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Oil content, fatty acid composition and tocopherol concentration in the Spanish almond genebank collection

O. Kodad[a,b], G. Estopañán[c], T. Juan[c], J.M. Alonso[a], M.T. Espiau[a], R. Socias i Company[a,*]

[a] Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Av. Montañana 930, 50059 Zaragoza, Spain
[b] Department of Pomology, National School of Agriculture Meknès, BP S/40, Meknès, Morocco
[c] Unidad de Calidad y Seguridad Alimentaria, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Av. Montañana 930, 50059 Zaragoza, Spain

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ABSTRACT

Total oil content and concentrations of the major fatty acids and of the tocopherol homologues were determined in the kernel oil of 44 local almond cultivars originated in the different Spanish growing regions. The ranges of variability for the different parameters was similar to those already reported for almond, such as 50.58–64.95% of dry matter (DM) for oil content, 64.97–79.59% of oleic acid in the total oil, and 313.0–616.1 mg/kg oil for α-tocopherol. Despite these ranges of variability, extremely high values were obtained in some genotypes, such as very high content of oleic acid in 'Muel' and of α-tocopherol in 'Araguayo-2'. These ranges of variability indicate that this collection reflects a high level of diversity and the importance of maintaining and characterising such a diverse germplasm collection. The knowledge of this diversity may be extremely useful in identifying interesting parents to be included in a breeding programme as a response to new selection objectives, such as the improvement of the chemical quality of the almond kernel.

1. Introduction

Almond [*Prunus amygdalus* Batsch syn. *P. dulcis* (Mill.) D.A. Webb] is the most important nut tree crop worldwide in terms of commercial production (FAOSTAT, 2012). Spain is the second world producer, with an average production of 223.431 tm in shell fruit (Socias i Company et al., 2011). Traditional almond culture utilized open-pollinated seedlings (Socias i Company et al., 2012) 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, allowing in each growing region the selection of almond cultivars well adapted to this area (Grasselly and Crossa-Raynaud, 1980; Socias i Company et al., 2012). In Spain, almond is produced under different climatic conditions: from inland regions characterized by high frost risks at blooming time or soon after (Ebro Valley, Castilla-La Mancha), to coastal regions with mild winters and hot and dry summers (Andalusia, Murcia), and the Balearic and Canary islands with high relative air humidity. The high climatic diversity of the different producing regions in Spain forced the local farmers to select genotypes in each region in order to avoid the harsh environmental conditions causing decreases in production. More than 70 traditional Spanish almonds cultivars were identified and introduced in the national almond collection maintained at the CITA of Aragón in Spain (Espiau et al., 2002). Local cultivars and landraces were selected over centuries of almond growing due to improved local performance in terms of production, resistance and/or quality traits. These highly selected traits represent very valuable germplasm for addressing future challenges and so need to be preserved, characterised, and incorporated into advanced breeding lines (Socias i Company et al., 2012).

During the last decades different breeding programmes have released new cultivars mainly characterised by late-flowering, self-compatibility and high nut quality (Socias i Company et al., 2009). These new cultivars have provoked a sharp decrease in overall diversity of the Spanish orchards since large numbers of local cultivars and landraces have been replaced with the commercially preferred breeding releases. The release of 'Guara' (Felipe and Socias i Company, 1987) represented a complete change of the almond organization due to its commercial success, favoured by its self-compatibility, having represented more than 50% of new plantings (Socias i Company et al., 2011). This situation has been general in most Mediterranean countries where an effective replacement of

* Corresponding author. Tel.: +34 976716313; fax: +34 976716335.
E-mail address: rsocias@cita-aragon.es (R. Socias i Company).

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cultivars began with the expansion of the late-blooming Puglia cultivars and later, with the release of the high market quality French cultivar 'Ferragnès' (Grasselly and Crossa-Raynaud, 1980). This general trend has been also favoured by the industry demands, since the increasingly global market gives a premium prize to crop consistency and product uniformity. It has been well demonstrated in other "improved" agronomic crops that such market-driven cultivar changeovers can dramatically reduce crop genetic diversity and thus increase vulnerability to later large-scale crop failures from pest, disease and/or climate changes (Socias i Company et al., 2003). An interesting example of such vulnerability is the recent findings by Alonso and Socias i Company (2007) that the extensive use of 'Tuono' as a source of self-compatibility in most European breeding programmes appears to result in inbreeding depression and reduced productivity in at least some of the new cultivar releases.

Recently, the determination of kernel oil quality, taking into account both its content and composition, is an imperative step in the evaluation of new cultivars before being released (Socias i Company et al., 2008). In fact, the modern almond industry demands commercial cultivars characterised by kernels with high quality attributes and well differentiated, since the best end-use for each cultivar is a function of its chemical composition (Berger, 1969). Additionally, the best quality attributes may avoid the use of synthetic additives according to the consumers' trend for foods without any additive (Krings and Berger, 2001). Consequently, the CITA has incorporated the chemical quality criteria as an objective in its almond breeding programme (Socias i Company et al., 2012).

The high nutritive value of almond kernels arises mainly from their high lipid content, which constitutes an important caloric source but does not contribute to cholesterol formation in humans. Almond oil has been reported to be very rich in mono-unsaturated fatty acids (MUFAs), especially in oleic and linoleic acids, whereas saturated fatty acids, especially palmitic, palmitoleic and stearic, are very low (reviewed by Yada et al., 2011). Kernel tendency to rancidification during storage and transport is a quality loss related to oxidation of the kernel fatty acids (Kester et al., 1993). Thus, oil stability and fatty acid composition, essentially the ratio of oleic to linoleic (O/L) acids are considered an important criterion to evaluate kernel quality (Socias i Company et al., 2008). Thus, low content of linoleic acid is correlated with high oil stability (Zacheo et al., 2000), whereas high content of oleic acid is considered a positive trait from the nutritional point of view (Socias i Company et al., 2008). Therefore, selection of parents for low linoleic acid and high oil content might be undertaken in a breeding programme for increased kernel quality.

Tocopherols are natural mono-phenols with anti-oxidant activities (Reische et al., 1998), with several homologues depending on the position and number of methyl groups. Tocopherols occur in plants as a family of four derivatives, named α-, β-, γ- and δ-tocopherol. These components are believed to be involved in a diversity of physiological, biological and biochemical functions, mainly due to their action as antioxidants but also by acting as membrane stabilizers (Azzi and Stocker, 2000). A large genotypic (Kodad et al., 2006; López-Ortiz et al., 2008) and environmental variability (Kodad et al., 2011a) has been reported for these components in almond oil. Information on tocopherol concentrations is important in determining the end-use of the kernels and for predicting their storage-life (García-Pascual et al., 2003; Senessi et al., 1996; Zacheo et al., 2000). Furthermore, Fourie and Basson (1989) studied variation in tocopherol concentrations in several nuts, and found that almond kernels, with a higher tocopherol concentration than other nuts, had longer storage ability. As a consequence, high tocopherol concentration, mainly α-tocopherol, has been considered as an objective in almond breeding programmes (Kodad et al., 2006).

The determination of the compositional variability of the oil from different cultivars or local genotypes could be imperative to choose the best genotypes to be included as parents in almond breeding programmes to improve the kernel quality and to avoid the inbreeding symptoms observed in the some of newly released almond cultivars. The information on the chemical composition of the almond kernels available at present is restricted to a reduced number of cultivars, mostly from the country where these cultivars originated or are grown (Kodad and Socias i Company, 2011). Therefore, the study of the chemical composition of a set of cultivars from different origin but grown in the same conditions was considered interesting, taking the opportunity of the almond collection belonging to the Spanish National Germplasm Network maintained at the CITA of Aragón (Espiau et al., 2002). Thus, our objective was the determination of the oil, fatty acid and tocopherol composition of 44 traditional Spanish almond cultivars considering their possible incorporation in an almond breeding programme.

2. Material and methods

2.1. Plant material

A total of 44 local Spanish almond cultivars were included in the analysis (Table 1). They were selected in order to represent the variability of the whole almond Spanish germplasm according to previous studies (Kodad et al., 2011b). The trees are maintained as living plants grafted on the almond × peach hybrid clonal rootstock INRA GF-677, using standard management practices (Espiau et al., 2002). Nuts from open pollination were harvested during three consecutive years (2008–2010) at mature stage, when fruit mesocarp was fully dried and split along the fruit suture and peduncle abscission was complete (Felipe, 1977). Three samples of 20 fruit were collected for each treatment.

2.2. Oil and fatty acid determination

Oil content and fatty acid composition were determined during the three years of the study. Kernels were blanched, dried, and ground in a domestic electrical grinder until obtaining fine flour. Total oil content was determined with a 3 g sample in a Soxtec Avanti 2055 fat extractor (Foss Tecator, Höganäs, Sweden) using 70 ml of petroleum ether as solvent (boiling point range 40–60 °C) and maintaining the heat source at 135 °C for a period of 2 h because previous checks showed that extraction is then practically completed, with no differences with an extraction period of 4 h. Fat content was expressed as the difference in weight of the dried sample before and after extraction (% of DW). The relative percentage of the different fatty acids in the oil was determined by capillary gas chromatography of the fatty acid methyl esters (FAMEs). These FAMEs were prepared by trans-etherification with KOH according to the official method UNE-EN ISO 5509:2000 (ISO, 2000). The FAMEs were separated using a gas chromatograph HP 6890 and afterwards detected using a flame ionization detector, equipped with a capillary column (HP-Innowax 30 m × 0.25 mm i.d.) and 0.25 μm film thickness (Agilent Technologies, Waldbronn, Germany). The carrier gas was helium and the flow rate was 1 mL/min. The temperature of the injector and detector was maintained at 220 and 275 °C, respectively. The initial column temperature was 100 °C for 3 min. The oven temperature was gradually increased from 100 to 240 °C, as follows, from 100 to 150 °C at 2.5 °C/min ramp rate, from 150 to 200 °C at 3 °C/min ramp rate, and from 200 to 240 °C at 13 °C/min ramp rate and it was maintained at 240 °C for 4 min. Injection volume was 1.0 μL. FAME identification was based on retention times as compared with those of the standard FAME mixture (Sigma-Aldrich, Madrid, Spain). Three