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Original Article

Comparative study of tocopherol homologue content in four almond oil cultivars during two consecutive years

Carmen M. López-Ortiz, Soledad Prats-Moya*, Ana Beltrán Sanahuja, Salvador E. Maestre-Pérez, Nuria Grané-Teruel, María L. Martín-Carratalá

Analytical Chemistry, Nutrition and Food Science Department, University of Alicante, P.O. Box 99, Alicante, Spain

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Abstract

In this study, a quick and easy analytical method was optimised to obtain the tocopherol (T) profile of almond oil samples in less than 18 min including the sample preparation process. As the tocopherol determination is being carried out on the almond oil, a previous optimisation step to analyse moisture and fat is simultaneously carried out. Additionally, the method was applied to the analysis of four almond cultivars (Marcona, Guara, Garrigues and Butte) cultivated in different locations during the 2004 and 2005 growing seasons. α-T mean values for the almond samples included in the study varied from 8.5 to 19.4 mg/100 g of dry almond. β + γ-T values ranged from 0.141 to 0.838 mg/100 g of dry almond. Meanwhile, δ-T was found in trace quantities in all almonds. After applying an ANOVA analysis to the data, Marcona samples were found to be significatively different to the other three cultivars included in the analysis on the basis of α-T and β + γ-T content. Moreover, a study of degradation of tocopherol homologues over time was done showing that after a year of storage, shelled and unshelled almonds maintained more than 80% of the initial tocopherol meanwhile, greater losses and variabilities in β + γ-T and δ-T were observed.

Keywords: Almonds; *Prunus dulcis*; Marcona; Guara; Garrigues and butte cultivars; Tocopherol content; Vitamin E; Fatty acid profile of nuts; Highest α-tocopherol content; HPLC

1. Introduction

Nuts, and specifically almonds, are a very interesting food from a nutrition point of view mainly due to the components present in the fatty fraction. There is a widely held belief that the fatty acid profile of nuts helps prevent cardiovascular diseases and lower cholesterol plasma levels (Sabaté et al., 2000; Kris Etherton et al., 2001; Fraser, 1999) since it is a food rich in monounsaturated and polyunsaturated fatty acids and poor in saturated fatty acids (Grane Teruel et al., 2001; Prats Moya et al., 1999; Martín et al., 1998). However, the beneficial health effects of nuts, in particular of almonds, are not only attributed to the fatty acid profile but also to the so-called phytochemical components such as fibre, phytosterols and antioxidant compounds (Kris Etherton et al., 2001; Howard and Kritchevsky, 1997).

As regards antioxidant food components some studies have recently been carried out on the total antioxidant power of nuts (Blomhoff, 2005) and also, on some specific antioxidant components present in nuts such as polyphenols (Wijeratne et al., 2006) and Ts (Kormsteiner et al., 2006). From these studies we know that the walnut has the highest total antioxidant content, followed by other nuts such as hazelnuts and almonds. It is also important to point out that unpeeled nuts have a higher total antioxidant capacity than nuts without tegument. However, although walnuts have the highest whole antioxidant capacity, almonds stand out for being the nut with the highest α-T content. This explains why almonds have been included in the recommendations of *The Dietary Guidelines for Americans* (USDA, 2005) in the context of enhancing the intake of this vitamin.

*Corresponding author. Tel.: +34 965903777; fax: +34 965903527.
E-mail address: maria.prats@ua.es (S. Prats-Moya).

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There are eight naturally occurring vitamin E compounds. All are derivatives of 6-chromanol and differ in the number and position of the methyl groups on the ring structure. The four tocopherol homologues (α, β, δ, γ) have a saturated 16-carbon phytol side chain whereas the tocotrienols (α, β, δ, γ) have three double bonds on the side chain.

Since there is strong evidence that α-T can play an important role in the prevention of low density lipoprotein cholesterol oxidation (Spiller et al., 1998; Hyson et al., 2002), the knowledge of T homologue contents in foods and more specifically in food with a high T content such as nuts is becoming of utmost importance. Therefore, in recent years a number of articles have been published in the literature providing data on T and tocotrienol content in Walnuts (Martínez et al., 2006) and Hazelnuts (Amaral et al., 2005, 2006a, b).

However, in spite of all this, there is still scant information in the literature on T homologue composition, particularly in relation to different almond cultivars. Usually, only total T composition or at the most α-T is related to almonds in general (García Pascual et al., 2003; Maranz and Wiesman, 2004).

Our interest in almond composition stems from the fact that Spain is the second largest producer of almonds in the world after the US, and is also one of the major consumers of this nut as the whole fruit or as a major ingredient in confectionery. Nevertheless, in Spain a great number of cultivars are grown, hence it would be of agronomical and nutritional interest to obtain data on the most abundant and popular ones in order to see if there are almonds with different tocopherol content. Moreover, as the Marcona cultivar is the most expensive one, it is also interesting trying to find discriminant parameters to differentiate this cultivar from others with similar physical appearance or cheaper. Thus, the findings of two research studies have recently been published (López Ortiz et al., 2006; Kodad et al., 2006) providing T data on a certain number of Spanish almond cultivars. However, in both cases the almonds were grown in only one location, and in the first of these, there was no comparison made between years.

Additionally, another significant attribute of the almond includes its excellent storage properties over time. The classic way of storing almond nuts is to keep them unshelled following natural drying until their consumption or use in industry. Another possibility is to shell them and store them at temperatures below 4 °C and with controlled relative humidity. This last mode of preservation is applied to imported shelled almonds. By popular wisdom, it is normally accepted that post-harvest almonds can be stored unshelled for up to a year without suffering any significant organoleptic changes. As T prevents fats from undergoing oxidation processes and thus prevents the development of rancidity flavours, it will also be interesting to ascertain how this pattern evolves over the period of a year in four almond cultivars included in the analysis. Several research studies within similar parameters have been carried out (Senesi et al., 1996; Zacheo et al., 2000) confirming that the shelf life of almonds increases when the levels of antioxidants as T are high.

Moreover, the aim of this study was to optimise a quick analytical method for determining T homologues in almond oil in a routine way. Secondly, to study the T profile of four almond cultivars (Marcona, Guara, Garrigues and Butte) grown under various environmental conditions for two growing seasons (2004 and 2005), in order to evaluate if it is possible to determine compositional differences among the cultivars regardless of place and year of cultivation. Finally, a study of the evolution of the T homologues in almonds stored over a year was undertaken.

2. Materials and methods

2.1. Instrumentation

Chromatographic analyses were performed in a Waters multisolvent HPLC system equipped with a double piston pump and a Waters 600E flow controller (Waters, Milford, USA). Detection of Ts was carried out using a photodiode array detector (Waters, 996) connected in series with a fluorescence detector (Waters, 474). Chromatograms were recorded and processed by Millenium ver. 32 chromatography software (Waters). The sample homogenisation during the saponification process was carried out using an Inkubator 1000/Promax 1200 (Heidolph, Kelheim, Germany) set at 50 °C and at 3500 rpm. For the evaporation of the eluent a Buchi rotavapor R-114 (Brinkmann Instruments, Westbury, NY, USA) was employed.

2.2. Reagents

Butylated hydroxytoluene 99.0% (BHT), (+)-α-T acetate, (+)-δ-T, (+)-γ-T, (±)-α-T were purchased from Sigma (Steinheim, Germany). A sample of grape oil (Berinoix, Noyers sur Cher, France) and soybean oil (Biosan, Tarragona, Spain). Methanol, ethanol, *n*-hexane and acetonitrile for HPLC from Lab Scan (Unit T26, Stillorgan Ind. Park Co., Dublin, Ireland), potassium hydroxide and petroleum ether from Prolabo (Briare Le Canal, France), and 1-propanol and L (+)-ascorbic acid for analysis from Panreac (Barcelona, Spain).

2.3. Standard solutions

Stock standard solutions, α-T (500 mg/l), γ-T (20 mg/l) and δ-T (50 mg/l) and acetate T (100 mg/l) were prepared in 1-propanol, stirred in a Vortex mixer, filtered through a 0.22 μm nylon syringe membrane from Phenomenex (Torrance, CA, USA), flushed with nitrogen and, finally stored at −20 °C in glass vials protected from the light. Combined working standard mixtures, with concentrations similar to those present in the samples were prepared from the stock standard solutions. All standards contained