

Selection for Some Olive Oil Quality Components Through the Analysis of Fruit Flesh

Leonardo Velasco · Álvaro Fernández-Cuesta ·
Raúl De la Rosa · M. Victoria Ruiz-Méndez ·
Lorenzo León

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Abstract Selection for oil quality is commonly conducted at the latest stages of olive breeding programs, as oil quality traits are measured in extracted oils. At the initial stages of breeding, the number of genotypes is high and fruit production is low, which makes it difficult to conduct oil extraction. The objective of this research was to evaluate the feasibility of conducting selection for some important oil quality traits in olive by analyzing fruit flesh instead of extracted oils. Fatty acids, tocopherols, phytosterols, and squalene were measured in fruit flesh and extracted oils from 22 individual olive trees showing variability for oil quality traits. Correlation coefficients between analyses conducted on fruit flesh and extracted oils were $r = 0.98$ for the main fatty acids palmitic, oleic, and linoleic acid, $r = 0.96$ for tocopherol content, $r = 0.89$ for phytosterol content, $r = 0.97$ for squalene content, and $r = 0.91$ and 0.94 for the concentrations of the two main sterols β -sitosterol and Δ^5 -avenasterol, respectively. The results revealed that selection for the mentioned oil quality traits can be efficiently conducted through the analysis of fruit flesh instead of extracted oil, which facilitates selection on larger numbers of genotypes at the initial stages of olive breeding programs.

Keywords Fatty acids · Oil quality · *Olea europaea* · Phytosterol content · Selection · Squalene content · Tocopherol content

Introduction

Olive oil is highly valued for its nutritional quality and its role in disease prevention [1, 2]. Olive oil quality is mainly determined by its high oleic acid content together with the presence of a myriad of biologically active minor components, which include polar phenolic compounds, squalene, tocopherols, and sterols [3]. Polar phenolic compounds or polyphenols are a mixture of compounds that belong to different classes such as phenolic acids, phenyl ethyl alcohols, hydroxyl-isochromans, flavonoids, lignans, and secoroids [4]. They have great importance for olive oil stability, nutritional value, and sensorial properties [3, 5]. Polar phenolic compounds usually range between 100 and 300 mg kg⁻¹ in olive oil [3]. Squalene is a terpenoid hydrocarbon that accounts for more than 50 % of the unsaponifiable matter of virgin olive oil, typically ranging between 200 and 7,500 mg kg⁻¹ [3]. Squalene is an important compound from a nutritional perspective, since several studies have suggested its chemoprotective role in cancer and aging [6]. Phytosterols or plant sterols are a group of compounds with similar chemical structure and biological function as cholesterol [7]. They play an important nutritional role by reducing cholesterol absorption and consequently blood cholesterol levels [8]. Virgin olive oil has a typical sterol content between 1,000 and 2,000 mg kg⁻¹, with predominance of β -sitosterol (75–90 % of sterols) followed by Δ^5 -avenasterol (5–20 %) [9]. Tocopherols are lipid-soluble antioxidants that act as free radical scavengers both in vivo (vitamin E) and

L. Velasco (✉) · Á. Fernández-Cuesta
Instituto de Agricultura Sostenible (IAS-CSIC), Alameda del
Obispo s/n, 14004 Córdoba, Spain
e-mail: lvelasco@ias.csic.es

R. De la Rosa · L. León
IFAPA Centro Alameda del Obispo, Avda. Menéndez Pidal s/n,
14004 Córdoba, Spain

M. Victoria Ruiz-Méndez
Instituto de la Grasa (IG-CSIC), Avda Padre García Tejero 4,
41012 Seville, Spain

in vitro. In vivo, they protect cellular tissues from oxidative, proliferative, and inflammatory damage [10]. In vitro, tocopherols protect unsaturated fatty acids of the oil from oxidative deterioration, particularly at high temperatures [11]. Among the four naturally occurring tocopherols, α -tocopherol has the maximum vitamin E activity [12], whereas γ - and δ -tocopherol exert maximum in vitro thermoxidative protection [13, 14]. Usual values of tocopherols in virgin olive oil are between 100 and 250 mg kg⁻¹ oil, with around 90 % of them being in the α -tocopherol form [3].

In olive, as in other fruit crops, effective selection at early generations increases the overall efficiency of the breeding process and allows a drastic reduction of genotypes in the early steps of selection, reducing the cost of breeding programs [15]. However, oil quality traits are only recorded in advanced selections of olive breeding programs [15, 16]. The main reason is that important oil quality traits for olive breeding such as fatty acids, phytosterols, tocopherols, and squalene are commonly measured in extracted oils [16, 17]. At seedling stage, the number of genotypes is very high and the olive yield is very low, making it extremely difficult to conduct oil extraction. Alternatively, some studies have focused on the analysis of oil quality traits in fruits instead of oils. For analysis of fatty acid methyl esters, a method for simultaneous lipid extraction and methylation from fresh tissues was developed and tested in olive fruits [18]. The method has been applied to the analysis of olive fruits in breeding programs [19]. Tocopherols [20], phytosterols, and squalene [21] have also been analyzed in olive fruits. However, the correlation between fruit and oil analyses for these traits has not been determined. For selection purposes, it is important that the analysis of oil quality traits in fruits be correlated with the corresponding analyses in oils. Therefore, the present research was aimed at determining the correlation between analyses conducted on fruit flesh and extracted oils for fatty acid composition, tocopherol, phytosterol, and squalene contents, and phytosterol composition in olive.

Materials and Methods

Plant Materials

The study was based on 22 individual olive trees grown in Córdoba, Spain, in a field trial established in open field in June 2007 at 6 × 5-m spacing. Trees were trained as a single trunk vase with 3–4 main branches, and minimal pruning was carried out to allow early bearing. The trees corresponded to the cultivars ‘Picual’ and ‘Arbequina’ as well as to selections from crosses between them. A wide

variability was expected for the selected genotypes based on previous analyses for fatty acid composition, tocopherol content, phytosterol content and composition, and squalene content [21, 22]. Samples of 4 kg fruits per tree were collected on 14 November 2011. Ripening index in the samples ranged from 1.39 to 3.29.

Sample Processing and Oil Extraction

Twelve fruits were randomly chosen from each sample collected from individual trees. They were stored at -80 °C shortly after harvest and lyophilized. After lyophilization, the stones were removed and the flesh was milled in a laboratory ball mill. The samples were then stored at -20 °C till analysis, usually within 48–72 h. Olive oils were extracted from the remaining fruits in an Abencor laboratory oil mill (MC2 Ingenierias y Sistemas, Sevilla, Spain), a laboratory-scale system that simulates a standard olive mill consisting of a hammer crusher, a malaxer, and a paste centrifuge [23]. It is important to remark that the analyses of quality parameters on fruit flesh and extracted oils described below corresponded to the same olive ripening index.

Analysis of Fatty Acid Composition

The fatty acid composition of the oil was analyzed by simultaneous oil extraction and fatty acid methylation of lyophilized olive flesh or direct fatty acid methylation of extracted oil [18], followed by gas-liquid chromatography (GLC) on a PerkinElmer Clarus 600 GC (PerkinElmer Inc., Waltham, MA, USA) equipped with a BPX70 30 m × 0.25 mm internal diameter × 0.25 μ m film thickness capillary column (SGE Analytical Science Pty, Ltd., Ringwood, Australia). Hydrogen was used as carrier gas at a constant flow of 0.8 ml min⁻¹. A split injector and flame ionization detector were maintained at 300 °C. The initial oven temperature was 140 °C maintained for 2 min, followed by a rate increase of 20 °C min⁻¹ up to 250 °C, maintained for 2 min.

Analysis of Tocopherols

Tocopherol extraction, separation by high-performance liquid chromatography (HPLC), and quantification was done on around 100 mg of extracted oil or lyophilized olive flesh following previously reported procedures [24] using a fluorescence detector (Waters 474) at 295-nm excitation and 330-nm emission and iso-octane/tert-butylmethylether (94:6) as eluent at an isocratic flow rate of 0.8 ml/min. Chromatographic separation of the tocopherols was performed on a LiChrospher 100 diol column (250 mm × 2 mm I.D.) with 5- μ m spherical particles, connected to a silica guard column (LiChrospher Si 60, 5 mm × 4 mm I.D.). Quantitative

determination of tocopherols was done by using external calibration curves obtained for each of the tocopherol homologs α -, β -, γ -, and δ -tocopherol using tocopherol standards (Calbiochem Tocopherol Set, catalog no. 613424, Merck KGaA, Darmstadt, Germany). Total tocopherol content was calculated as the sum of α -, β -, γ -, and δ -tocopherol contents, expressed as mg kg^{-1} seed.

Analysis of Phytosterols and Squalene

Sterols and squalene contents in extracted oil or lyophilized olive flesh samples were analyzed by GLC of the unsaponifiable fraction following silylation, without preliminary thin-layer chromatography (TLC) fractionation, as reported by Fernández-Cuesta et al. [21] based on the method proposed by Giacometti [25]. This methodology for analysis of phytosterols has been validated in sunflower against the conventional analysis of oils including TLC fractionation [26]. However, the analysis of squalene without previous fractionation has not been validated. Accordingly, the set of 22 oils used in the study were also analyzed with previous fractionation of hydrocarbons using solid-phase extraction on silica-gel cartridges [27, 28] and the results were compared.

Statistical Analysis

Pearson's correlation coefficients were computed using IBM SPSS Statistics 19.0.

Results and Discussion

The fatty acid composition of the oil measured in olive fruit flesh and extracted oil showed correlation coefficients of $r = 0.98$ for the main fatty acids palmitic, oleic, and linoleic acid, which together accounted for more than 95 % of the total fatty acids (Fig. 1). The variability of those fatty acids was comparable to that observed in the oil analysis of other breeding selections [16, 17]. Similar results were obtained for the tocopherol content ($r = 0.96$; $P < 0.01$; Fig. 2) and phytosterol content ($r = 0.89$; Fig. 3a). The genotypes used for the study showed low variation for tocopherol composition, as expected in olive oil [3], with alpha-tocopherol accounting in all cases for more than 94 % of the tocopherols. However, they showed large variation for phytosterol composition, mainly for the concentration of β -sitosterol and Δ^5 -avenasterol, which together accounted for more than 90 % of the phytosterols. The analysis of these compounds in fruit flesh and extracted oils showed correlation coefficients of $r = 0.91$ for β -sitosterol ($P < 0.01$; data not shown) and $r = 0.94$ for Δ^5 -avenasterol ($P < 0.01$; Fig. 3b).

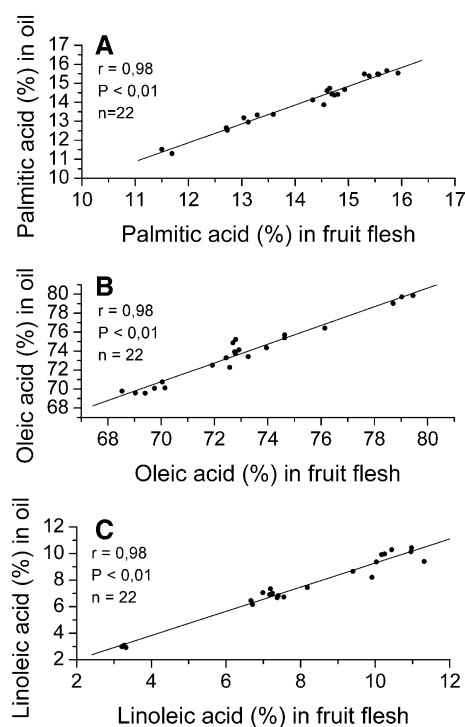


Fig. 1 Scatter plots of **a** palmitic acid, **b** oleic acid, and **c** linoleic acid contents (% total fatty acids) in fruit flesh and extracted oil from 22 individual olive plants

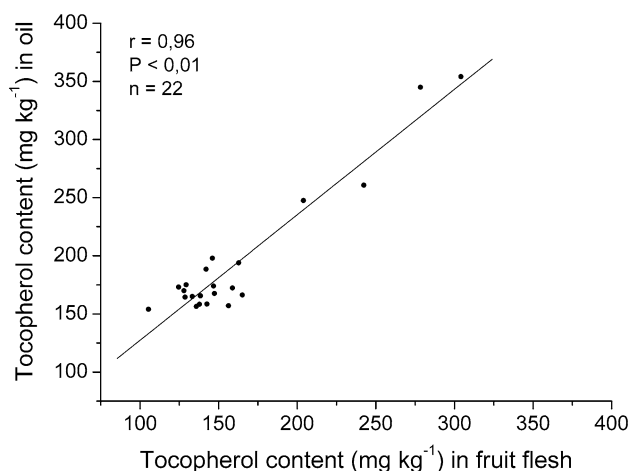


Fig. 2 Scatter plot of tocopherol content (mg kg^{-1}) in fruit flesh and extracted oil from 22 individual olive plants

For squalene content, in a first step we compared the analysis of squalene in the unsaponifiable fraction by GLC without previous fractionation and the analysis after fractionation [27, 28] in the set of 22 extracted oils. The results showed a correlation between both methods of $r = 0.97$ ($P < 0.01$; Fig. 4). Squalene content was slightly higher in the case of analysis without fractionation, with an average squalene content of $5,285 \text{ mg kg}^{-1}$ compared to

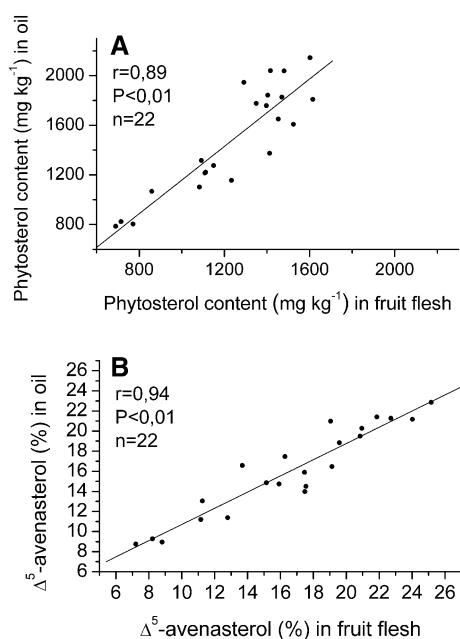


Fig. 3 Scatter plots of **a** phytosterol content (mg kg^{-1}) and **b** Δ^5 -avenasterol content (% total phytoesters) in fruit flesh and extracted oil from 22 individual olive plants

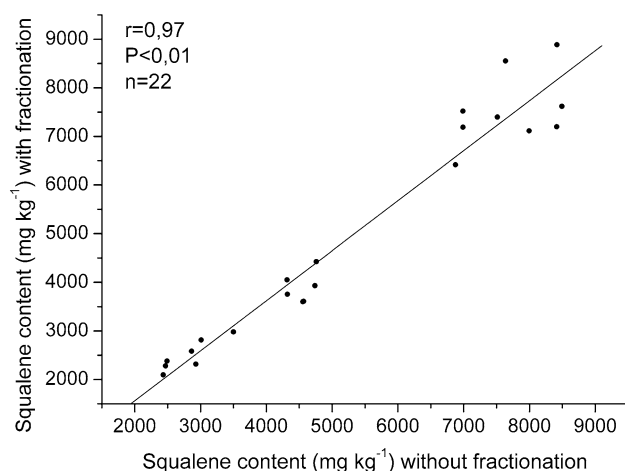


Fig. 4 Scatter plot of squalene content (mg kg^{-1}) in extracted oil from 22 individual olive plants analyzed with and without fractionation of hydrocarbons previous to gas chromatographic analysis

4,942 mg kg^{-1} when previous fractionation was applied. The results might indicate the presence of other hydrocarbons in the squalene peak in the case of absence of fractionation or simply small differences attributable to the use of different methods of analysis. However, as the main objective of the research was to develop a methodology for use in breeding programs, such a small difference was compensated by the significant correlation between both methods, taking into account that the absence of the

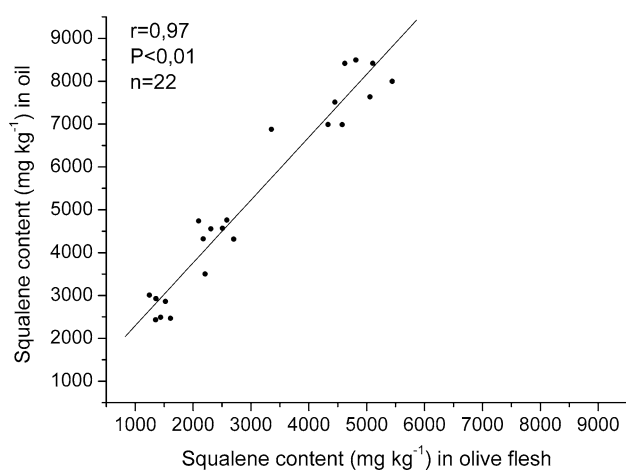


Fig. 5 Scatter plot of squalene content (mg kg^{-1}) in fruit flesh and extracted oil from 22 individual olive plants

fractionation step considerably reduces the analytical efforts, as squalene is analyzed simultaneously to sterols [25]. Comparison of squalene analysis in fruit flesh and oil, using in both cases the same method of analysis based of GLC of the unsaponifiable matter following silylation, revealed a close correlation between both procedures ($r = 0.97$; Fig. 5).

Oil quality is considered one of the main objectives in olive breeding programs. However, due to the need for oil extraction for quality assessment, selection for oil quality is commonly conducted at the latest stages of breeding when only a reduced number of genotypes previously selected for other characters are under evaluation [16, 29, 30]. Early evaluation of large numbers of genotypes at the initial breeding steps is required when selection of olive genotypes with modified levels of oil quality constituents represents the main goal of the breeding program. At this stage, a wider variability for any specific quality component is available and therefore a higher efficiency for selection should be expected. However, the need for oil extraction to conduct oil quality analyses is a bottleneck due to both the large number of samples and the reduced availability of fruits per tree. Accordingly, conducting oil quality assessment on fruits instead of oils has obvious advantages, provided that fruit analyses correlate with oil analysis. Our results revealed that analyses conducted on fruit flesh and extracted oils for oil quality traits such as fatty acid composition, tocopherol, phytosterol, and squalene contents, and phytosterol composition were closely correlated. Other important constituents for olive oil quality, e.g., polyphenols and volatiles, were not included in the present research. Further research will therefore be required to assess the feasibility of selection for these compounds at the fruit level. Finally, it is important to

mention that the fruits and oils analyzed in this research corresponded to the same olive ripening index. Previous studies have shown changes in fruit and oil quality associated with fruit maturation [21, 31, 32].

Conclusion

Selection for oil quality traits can be efficiently conducted through analysis of fruit flesh, which facilitates selection from large numbers of genotypes at the initial stages of olive breeding programs. Further research is required to incorporate other oil quality traits such as phenolic compounds and volatiles to this selection scheme.

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