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Simultaneous HPLC quantification of total cholesterol, tocopherols and β-carotene in Barrosã-PDO veal

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Abstract

A simple, rapid and sensitive procedure for the simultaneous determination of total cholesterol, tocopherols and β-carotene in meat is described. The method involves a direct saponification of the meat, a single *n*-hexane extraction and the analysis of the extracted compounds by normal-phase HPLC, using fluorescence (tocopherols) and UV–Vis photodiode array (cholesterol and β-carotene) detections in tandem. Rates of recovery of spiked meat samples were 93% for cholesterol, 83–86% for (α-, β- and -γ) tocopherols and 89% for β-carotene. Repeatabilities were high (CV < 6%) for all determined compounds, except for δ-tocopherol. This tocopherol, which is not usually present in meat, showed a much lower recovery percentage (73%) and repeatability (12.8%). This methodology was applied for the quantification of total cholesterol, tocopherols and β-carotene in three muscles (*longissimus thoracis*, *longissimus lumborum* and *semitendinosus*) of the Portuguese traditional Barrosã-PDO veal, obtained from autochthonous calves fed extensively during summer (with the least abundant green pastures) and slaughtered in early autumn (October). Barrosã-PDO veal showed median contents of total cholesterol (0.50–0.56 mg/g) and, depending on the analysed muscle, moderate to high contents of α-tocopherol (3.3–3.9 μg/g) and β-carotene (0.07–0.09 μg/g), suggesting an high sensorial and hygienic quality.

Keywords: Cholesterol; Tocopherols; β-Carotene; HPLC analysis; Barrosã-PDO meat; Veal

1. Introduction

It is generally accepted that, apart from microbial spoilage, lipid oxidation is the primary cause of quality deterioration in muscle foods (Buckley, Morrissey, & Gray, 1995; Monahan, 2000). D-α-, D- β-, D-λ- and D-δ-Tocopherols, together with the corresponding tocotrienols (AOAC, 2000), are the natural compounds with vitamin E activity, which is the primary lipid-soluble antioxidant in biological systems (Eldin & Appelqvist, 1996; Kerry, Buckley, & Morrissey, 2000). Although the major form of vitamin E in meat is α-tocopherol, minor amounts of other vitamin E homologues also exist (Pyrenean, Syvaoja, Varo, Salminen, & Koivistoinen, 1985). Considering that the various vitamin E forms have different antioxidant potencies and biological activities (Abidi, 2000), the quantification of all vitamin E molecules in foods is usually required. β-Carotene, a pro-vitamin A compound, is the predominant carotenoid

Abbreviations: ANOVA, analysis of variance; BHT, 2,4-di-*tert*-butyl hydroxytoluene; CHR, cholesterol; β-CT, β-carotene; CV, coefficient of variation; HPLC, high-performance liquid chromatography; LL, *longissimus lumborum*; LT, *longissimus thoracis*; LSD, least significant difference; PDO, protected designation of origin; ST, *semitendinosus*; TF, tocopherols; UV, ultraviolet.

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in meat and meat products (Indik, 1988; Mortensen & Skibsted, 2000). This carotenoid has been suggested to function as a dietary lipid-soluble antioxidant, with an important role in controlling oxidatively induced diseases, such as cancer and atherosclerosis (Decker, Livisay, & Zhou, 2000; Palozza & Krinsky, 1992). Other carotenes, such as α-carotene, are absent or present in such low quantities in meat that may be ignored as a vitamin A source (Torrissen, 2000). Meat provides from one third to half (Chizzolini, Zanardi, Dorigoni, & Ghidini, 1999) of the daily-recommended cholesterol intake (300 mg, World Health Organisation). Epidemiological and clinical studies have suggested that cholesterol intake is directly associated to a greater risk of obesity and hypercholesterolemia, conditions that predispose to several chronic diseases of the circulatory system (Ganji, Kamanna, & Kashyap, 2003; Stark, 1996). Moreover, cholesterol oxidation products in food, exhibiting mutagenic, carcinogenic and cytotoxic properties (Guardiola, Codony, Addis, Rafecas, & Botella, 1996), are strongly dependent on cholesterol concentrations (Engeseth & Gray, 1994).

It is clear, from the discussion above, that the quantification of total cholesterol and lipid-soluble antioxidant vitamins (tocopherols and β-carotene) could provide valuable information relating to meat quality and safety. To our knowledge, there are no methods in the literature describing the simultaneous determination of these compounds in meat. However, some HPLC methods for the quantification of α-tocopherol in meat, matrix in which the higher levels of protein interfere in the process of extraction (Abidi, 2000), have been developed (Arnold, Scheller, Arp, Williams, & Schaefer, 1993; Liu, Scheller, & Schaeffer, 1996). The method proposed by Liu et al. (1996) is simple and rapid, allowing the analysis of a large number of samples per day (Katsanidis & Addis, 1999). This method involves a direct saponification of the meat followed by a one-step isooctane extraction of the saponified samples. Katsanidis and Addis (1999) have simultaneously determined tocopherols, tocotrienols, and cholesterol in meat by using a modification of the method described by Liu et al. (1996). More recently, Cayuela, Garrido, Banón, and Ros (2003) have also introduced modifications on the method reported by Liu et al. (1996) in order to determine α-tocopherol and cholesterol in fresh pig meat. Finally, it has been proposed, by Eldin, Gorgen, Petterson, and Lampi (2000), that silica columns and hexane-based mobile phases are the most appropriated for the separation of tocopherols and tocotrienols by normal-phase HPLC. Cholesterol, tocopherols and β-carotene are all nonpolar compounds that absorb in the UV–Vis range. Therefore, it is possible that these compounds could be analysed simultaneously, on the same normal-phase HPLC experiment, using the method of Liu et al. (1996) with the improvements introduced by Katsanidis and Addis (1999), Eldin et al. (2000) and Cayuela et al. (2003).

Traditional meats with protected designation of origin (PDO), derived from local extensive production systems and animal breeds, are certified by European Union legislation and are supposed to present unique quality and organoleptic characteristics, especially associated with the specific properties of its lipid fraction (Council Regulation No. 2081/92 of 14/7, EEC). Quality and nutritive value of pasture biomass is highly dependent on cultural practices, season and geographical factors (Moloney, Mooney, Kerry, & Troy, 2001; Oltjen & Beckett, 1996). Therefore, meat from grazing ruminants is expected to reflect these variabilities. However, the investigations reporting tissue contents of tocopherols and carotenoids in pasture-fed cattle are scarce (Yang, Brewster, Lanari, & Tume, 2002). According to Roseiro, Costa, and Santos (2002), meat from autochthonous bovine breeds, produced under traditional handling systems based on pastures, has been progressively introduced in Portuguese diets. One of such examples is Barrosã-PDO veal (Commission Regulation No. 1263/96 of 1/7, EC), which is obtained from Barrosã breed calves, produced in a traditional production pastured-based system in Minho Highlands and Terras do Barroso (North of Portugal). However, there are no reports on the composition of this Portuguese traditional veal on tocopherols and β-carotene.

The goal of this work was, therefore, to develop a simple, rapid and sensitive method for the simultaneous determination of total cholesterol, tocopherols and β-carotene in meat. Additionally, since the information relating the composition of meat from Portuguese traditional cattle is scarce, the novel methodology was used for the quantification of total cholesterol, tocopherols and β-carotene in Barrosã-PDO veal. The animals were fed in an extensive production system with the least abundant green pastures of summer and were slaughtered in early autumn.

2. Materials and methods

2.1. Reagents and standard solutions

General pro-analysis grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany) and absolute ethanol (99.8% v/v) from AGA (Lisbon, Portugal). *n*-Hexane, isopropanol (Merck Biosciences, Darmstadt, Germany) and Milli Q water were of HPLC-grade. High-purity nitrogen gas (R grade) was acquired from Air Liquide (Lisbon, Portugal). DL-α-, D-β-, D-γ- and D-δ-Tocopherols standards were obtained from Calbiochem (Merck Biosciences, Darmstadt,

Germany), and all-*trans*–carotene and cholesterol standards from Sigma Chemical Co. (St. Louis, MO, USA).

The standard stock solutions of cholesterol in *n*-hexane (1.0 mg ml^{-1}) were prepared monthly and stored at −20 °C. Working standard solutions (0.01–1 mg ml^{-1}) were obtained by serial dilutions of the stock solution with *n*-hexane. Standard stock solutions of tocopherols and β-carotene were prepared to a concentration of approximately 4 mg ml^{-1} in absolute ethanol and 20 μg ml^{-1} in *n*-hexane, respectively, and stored in amber vials at −20 °C. Working standard solutions of these vitamins (approximately from 0.1 to 1.25 mg ml^{-1}) were obtained by diluting the stock solutions with *n*-hexane and their exact concentrations determined spectrophotometrically using the specific extinction coefficients for tocopherols (Frolik & Olson, 1984) and β-carotene (De Ritter & Purcell, 1981).

2.2. Animals and meat samples

Barrosã breed calves ($n = 17$) were maintained following a traditional production pasture-based system according to the rules established in the Barrosã-PDO product specifications. Briefly, the calves were reared on pasture with their dams until weaning at 6 ± 0.5 months of age. After weaning, calves were raised on a summer grass pasture until slaughter in October 2003, at 8.5 ± 0.5 months of age (live body weight: 205 ± 24 kg).

Meat samples were collected from the ribeye and loin portions of *longissimus dorsi* muscle (T1–T3 *longissimus thoracis* muscle, LT, and L4–L6 *longissimus lumborum* muscle, LL, respectively) and from the distal region of *semitendinosus* muscle (ST), 2–3 days after slaughter (+1 °C). All meat samples were ground using a food processor (3 × 5 s), vacuum packed and stored at −80 °C until analysed.

2.3. Saponification and extraction

For saponification, 0.75 g of homogenised meat sample was placed in a screw teflon-lined cap tube, in duplicate, to which 0.2 g L-ascorbic acid and 5.5 ml saponification solution were added. The saponification solution, freshly prepared each week, contained 11% w/v potassium hydroxide in a mixture of 55% v/v absolute ethanol and 45% v/v distilled water. The sample was then immediately vortexed in order to avoid meat agglomeration. After vortexing, the air was eliminated from the reaction, by displacement with nitrogen gas and the sample was further shaked until the ascorbic acid was completely dissolved. The saponification was carried out in a shaking water bath (200 rpm) at +80 °C for 15 min.

After saponification, samples were cooled in tap water for 1 min. Following cooling 1.5 ml of distilled water and 3 ml of 25 μg/ml BHT solution in *n*-hexane were added (final proportions of 4.5 ml H_2O:3 ml ethanol:3 ml *n*-hexane; the meat sample was assumed to contribute with 0.5 ml H_2O). The samples were vigorously vortexed for 2 min and centrifuged at 1500*g* for 5 min, in order to accelerate phases separation. An aliquot of the upper layer (*n*-hexane) was transferred into a small screw teflon-lined cap tube and a spatletip of anhydrous sodium sulphate was added. Finally, the tube was briefly shaken and an aliquot of the *n*-hexane layer was filtered through a 0.45-μm hydrophobic membrane into an amber screw-cap vial with teflon septa.

2.4. HPLC analysis

The HPLC system used was an Agilent 1100 Series (Agilent Technologies Inc., Palo Alto, CA, USA) composed by a G1311A Agilent quaternary pump, a G1322A Agilent vacuum solvent delivery degasser, a G1316A Agilent thermostatted column compartment with cooling, a G1313A Agilent autosampler, a G1315B Agilent UV–Vis photodiode array detector, and a G1321A Agilent fluorescence detector. The liquid chromatographic system was controlled and the data collected and processed by the HP ChemStation for LC 3D software (Rev. A.09.01, Agilent Technologies Inc., Palo Alto, CA, USA).

The simultaneous analysis of cholesterol, tocopherols and β-carotene in meat were performed using a normal-phase silica column (Zorbax RX-Sil with the corresponding 12.5 mm analytical guard column, 4.6 mm ID × 250 mm, 5 μm particle size, Agilent Technologies Inc., Palo Alto, CA, USA), with fluorescence detection for tocopherols (excitation wavelength of 295 nm and emission wavelength of 325 nm) and UV–Vis photodiode array detection for cholesterol (202 nm) and β-carotene (450 nm) in series. The solvent (1% v/v isopropanol in *n*-hexane) flow rate was 1 ml/min, the run last for 17 min and the temperature of the column oven was adjusted at +20 °C. The injection volumes used varied between 20 and 100 μl in order to get values inside the linearity range of the standard curves.

The contents of total cholesterol, tocopherols and β-carotene in meat were calculated, in duplicate for each muscle sample (values accepted for CV < 6%), based on the external standard technique, from a standard curve of peak area vs. concentration.

2.5. Statistical analysis

Standard curves for total cholesterol, tocopherols and β-carotene were obtained by regression analyses using seven different concentrations of standard

solutions in triplicate. Correlation coefficients were calculated using the Pearson method of Statistix for Windows version 8 (Analytical Software, Tallahassee, FL, USA) and their significance red on statistical tables (Fisher, Ronald, & Yates, 1963). The validation of the analytical procedure was done in agreement with the guidelines described in Directive (1996). The detection limits of the method, for the different analytes, were expressed as the ratio between 3.3 times the standard deviation of the responses, with estimations based on the standard curves, and the slope of the standard curves (Directive, 1996).

Statistical treatment of data were conducted by ANOVA, at a significance level of 5% (H_0: $p < 0.05$), using the one-way ANOVA procedure of Statistix for Windows. When the *F*-test was significant, the comparison of means were assessed by the LSD method also at a significance level of 5%. The coefficient of variation (CV) is the ratio between the standard deviation and the average value in percentage.

3. Results and discussion

3.1. Validation of the proposed method for the simultaneous determination of cholesterol, tocopherols and β-carotene in meat

Following the method proposed here, total cholesterol and lipid-soluble antioxidant vitamins (tocopherols and β-carotene) in meat were subjected to a direct saponification (without previous fat extraction) and extracted in a single step with *n*-hexane. The simultaneous quantification of cholesterol, tocopherols and β-carotene in meat were performed by normal-phase HPLC using fluorescence (tocopherols) and UV–Vis photodiode array (cholesterol and β-carotene) detections (Fig. 1). The saponification and extraction procedures used were based on the method developed by Liu et al. (1996), for α-tocopherol determination in beef, including the modifications proposed by Katsanidis and Addis (1999) and Cayuela et al. (2003), using *n*-hexane as the

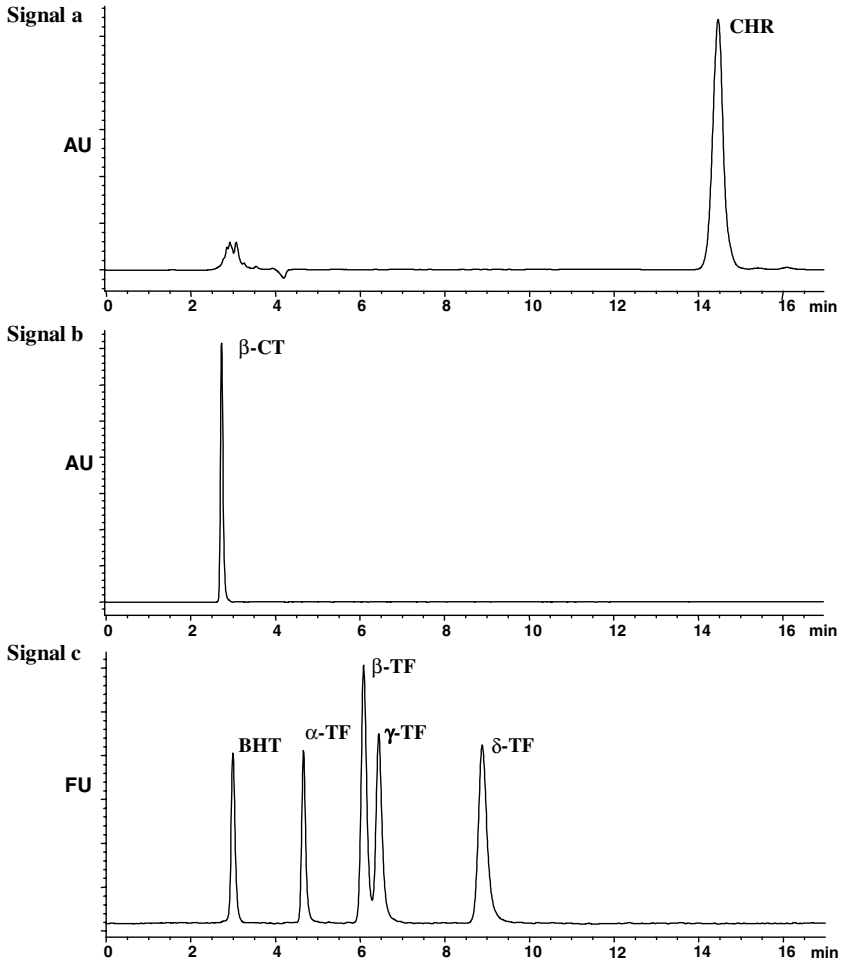

Fig. 1. HPLC chromatogram of a mixture of cholesterol, tocopherols and β-carotene standards: signal (a) cholesterol (CHR) detection (UV, 202 nm); signal (b) β-carotene (β-CT) detection (visible, 450 nm); signal (c) α-, β-, γ- and δ-tocopherols (TF) detection (fluorescence, excitation at 295 nm and emission at 325 nm). BHT (25 μg/ml) was added to the standard solutions as antioxidant.

Table 1
Evaluation of the proposed method for the simultaneous HPLC determination of cholesterol, tocopherols and β-carotene in meat

	Rates of recovery[a] (%)	Repeatability[b] (CV)	Limits of detection[c] (ng/injection)	Linearity[d] (up to, ng/injection)
Cholesterol	93	5.1	0.5 μg	20 μg
α-Tocopherol	86	4.2	1.4	25
β-Tocopherol	83	4.5	1.3	25
γ-Tocopherol	84	4.4	1.1	25
δ-Tocopherol	73	12.8	2.0	25
β-Carotene	89	2.8	0.3	30

[a] Average of quadruplicate determinations of spiked meat samples with three different concentrations of cholesterol (0.03, 0.3 and 3 mg), tocopherols and β-carotene (0.3, 1.5 and 3.0 μg).
[b] Average coefficients of variation (CV) of quadruplicate determinations of spiked meat samples with the three different concentrations analysed.
[c] Calculation based on the standard curves, using seven different concentrations of standard solutions in triplicate, as described in Section 2.5.
[d] Established from the preparation of the standard curves, using seven different concentrations of standard solutions in triplicate.

extracting solvent. Saponification was necessary to achieve complete hydrolysis and release of the molecules to be analysed from their membranal location. The addition of L-ascorbic acid previous to the saponification step was necessary to prevent tocopherol degradation by the alkaline solution (Liu et al., 1996). Cayuela et al. (2003) proposed the use of an inert nitrogen atmosphere during the saponification step and the addition of an antioxidant (BHT) in the organic extraction phase to enhance the stability of the α-tocopherol. In parallel, Katsanidis and Addis (1999) suggested the addition of water to increase the polarity of the aqueous phase and improve the partitioning of nonpolar compounds into the organic phase (*n*-hexane). The suitability of silica columns and hexane-based mobile phases for the separation of tocopherols and tocotrienols, by normal-phase HPLC, has been previously demonstrated by Eldin et al. (2000).

The recovery capacities of the method, assessed in quadruplicate by spiking meat samples with three different concentrations of cholesterol (0.03, 0.3 and 3 mg), tocopherols (0.3, 1.5 and 3.0 μg) and β-carotene (0.3, 1.5 and 3.0 μg) standards before saponification, are shown on Table 1. The average recovery of cholesterol (93%) from spiked meat samples was slightly lower than the achieved by the modified method of Cayuela et al. (2003) (97%) but higher than the reported by Katsanidis and Addis (1999) (84%). The direct saponification of the meat followed by the one-step *n*-hexane extraction of the saponified molecules was shown to be effective for extracting α- (86%), β- (83%), γ- (84%) and δ- (73%) tocopherols, as well as β-carotene (89%). These average recovery levels are lower than those described by Katsanidis and Addis (1999), for α- (95%), β- (95%), γ- (94%) and δ- (80%) tocopherols, as well as those presented by Liu et al. (1996) for α-tocopherol (91%), but higher than the recovery percentages obtained by Cayuela et al. (2003) for α-tocopherol (78%). The apparent high susceptibility of δ-tocopherol to degradation during alkaline saponification following the method described here is in agreement with the results of Katsanidis and Addis (1999). Nevertheless, this vitamin E homologue is not usually present in meat.

The average of the coefficients of variation, expressing the repeatability of the method, assessed in quadruplicate with mixtures of standards and meat samples, were 5.1% for cholesterol, 4.2% for α-tocopherol, 4.5% for β-tocopherol, 4.4% for γ-tocopherol, 12.8% for δ-tocopherol, and 2.8% for β-carotene (Table 1). The repeatability of the proposed method for cholesterol and α-tocopherol determinations was comparable to those reported by Liu et al. (1996), which was 3.1% for α-tocopherol, and Cayuela et al. (2003), which were 5.6% for α-tocopherol and 5.9% for cholesterol. The detection limits of the method were 0.5 μg/injection for cholesterol, 1.4 ng/injection for α-tocopherol, 1.3 ng/injection for β-tocopherol, 1.1 ng/injection for γ-tocopherol, 2.0 ng/injection for δ-tocopherol, and 0.3 ng/injection for β-carotene (Table 1). The linear response of the detectors was found for concentrations up to 20 μg/injection for cholesterol, 25 ng/injection for tocopherols and 30 ng/injection for β-carotene (Table 1). The values of correlation coefficients were very highly significant (H_0: $p < 0.001$) for all the compounds analysed (0.9989 for cholesterol, 0.9981 for α-tocopherol, 0.9984 for β-tocopherol, 0.9981 for γ-tocopherol, 0.9963 for δ-tocopherol, and 0.9999 for β-carotene). The silica column, the hexane-based mobile phase and the two detectors in series showed to be efficient for the complete separation and sensitive quantification of these substances in meat. The serial combination of fluorescence and UV detectors for the analysis of lipids was previously used by Murphy, Rosenberger, and Horrocks (1996) and Cayuela et al. (2003).

3.2. Contents of cholesterol, tocopherols and β-carotene in Barrosã-PDO veal obtained from calves slaughtered in early autumn

Fig. 2 displays a typical HPLC chromatogram of the Barrosã-PDO veal obtained from calves slaughtered in

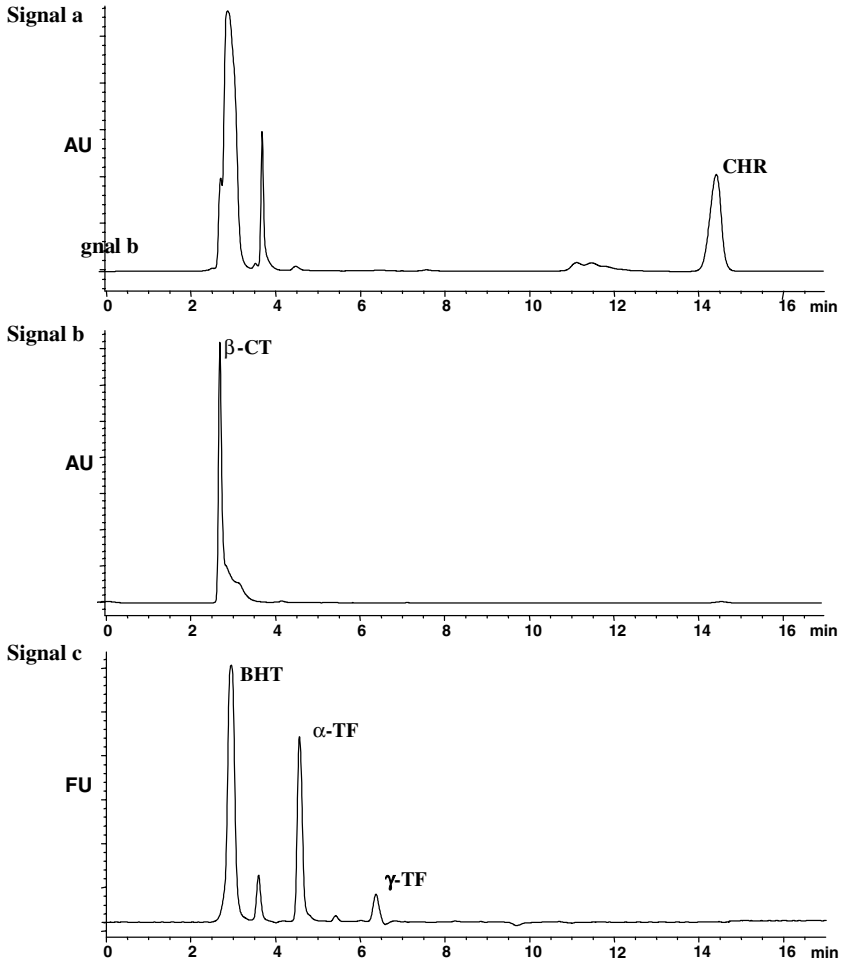

Fig. 2. Typical HPLC chromatogram of a Barrosã-PDO veal sample, obtained from calves slaughtered in early autumn: signal (a) cholesterol (CHR) detection (UV, 202 nm); signal (b) β-carotene (β-CT) detection (visible, 450 nm); signal (c) α- and γ-tocopherols (TF) detection (fluorescence, excitation at 295 nm and emission at 325 nm). BHT (25 μg/ml) was added to the meat samples as antioxidant.

Table 2
Contents (means ± standard deviation, $n = 17$) of cholesterol, tocopherols and β-carotene, in different muscles (*longissimus thoracis*, LT; *longissimus lumborum*, LL; and *semitendinosus*, ST) of Barrosã-PDO veal, obtained from calves slaughtered in early autumn

	LT muscle	LL muscle	ST muscle	*F*-test	Probability for H_0
Cholesterol (mg/g)	$0.56 \pm 0.068^{a*}$	0.52 ± 0.048^{b}	0.50 ± 0.038^{b}	6.61	$p < 0.05$
α-Tocopherol (μg/g)	3.9 ± 1.11	3.3 ± 1.29	3.6 ± 1.32	1.06	ns
γ-Tocopherol (μg/g)	0.15 ± 0.081	0.13 ± 0.053	0.14 ± 0.064	0.47	ns
β-Carotene (μg/g)	0.09 ± 0.044	0.07 ± 0.027	0.08 ± 0.039	2.10	ns

* Means within the same row with distinct superscript letters are significantly different (H_0: $p < 0.05$); ns, means not statistically significant (H_0: $p > 0.05$).

early autumn. The contents of total cholesterol in the different muscles of Barrosã-PDO veal are presented in Table 2. The values of total cholesterol in LT muscle (0.56 mg/g) were significantly higher (H_0: $p < 0.05$) than those obtained in LL (0.52 mg/g) and ST (0.50 mg/g) muscles. According to Chizzolini et al. (1999), differences in the cholesterol content observed on different muscles of the same animal species might be explained by differences in fibre composition. This metabolic hypothesis results from the observation that oxidative muscles are known to be richer in phospholipids and that there is a direct relation between the contents of phospholipids and cholesterol. The direct relationship between phospholipids and cholesterol (60–80% in the membrane component of muscle) seems to be necessary to maintain membrane fluidity in a narrow range (Alasnier, Rémignon, & Gandemer, 1996). In contrast, cholesterol provided by the intramuscular adipose tissue in muscles (marbling) only slightly contributes (about 0.02 mg/g) to the total cholesterol of meat (Browning,

Huffirian, Egbert, & Jungst, 1990; Chizzolini et al., 1999). The values for total cholesterol in Barrosã-PDO veal are similar to those described for *longissimus dorsi*, *biceps femoris* and *supra spinatus* muscles of the same veal (Roseiro et al., 2002), as well as to those reviewed by Chizzolini et al. (1999) for beef.

The contents of the three muscles of Barrosã-PDO veal in vitamin E compounds are depicted on Table 2. α- and γ-Tocopherols, the last one being present in small amounts, were the only vitamin E homologues detected in Barrosã-PDO veal. β- and δ-Tocopherols were not detected in any of the muscles analysed. The prevalence of α-tocopherol in meat is well known and is due to the more than tenfold preference of the tocopherol-binding protein for α-tocopherol, relatively to γ-tocopherol, which is the most common vitamin E homologue in plant foods (Decker et al., 2000). The results showed no significant differences (H_0: $p > 0.05$) on the contents of α- and γ-tocopherols among LT, LL and ST muscles. However, LT muscle tended to have higher mean concentrations of α-tocopherol, ST muscle the intermediate mean values and LL muscle the lower mean values. The levels of α-tocopherol in Barrosã-PDO veal (mean values varied from 3.3 to 3.9 μg/g, depending on the muscle) are close to the values reported in meats originated on pasture-fed cattle (4.4–5.8 μg/g) and on grain-fed cattle receiving supra-nutritional doses of vitamin E (4.3–6.0 μg/g). However, these values are much higher than those reported for meat derived from grain-fed cattle (1.8–2.4 μg/g) (Yang et al., 2002). The α-tocopherol values of Barrosã-PDO veal are also similar to those reported by Kerry et al. (2000) in meat (*gluteus medius* muscle) from summer-pastured crossbreed steers (3.5 μg/g). However, West, Young, and Agnew (1997) mentioned contents of α-tocopherol in the *longissimus lumborum* of pastured cattle varying between 3.7 and 7 μg/g, suggesting that there may be a difference in the α-tocopherol levels found in pastures with different biomass compositions. According to the results reported by Yang et al. (2002), the supplementation of pasture-fed cattle with vitamin E did not increase the levels of α-tocopherol in meat (4.3–6.1 μg/g). In fact, there appears to exist a limit for the accumulation of α-tocopherol in muscle tissues (Arnold, Arp, Scheller, Williams, & Schaefer, 1993; Yang et al., 2002), which in the *longissimus dorsi* should be around 7 μg/g (Arnold et al., 1993).

It is well known that, in cattle, β-carotene is essentially the only carotenoid absorbed at the level of the intestine and is, therefore, the predominant carotenoid form found in meat (Yang, Larsen, & Tume, 1992; Yang et al., 1993). The values of β-carotene in the Barrosã-PDO veal were not significantly different (H_0: $p > 0.05$) among LT, LL and ST muscles (Table 2). These contents of β-carotene (mean values varied from 0.07 to 0.09 μg/g, depending on the muscle) reached the lower limit of the range described for β-carotene in meat from cattle grazed on good green pasture (0.09–0.22 μg/g), which is rich in tocopherols and carotenoids (Yang et al., 2002). According to the same authors, the levels of β-carotene in meat from grain-fed cattle are much lower (0.01–0.03 μg/g).

Taken together, the data suggest that calves were grazed on a good quality summer pasture, moderate to rich in tocopherols and carotenoids. The good quality of this summer pasture can be explained by the maritime climate of Minho Highlands and Terras do Barroso (North of Portugal), with relatively high values of precipitation and temperature at this time of the year. Based on the moderate to high contents of α-tocopherol and β-carotene and on their synergistic antioxidant effect (Haila & Heinonen, 1994; Mortensen & Skibsted, 2000), it seems that Barrosã-PDO veal, obtained from calves slaughtered in early autumn, has a good lipid stability and hence an high sensorial quality, nutritional value and healthiness. In order to investigate the seasonal variability of tocopherols and β-carotene contents in Barrosã-PDO veal, we are currently determining these compounds in meat originated from calves fed extensively with the most abundant green pastures of winter-spring and slaughtered in late spring (late May–June).

4. Conclusions

The methodology described here provides a simple, rapid and sensitive approach for the simultaneous determination of total cholesterol, tocopherols and β-carotene in meat. With the exception of δ-tocopherol, which is not usually present in meat, the direct saponification single extraction procedure showed to be efficient in achieving high repeatable recovery percentages for all analysed compounds. Together, the modifications introduced to the original method for α-tocopherol determination in beef, enabled the simultaneous and accurate determination of total cholesterol and unstable lipid-soluble antioxidant vitamins in meat. Barrosã-PDO veal obtained from calves slaughtered in early autumn (October), fed in an extensive production system with the least abundant green pastures of summer, showed median contents of total cholesterol and, depending on the analysed muscle (LT, LL and ST), moderate to high contents of α-tocopherol and β-carotene. Based on the synergistic antioxidant effect between α-tocopherol and β-carotene, these findings suggest that Portuguese Barrosã-PDO veal obtained from calves slaughtered in early autumn seems to have a good lipid stability and, possibly, an high sensorial quality, nutritional value and safety for human health.

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