Journal of Food Composition and Analysis 33 (2014) 161–165

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Tocopherol concentration in almond oil from Moroccan seedlings: Geographical origin and post-harvest implications

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ARTICLE INFO

Article history:
Received 9 July 2012
Received in revised form 20 December 2013
Accepted 23 December 2013

Keywords:
Prunus amygdalus Batsch
Almond
Seedling
Food analysis
Food composition
Food quality
Biodiversity and nutrition
Genetic resources
Geographical origin
Terroir
Breeding
Tocopherol

ABSTRACT

The concentration of the three main tocopherol homologs was determined in oil samples of almond kernels of four different seedling populations from Morocco. The concentration of α-tocopherol ranged from 300.9 to 656.7 mg/kg oil, showing higher concentrations than what has previously been reported for almond. The concentration of δ-tocopherol ranged from 0.22 to 2.2 mg/kg oil, similar to that of other almond genotypes, and that of γ-tocopherol from 4.4 to 33.4 mg/kg oil, a wider range than has previously been determined in almond. Although the effect of harvest year was significant, tocopherol variability was due mainly to the genotype. The geographical origin was also significant, with the mountain populations showing higher concentrations of tocopherol, probably due to empirical selection for increased storage life, as tocopherol retards rancidification. These results may help to determine geographical areas of almond production with defined characteristics in order to increase the value of the almond products and the revenue of the local growers. Additionally, some seedlings could be incorporated as parents in a breeding program for increasing tocopherol concentration.

1. Introduction

Almond (*Prunus amygdalus* Batsch) is the most important tree nut crop in terms of commercial production. This production is limited to areas characterized by a Mediterranean climate (Kester and Asay, 1975), including regions in the Mediterranean countries, the Central Valley of California, the Middle East, and some equivalent areas in the Southern Hemisphere. Traditional almond culture utilized open-pollinated seedlings (Grasselly, 1972; Rikhter, 1972) which, together with self-incompatibility, produced a very high heterozygosity in this species (Kester et al., 1990; Socias i Company and Felipe, 1992). This large variability has provided a useful genetic pool for almond evolution, enabling in each growing region the selection of almond cultivars well adapted to the area (Kester et al., 1990). In Morocco, almond is grown in several regions from North to South, under different environmental conditions, mostly on non-irrigated areas of poor soils and receiving little attention from farmers. The climate is primarily Mediterranean, becoming more extreme toward the inland regions and Saharian in the South. The resultant variability in environment and climate has turned into an extensive diversity of almond genotypes in each producing region, due to the fact that about 50% of the almond trees grown in Morocco are seedlings, located primarily in the north and the south (Lansari et al., 1998; Ministère de l'Agriculture et de la Pêche Maritime, 2011). Several studies have been undertaken to evaluate the genetic diversity and nut quality of the Moroccan almond population using morphological and phenological criteria (Laghezali, 1985; Lansari et al., 1994), but studies on the chemical composition of the kernels are limited.

The kernel is the edible part of the almond fruit, being of high nutritive value The knowledge of its chemical composition would enable to establish not only quality criteria, but also consumption criteria, due to the incidence of some compositional parameters on the nutritional and health values of almond kernels (Socias i Company et al., 2010). Kernel tendency to rancidity during storage and transport is a quality loss and is related to oxidation of the kernel fatty acids (Senessi et al., 1996). The presence of natural anti-oxidants in almond kernels is an important determinant of almond quality (Socias i Company et al., 2008). Tocopherols are natural benzopyranols with a creomanol ring and a lateral

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http://dx.doi.org/10.1016/j.jfca.2013.12.010

hydrophobic chain of 16 C atoms showing anti-oxidant activities (Reische et al., 1998). They are found as several homologs depending on the position and number of methyl groups, being the most relevant in almond α-tocopherol (5,7,8-trimethyl), γ-tocopherol (7,8-dimethyl), and δ-tocopherol (8-methyl).

A high concentration of tocopherol has also been shown to be very important in the human diet, due to its vitamin E activity (Kamal-Eldin and Appelqvist, 1996). Thus tocopherol content in seed oil is considered as a value-added characteristic (Marwede et al., 2004). The recent studies also point out that almond is widely consumed in the Mediterranean countries (Romero et al., 2002), including Morocco, and could be considered as an important complementary source of α-tocopherol in this region. Taking into account all the studies on the positive properties of the almond consumption, it can be considered a genuine health food (Kamil and Chen, 2012).

No information is available on the variability of tocopherol concentration in local almond populations, mainly if these were originated by seedling propagation. This information is crucial not only in order to increase the knowledge of the almond diversity based on the kernel chemical components, but also on the nutritional and health value of these kernels, establishing the basis for determining the kernel quality of these local populations and their valorization. The kernels produced by the local Moroccan almond populations are generally of poor physical quality (small, roughly, wrinkled and unattractive), thus considerably reducing their market value. Consequently, their valorization implies their transformation into different commodities which would increase their market value and improve the income of local growers. As the authenticity and traceability of the almond kernels and their by-products are of great interest for the protection of the consumer, the determination of the compositional variability of the kernels from different countries, locations or cultivars could be imperative for the proper classification of the product and the protection of its authenticity in the market.

Therefore, the main objective of the present work was the evaluation of the tocopherol concentration in the kernel oil of the main important local almond populations in Morocco for their possible utilization to improve the commercial value of the final product and their possible incorporation in almond breeding programs.

2. Materials and methods

2.1. Plant material

This study was carried out in four different regions with wealthy almond genetic resources: Aknoul situated in the Rif Mountains (North of Morocco), Azilal in the high Atlas Mountains (Central Morocco), and two valleys in Central Morocco: Saïs, around Bni Mellal, and Tadla, around Sfasif. A total of 41 local genotypes from different zones of each region were selected based on the general status of the plant (vigor, ramification, foliar density and appearance), physical quality of the kernel, late blooming and appreciation of their kernels by the local population. These genotypes were unique seedlings, therefore each genotype is a single tree. These genotypes were marked and fruits were randomly collected in summer (7–10 August) during two consecutive years (2009 and 2010). The nuts were collected in lots of about 1 kg when the fruit mesocarp had fully dried and split along the fruit suture, and peduncle abscission was complete (Felipe, 1977). Then the mesocarp was removed and the nuts were dried and stored at ambient temperature for three weeks. From each lot, representing each genotype, two independent samples of 30 fruits were randomly removed for analysis. After cracking, seed coats were removed by blanching. Kernels were dried at room temperature for ten days and ground in an electrical grinder.

2.2. Oil and tocopherol determination

Once the kernels were ground, the oil was extracted from 4 to 5 g of each sample in duplicate in a commercial fat-extractor (Selecta, Barcelona, Spain) for 2 h using petroleum ether and keeping the heating source at 135 °C. The fat was maintained in dark vials at 4 °C until analysis. Tocopherol concentrations were determined in each duplicate sample according to the method described by López-Ortiz et al. (2008). Samples of 0.30 g of almond oil were dissolved in 2 ml of 1-propanol by shaking at air temperature for 3 min. The resulting mixture was then filtered through a 0.45 μm nylon syringe membrane (Phenomenex; Torrance, CA, USA) before measurement. The final extract was kept in a dark vial in a refrigerator at 2–4 °C until chromatographic analysis. Tocopherol homolog determinations were performed using a Kontron HPLC 360 equipped with a double piston pump (Kontron, Eching, Germany). Tocopherol homologs were detected using Kontron 440 photodiode array detector and Sfu 25 fluorescence detector. The chromatographic conditions finally selected for the simultaneous determination of all tocopherol homologs were as follows: 20 μl of sample was injected into the chomatographic column Phenomenex Luna C18 (0.5 mm × 4.6 mm, 5 μm particle size, 100 Å pore size). The mobile phase consisted of an acetonitrile:methanol (30:70) mixture at a flow rate of 1.2 ml min^{-1} and maintained at 30 °C. Tocopherol homologs were detected at 295 nm. Peak areas were measured, and retention times were compared with standards of the three homologs (Sigma–Aldrich, Madrid, Spain) with a purity of 95% for α-tocopherol, 96% for γ-tocopherol, and 90% for δ-tocopherol. The values were confirmed by their characteristic spectra using the photodiode array detector, which also confirmed their purity. To quantify all the homologs, calibration curves were drawn. Standard linearity was verified in each case by analysis of six standards in triplicate each containing 20–200 mg/kg oil for α-tocopherol, 0.1–8 mg/kg oil for γ-tocopherol and 0.05–5 mg/kg oil for δ-tocopherol. With this method, the recovery of the three tocopherol homologs was approx. 98%. Under these experimental conditions the limits of detection calculated from the residual error of the calibration curves were: 5.5 mg/kg oil for α-tocopherol, 0.2 mg/kg oil for γ-tocopherol and 0.1 mg/kg oil for δ-tocopherol. Tocopherol compositions were the mean values of three replicates ($n = 3$) from each duplicate sample and were expressed as mg/kg oil.

2.3. Statistical analysis

All statistical analyses were performed with the SAS program (SAS, 2000). Hierarchical lineal modeling analysis was chosen because the number of genotypes from the different populations is different, and we were interested in determining whether the means differed among the populations. The analysis of variance was performed using the General Lineal Model procedure with a three random factors design. Year and geographical origin were orthogonal factors whereas the factor genotype was hierarchical to the geographical origin factor because the genotypes were not repeated between sites. For a general conclusion among the four almond locations, the population was considered as a random effect (Steel and Torrie, 1960). The mean separation was calculated with the Least Significant Difference test at a $P < 0.05$.

3. Results and discussion

3.1. Genetic variability

The tocopherol profile in analyzed Moroccan almonds showed that α-tocopherol is the major homologue, followed by γ-tocopherol and δ-tocopherol, confirming the previous results reported in other almond cultivars and selections (Kodad et al.,