively control and adjust meat characteristics to consumer's wishes. Such strategies could be oriented with respect to three main goals: preventing or limiting the presence of certain potentially harmful compounds including growth promoters; increasing certain desirable nutrients, such as conjugated linoleic acids (CLA) and long-chain polyunsaturated fatty acids (LC-PUFA) with potential beneficial effects on human health; and offering consistently high sensory quality standards. Although recent studies have shown that sex and slaughter season influence Barrosã meat fat composition (Costa et al., 2006; Alfaia et al., 2007), the effects of other production factors, including farm location in Barrosã-PDO meat nutritional value, remain unknown.

Taking into consideration the above discussion, the objective of this study was to characterize intramuscular fatty acid composition (IMF), cholesterol and α-tocopherol contents of Barrosã-PDO meat produced in lowland (<400 m), ridge (400–700 m) and mountain (>700 m) areas. In addition, a canonical discriminant analysis (CDA) was applied to FA profile in order to classify and predict the origin of the meat. The study was conducted in natura under the usual conditions of farming and management applied to produce Barrosã-PDO meat.

2. Material and methods

2.1. Animals and meat samples

Meat samples were obtained from 45 (25 males and 20 females) purebred Barrosã calves (produced according to PDO guidelines in farms located in Minho and Trás-os-Montes, the two geographic sub-regions legally defined for meat certification (Council Regulation no. 2081/92 of 14/7, EEC), at three distinct altitudes (15 animals from each altitude): lowlands (<400 m), ridges (400–700 m) and mountains (>700 m). The farms chosen in order to represent Barrosã-PDO veal available in the market were geo-referenced by global positioning system (GPS), and the respective altitude was determined using the program ArcGIS (2004).

Calves (7.6 ± 1.0 months of age and 99.4 ± 14.1 kg live weight; mean ± standard deviation) were slaughtered in the industrial abattoir of Penafiel. During the day after slaughtering, samples for lipid, cholesterol and α-tocopherol determinations were taken from the distal regions of biceps femoris (Bf) and supraspinatus (Ss) muscles and from the middle superficial layer of the longissimus dorsi (Ld) (L4–L6) and stored under vacuum at −20 °C, until analysis. Muscles were selected due to their divergent growth patterns and functionalities in vivo and also, because they represent meat cuts of different expected eating quality and economical value (round, loin and chuck).

2.2. Fatty acid analysis

Intramuscular fat was extracted with chloroform/methanol (2:1) (Folch et al., 1957) in duplicate from 20 g of muscle, trimmed of visible adipose and connective tissues. Due to the fact that FA compositions of neutral and polar lipids have different features and pattern of changes, a separate evaluation of both classes of lipids was performed to afford a more precise analysis and to answer how PDO meat FA composition is affected by farm location. Thus, separation into neutral lipids (NL) and phospholipids (PL) was performed through a Waters Sep-Pak silica cartridge (Millipore, Waters Chromatography Division, Milford, MA, USA) as described by Juaneda and Roquelin (1985). Lipid extracts were esterified with KOH (2 N) in methanol (ISO 5509, 2000). FA methyl esters (FAME) were analysed by gas–liquid chromatography (GLC) using a HRGC 5160 gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with a flame ionization detector (FID) and a 60 m long DB 23 capillary column (50% cyanopropyl–methylpolysiloxane). The oven temperature was raised from 70 to 195 °C at 5 °C/min for the NL fraction and from 70–195 °C (10 min) to 220 °C (60 min) for the PL fraction. Injector and detector temperatures were set at 220 and 280 °C, respectively. The identification of FA was based on the comparison of retention times of individual FA with standard mixtures (Supelco and Nuchek GLC reference standard FAME mixture). Due to the fact that some peaks overlapped and became difficult to be properly identified, some samples were separated on a SPTM-2560 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness, Supelco Inc., Bellefonte, PA, USA), coated with cyanopropyl polysiloxane stationary phase and confirmed by GC–MS (Saturn 2200, Varian, Walnut Creek, CA, USA). The FA was expressed as weight percentage.

2.3. Cholesterol analysis

Cholesterol was quantified by high performance liquid chromatography (HPLC) according to Costa et al. (2006), using a Spectra-Physics Model Spectra 100 equipped with variable wavelength UV detector set at 206 nm and a Spherisorb S5W silica cartridge with 5 μm and 4.0 mm × 125 mm (Waters PSS 845549). The mobile phase was hexane/2-propanol (97:3 v/v) at a flow rate of 1.0 mL/min. The cholesterol content was expressed as mg/100 g of fresh muscle.

2.4. α-Tocopherol analysis

α-Tocopherol was determined by HPLC, according to Costa et al. (2006). Briefly, 20 g of sample was weighed into a dark flask and saponificated with 60% potassium hydroxide solution and 150 mL of absolute ethanol. The mixture was refluxed for 40 min at 100 °C and then cooled and extracted with petroleum ether. After the solvent evaporation, the residue was redissolved in 5 mL of hexane, filtered and injected in an HPLC system (Spectra-Physics, model Spectra 100) equipped with variable wavelength UV detector set at 292 nm and a Spherisorb S5W silica cartridge with 5 μm and 4.0 mm × 125 mm (Waters PSS 845549). The mobile phase was hexane:1,4-dioxane (99:1) at a flow rate of 0.8 mL/min. The α-tocopherol content was expressed as mg/100 g of fresh muscle.

2.5. Statistical analysis

The influence of geographic origin on the FA composition was evaluated within Bf, Ld and Ss muscles by analysis of covariance (ANCOVA) using a general linear model (GLM) procedure and Fisher's test at a 5% of significance level with the STATISTICA 7.0 software (Statistica, 2004). The model included the effect of farm location (lowland vs. ridge vs. mountain) and age as covariate, to avoid its confounding effects. Canonical discriminant analysis was applied to FA from Bf, Ld and Ss muscles in order to classify and predict the meat origin. The selection of variables for CDA was