

Short paper

Determination of α -tocopherol in pork with high intramuscular fat content

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RESUMEN

Determinación de α -tocoferol en carne de cerdo con alto contenido en grasa intramuscular

Se han utilizado tres métodos de cuantificación de α -tocoferol en muestras de tejido muscular de cerdos con un contenido en grasa intramuscular del 3% (LF) y del 9% (HF). Dos de los métodos saponifican la muestra antes de extraer el α -tocoferol, y el tercero utiliza una extracción directa con solventes. Antes del análisis se añadieron cinco cantidades de α -tocoferol en cada caso. La recuperación del α -tocoferol en las muestras de bajo contenido en grasa intramuscular fue en todos los casos alrededor del 85-95%, sin encontrarse diferencias según el método utilizado. En las muestras con alto contenido en grasa intramuscular la recuperación fue mucho menor cuando se utilizaron los métodos que saponifican la muestra (50-60%). Al añadir mayor concentración de KOH no se mejoró sustancialmente la recuperación. El método basado en extracción directa con solventes proporcionó una recuperación superior (85-92%) y es por tanto el que se recomienda para muestras con alto contenido en grasa intramuscular.

PALABRAS-CLAVE: α -tocoferol (determinación) – Comparación de métodos – Grasa – Tejido animal.

SUMMARY

Determination of α -tocopherol in pork with high intramuscular fat content

Extraction of α -tocopherol from pork samples with low (3%) (LF) or high (9%) (HF) amount of intramuscular fat have been carried out by three different methods, two of them based in saponification plus extraction of α -tocopherol and the other one without saponification. All samples were spiked with five different amount of α -tocopherol prior to analysis. In LF samples, recovery was in the range 85-95% in all cases, with not significant differences between methods. Recovery was much lower in HF samples when using methods which involve prior saponification of muscle samples (50-60%). Changes in KOH concentration did not improve markedly the recovery. The method based on direct extraction provided much better recovery in HF samples (85-92%) and consequently is recommended for samples high in fat.

KEY-WORDS: α -tocopherol (determination) – Animal tissue – Comparison of methods – Fat.

1. INTRODUCTION

There is increasing interest in the involvement of in vivo lipid peroxidation and its links to some associated diseases, with special attention being paid to the effect of certain dietary compounds, particularly α -tocopherol, in retarding some degenerative processes (Parker, 1981, Burton and Traber, 1990). Moreover, oxidation may be reduced in foods from animal origin if α -tocopherol is included at appropriate dose in the feed and is administered for enough time, due to stabilization and prevention of oxidative damage in cellular and subcellular membranes (Ashgar *et al.*, 1989, Monahan *et al.*, 1992, López Bote *et al.*, 1996). Quantitation of tocopherol in animal tissues is an important issue. However, due to difficulty in analytical methodology, specially for muscle tissues, data are disperse and authors complain of low reproducibility (Ang *et al.*, 1990, Roy *et al.*, 1995). Among other reasons because of the sensitivity of α -tocopherol to heat and light (Kochhar and Rossel, 1990).

Earlier methods for the analysis of vitamin E in tissues include colorimetry, spectrophotometry and fluorimetry, usually after isolating a vitamin E-rich fraction by saponification. Tissue analyses for vitamin E based on gas-liquid chromatography have also been developed but degradation may occur due to high temperatures. Else, this method require complex sample preparation procedures to remove interfering lipids with consequently low recovery (Shearer, 1986).

Recently tocopherols in food, feeds, blood and tissues have been determined by HPLC. As a sample pretreatment for HPLC, unsaponifiable matter is commonly extracted with an organic solvent after saponification (Cohen and Lapointe, 1980, Chow and Omaye, 1983, Fukuba and Murota, 1985). Extracted tocopherols are separated using either a normal phase or a reverse phase column and quantified with a fluorometric, ultraviolet or electrochemical detector. In either case, saponification is usually carried out by adding concentrated KOH to tissue homogenates and maintain in a hot environment (around 70°C) for at least 30 minutes. However, attention should be paid because α -tocopherol is rapidly oxidized in alkaline environment

(Egan *et al.*, 1981). Moreover, saponified material may interfere quantification of tocopherols (Ueda and Igarashi, 1987b). Methods developed to avoid saponification have also been proposed but its use is much limited (Egan *et al.*, 1981, Erickson, 1991).

In the present study we compare the recovery of α -tocopherol from muscle tissues low or high in intramuscular fat following different methods of extraction.

2. EXPERIMENTAL

Longissimus dorsi samples from Iberian pigs slaughtered at 160 kg live weight (9% intramuscular fat) (high fat meat) and from Landrace x Large White hybrids slaughtered at 90 kg live weight (3% intramuscular fat) (low fat meat) were used for analysis. In all cases animals received diets which incorporate low levels of α -tocopherol (10 mg/kg feed). Three different methods were used to analyze α -tocopherol concentration in tissues. Method A (Buttriss and Diplock, 1984) and B (Ueda and Igarashi, 1987a) include previous saponification of samples and method C direct extraction and injection with no saponification. This method is an adaptation of a methodology used in the Department of Animal Science Iowa State University, Ames, Iowa, (Laboratory of Dr. Sell) and is fully described by Mallarino (1992). Analysis were carried out six times from each sample. In each case, 0.5, 1, 2, 5 and 10 μ g α -tocopherol (Sigma, St Louis, USA) were spiked into homogenized samples prior to extraction. The final volume of extraction was adjusted in all cases to 200 μ l of absolute ethanol. Additionally a standard curve was done by addition of the same amounts of α -tocopherol to vials that were brought to a final volume of 200 μ l. Briefly, extraction methods used were as follows:

Method A.- A 20% tissue homogenate was prepared using 1.15% KCl. One ml of homogenate was placed in a tube containing 2 ml of 1% ethanolic pyrogallol (Sigma, St Louis, USA) and 0.3 ml of 50% KOH were also added. The tube was capped and maintained in a water bath at 70°C for 30 minutes. The tubes were then cooled and extraction with 4 ml of hexane (containing 0.05% BHT) was done in duplicate. After thoroughly mixing and centrifugation, the upper hexane layer was transferred to a tube, evaporated under nitrogen stream, redissolved in 200 μ l HPLC grade ethanol and injected in a HPLC.

Method B.- 100 mg of muscle homogenates were weighed accurately in a screwcapped centrifuge tube. Immediately, 100 μ l of a 1% NaCl solution, 1 ml of 6% ethanolic pyrogallol and 0.2 ml of 60% KOH were added and the tubes were heated in a water bath fixed at 70°C for 60 minutes. 4.5 ml of a 1% NaCl solution were then added and the whole mixture extracted with n-hexane/ethyl acetate solution (3ml). The saponified

extracts were centrifuged at 1500 xg for 5 min, and n-hexane was pipetted in another tube, evaporated under a nitrogen stream, redissolved in 200 μ l HPLC grade ethanol and injected in a HPLC.

Method C.- 0.8 g of tissue were cutted into small pieces (<ca. 1 mm) and 3 ml of 0.054 M phosphate buffer/HCl (pH 7) were placed in a homogenizer tube and homogenized for 30 sec. Homogenate was pour into a 10 ml volumetric flask and bring to volume with buffer after successive washes of the homogenizer tube. 3 ml of absolute ethanol and 3 ml of muscle homogenate were added to a culture tube and mixed. Additionally 1 ml of hexane was added and mixed. After centrifugation, hexane was pipetted in another tube evaporated under a nitrogen stream, redissolved in 200 μ l HPLC grade ethanol and injected in a HPLC.

25 μ l of extracts and standard solution were injected in all cases. HPLC analysis was carried out on a Hewlett Packard (Series 1050) HPLC isocratic pump. The column used was a Lichrocart PR 18 endcapped column (250x4-mm i.d., 5 μ m particle size) (Merck, Darmstad, Germany). The eluting solvent was methanol: water (97:3) at a flow rate of 2 ml/minute. Detector took place at a wavelength of 292 nm on a Hewlett Packard (Series 1050) absorbance detector. Chromatograms were recorded on a HPChem software package (Hewlett Packard). The recovery of α -tocopherol of spiked samples after different methods of extraction was determined by comparison of peak highness with those obtained for the standard curve of α -tocopherol.

Regression analysis was done to estimate the basal level of α -tocopherol in muscle samples. After discounting the calculated basal level to all the peaks, the highness of the peak corresponding to α -tocopherol in spiked samples at different concentrations and in injected pure solution were plotted and differences in recovery (due to each of the previously described extraction methods) were calculated. Statistical analyses were accomplished using software procedures contained in SAS (1988).

3. RESULTS AND DISCUSSION

Foods from animal origin may have a wide range of fat content depending on the system of production. A wide variety of methods for tocopherol quantification in food systems have been proposed (Buttriss and Diplock, 1984, Burton *et al.*, 1985, Ueda and Igarashi, 1987a, Rustan *et al.*, 1993, Delgado *et al.*, 1995, Roy *et al.*, 1995), but in general the practical use of some of these methods, especially in muscle systems produce low reproducible results. In a recent collaborative study within the scope of the European Union, eight different specialized laboratories analyzing the same samples each with their own home methods produced results rather disperse and with a

relatively high variation (CV ranging 3.2-29%) (Jensen *et al.*, 1996). Part of the variation may be due to lipid content of muscles. However, there is little conclusive information about the effect that the level of fat in the sample may have on the ability of different method to quantify tocopherol. This is a key issue, particularly in some mediterranean countries, where pigs with high fat content are raised to produce high quality meat products, particularly due to the current emphasis in the use of high levels of α -tocopheryl acetate in the feeding to control rancidity and off-flavor development along processing.

In our experiment, recovery of spiked α -tocopherol in muscle samples high or low in intramuscular fat is shown in Figure 1. In LF samples, recovery was in the range 85-95% in all cases, with not significant differences between the methods. In this cases, saponification of samples prior to analysis neither improve nor reduce recovery of spiked samples. Recovery of α -tocopherol in both cases were similar to

those reported by other authors (Buttriss and Diplock, 1984, Burton *et al.*, 1985, Ueda and Igarashi, 1987a, Erickson, 1991, Monahan *et al.*, 1992).

When HF samples where analyzed using the methods proposed by Buttriss and Diplock (1984) and Ueda and Igarashi (1987a), recovery of α -tocopherol was much lower (in the range 50-60% and 50-55% respectively) (Figure 1 A and B). Since total fat accumulation was of higher magnitude in HF samples (9% vs 3% in fresh muscle) and heat incubation with KOH saponify fat, we decided to assess different concentrations of KOH. Recovery was slightly higher (up to 74%) when double amount (2x) of KOH was added, but decrease again when higher amount were used (3x, 4x). Also longer (2x, 3x) incubation times were assessed without better results. Buttriss and Diplock (1984) reported that saponification at 70°C for 30 minutes can result in losses of tocopherol of the order of 5% (similar to those found in LF samples). There is not reason to suppose higher losses of α -

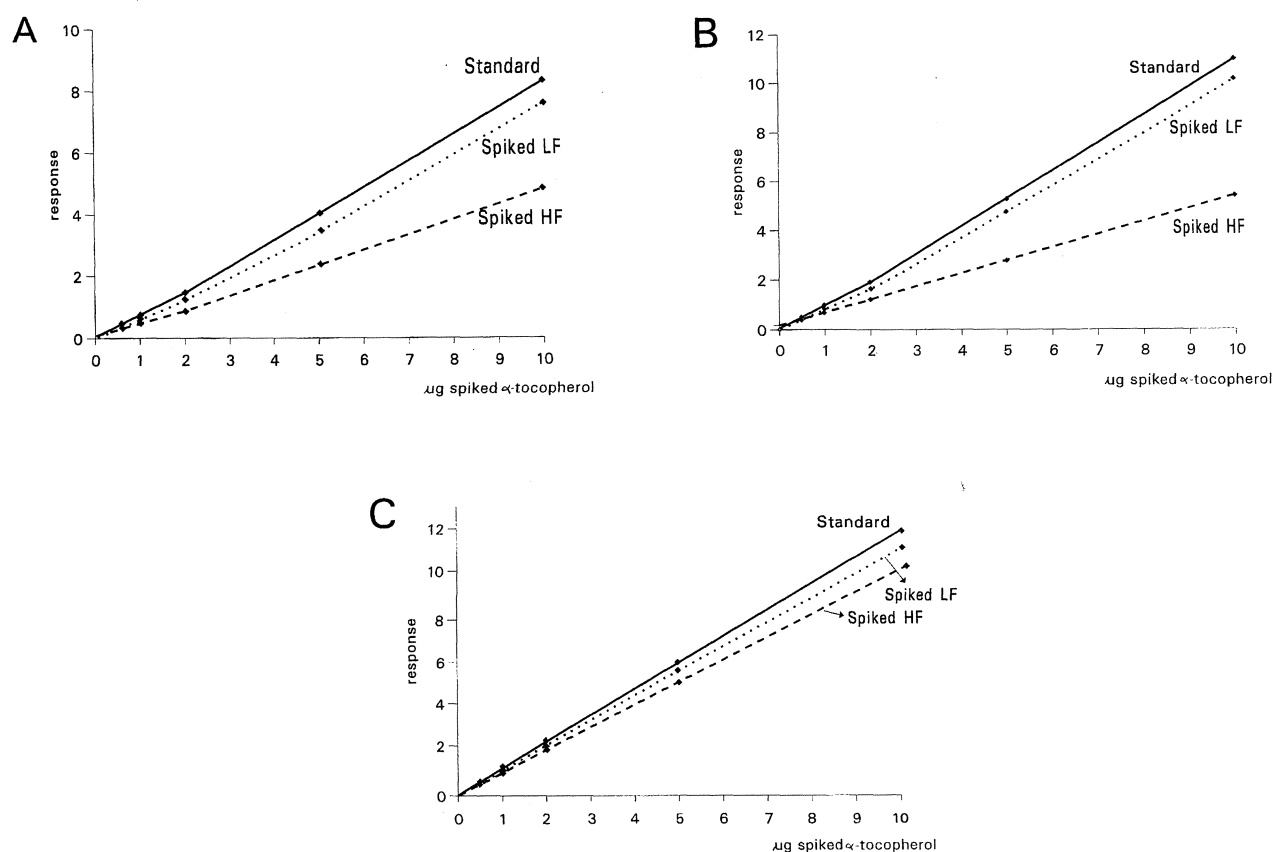


Figure 1

Recovery of α -tocopherol from meat samples high (9%) (HF) or low (3%) (LF) in intramuscular fat. (A) Method of Buttriss and Diplock (1984), (B) method of Ueda and Igarashi (1987) and (C) Adapted method of Mallarino (1992) and colleagues, without previous saponification of the sample. In each case, 0.5, 1, 2, 5 and 10 μ g α -tocopherol was spiked into homogenized samples prior to extraction. The standard was done by addition of the same amounts of α -tocopherol to vials that were brought to the same final volume

tocopherol in HF than in LF samples. Also, it has been proposed that saponified material may interfere quantification on samples (Ueda and Igarashi, 1987b). This could be an explanation for differences in recovery depending on the amount of intramuscular fat. Since HF have 3 times more fat than LF samples and consequently saponified material could interfere quantitation to a greater extent. On the other hand, the solvent extraction method without prior saponification showed no differences in recovery depending in the amount of intramuscular fat (Figure 1C) (85-92%) and consequently is recommended for samples high in fat.

The relatively high recovery of α -tocopherol in meat samples high in fat obtained with a method that involve no saponification may be of interest not only in fresh meat samples of Iberian and other pigs, but also in fish flesh high in fat and in some meat products, where quantitation of tocopherol have been scarce due to difficulties in analysis.

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