Assessment of the olive diversity for metabolites associated to the nutritional and sensory quality of virgin olive oil

A. Belaj¹, L. León¹, A.G. Pérez², R. de la Rosa¹ and C. Sanz^{2,a}

¹IFAPA, Centro Alameda del Obispo, Córdoba, Spain; ²Instituto de la Grasa (CSIC), Sevilla, Spain

Abstract

There is an increasing need for diversification of olive (*Olea europaea* L.) cultivars mainly because of the new intensive industry and the current trend of establishing olive productions in new areas outside the Mediterranean basin. Olive breeding has been traditionally focused on the improvement of agronomic traits. However, the nutritional quality of virgin olive oil (VOO) is being lately considered as a breeding target due to the growing number of scientific evidences supporting the positive impact of VOO on human health. Increasing VOO consumption through improvement of the nutritional and sensory properties will have in turn a positive effect on both the consumer's health and the olive producing areas. VOO metabolites responsible for its nutritional and sensory quality include components, such as phenolics and volatile compounds, which are synthesized during the oil extraction process by endogenous enzymes. Here, we aimed to investigate the olive diversity regarding the key VOO quality metabolites of olive accessions cultivated around the world. For such purpose, oils from 97 olive cultivars representative of the diversity found at the World Olive Germplasm Collection (CAP-UCO-IFAPA), located at IFAPA Centre "Alameda del Obispo" Cordoba, Spain, were obtained and the phenolic and volatile fractions were further analyzed. The components profiles were compared with those of a similar subset of seedlings arisen from the cross of two widely cultivated olive cultivars having contrasted oil compositions ('Arbequina' and 'Picual'). Results showed that a single cross provides enough degree of variability to produce new olive cultivars with improved quality. The set of cultivars from the collection displayed, in general, higher levels of variability than the progeny cross for the main groups of volatile compounds. However, the latter displayed a wider variability than the set of cultivars under study for most of the main classes of phenolic compounds in the oil but the lignans.

Keywords: *Olea europaea* L., virgin olive oil, phenolics, volatile compounds

INTRODUCTION

VOO represents one of the main features that distinguish the Mediterranean diet. Different scientific evidences suggest that VOO has a significant positive impact on human health (Konstantinidou et al., 2010; Estruch et al., 2013). Also, the long term dietary consumption of VOO rich in phenolics seems to be related to an attenuation of the inflammatory response and reduction of the associated risk of chronic inflammatory disease states (Visioli and Bernardini, 2011; Lucas et al., 2011). These phenolics are also responsible, together with the volatile compounds, for most of the VOO sensory properties. Phenolics are responsible for the bitter and pungent taste notes (Andrewes et al., 2003; Mateos et al., 2004) whereas the volatile compounds are responsible for the aroma of VOO. These compounds are produced, from substrates present in the olive fruit, by the action of different hydrolytic and oxidative enzymes after olive fruit is crushed during the olive oil extraction process. Thus, the composition and biochemical status of the olive fruit are the most important variables determining the synthesis of phenolic and volatile compounds during the oil extraction process. Furthermore, the edaphoclimatic conditions of the tree or

^a E-mail: carlos.sanz@ig.csic.es

the technological conditions used during olive oil processing may also have a significant impact on the oil phenolic and volatile profiles.

Represented by thousands of local and old cultivars, restricted to specific areas where they originally grew, the wide genetic heritage of the olive tree is one of the main attributes of this crop. Much emphasis has been laid on the collection and conservation of olive genetic resources leading to the establishment of germplasm banks. In this sense, the World Olive Germplasm Collection (CAP-UCO-IFAPA) (WOGC), located at IFAPA Centre “Alameda del Obispo” in Córdoba, Spain, is an international reference on olive germplasm due to the high number of accessions included and their high degree of identification and evaluation. Molecular markers and agronomical traits were used to study the patterns of genetic diversity and underlying genetic structure among olive accessions in the WOGC (Belaj et al., 2012). Therefore, considering the nutritional and sensory relevance of the VOO phenolics and volatile compounds, the purpose of the present work was to evaluate the phenolic and volatile fractions of oils produced by a subset of highly diverse cultivars from the WOGC.

MATERIALS AND METHODS

Plant material

Ninety-seven olive cultivars were studied, representing around one-fourth of the accessions of the WOGC (IFAPA Alameda del Obispo in Córdoba, Spain), being representative of the world genetic variability of the species *Olea europaea*. Trees, two per accession, were grown in the same edaphoclimatic conditions at the experimental WOGC orchards using drip irrigation and standard culture practices. Olive oil was produced from hand-picked fruits at the turning stage.

Olive oil extraction

Olive oil was extracted using an Abencor plant (Comercial Abengoa, S.A., Seville, Spain) that mimics the industrial process of VOO production at a laboratory scale (Martínez et al., 1975). Fruit was crushed using a stainless steel hammer mill operating at 3000 rpm and with a 5 mm sieve. Then, malaxation was carried out at 30 °C for 30 min. Finally, the kneaded paste was centrifuged at 3500 rpm for 1min. The oils were decanted, paper filtered, and stored under nitrogen at -20 °C until analysis. Data from the WOGC cultivars were compared to those from the oils of the cross Picual x Arbequina (Pérez et al., 2014; 2016).

Extraction and analysis of virgin olive oil phenolic compounds

Phenolic compounds were isolated from olive oils following a previously described procedure (Mateos et al., 2001) by SPE on a diol-bonded phase cartridge (Supelco, Bellefonte, PA). An aliquot (0.5 mL) of standard solution containing *p*-hydroxyphenyl-acetic acid (0.12 mg/mL) and *o*-coumaric acid (0.01 mg/mL) was added to oil samples (2.5 g) before phenolic extraction. Two phenolic extracts were obtained from each oil.

VOO phenolic extracts were analyzed in a Beckman Coulter liquid chromatographic system equipped with a diode array detector and a Mediterranean Sea 18 column (4.0 mm i.d.×250 mm, particle size 5 µm) (Teknokroma, Barcelona, Spain) under the system conditions previously described by Pérez et al. (2014). The identification of compounds was confirmed using the same chromatographic system and conditions but connected this time to a MAT95 magnetic sector mass spectrometer (Finnigan Mat, Bremen, Germany) equipped with an ESI-II electrospray ionization (ESI) interface.

Analysis of volatile compounds

Extraction and analysis of oil volatile compounds were performed according to Pérez et al. (2016). Samples for volatile analyses (0.5 g) were prepared in 10-mL vials and placed in a vial heater at 40 °C. After a 10 min equilibration, volatile compounds from the headspace were adsorbed onto SPME fiber DVB/Carboxen/PDMS 50/30 µm (Supelco Co., Bellefonte, PA). The sampling time was 50 min at 40 °C and desorption of volatile compounds trapped in the SPME fiber was performed directly into the GC injector. For

quantitative purposes, VOO volatile compounds were analyzed three times on a HP-6890 gas chromatography apparatus equipped with a FID detector (Agilent Technologies) using a DB-Wax capillary column and identified using a 7820A/GC-5975/MSD system (Agilent Technologies) as described in Pérez et al. (2016). The volatile compounds were clustered into different groups according to the number of carbons (six and five straight-chain carbons, C6 and C5 volatiles, respectively) and esters originated through the lipoxygenase (LOX) pathway and the branched-chain (BC) volatile compounds coming from the amino acid metabolism.

RESULTS AND DISCUSSION

Phenolic profiles

A high degree of variability for the content of phenolic compounds has been found for the set of cultivars under study (Figure 1). Phenolics mostly belong to four different groups, compounds derived from tyrosol, lignans, flavonoids, and phenolic acids. The tyrosol derivatives are the most abundant phenolics in the oils, especially those with a secoiridoid chemical structure such as the dialdehydic forms of decarboxymethyloleuropein and decarboxymethylligstroside aglycones and the aldehydic forms of oleuropein and ligstroside aglycones, which contain the phenolic alcohol tyrosol or its hydroxyl derivative hydroxytyrosol in their molecules (Pérez et al., 2014). The mean content of tyrosol derivatives was 431 µg/g oil with a range of variability from 13 to 1385 µg/g oil. However, quite surprisingly these figures were lower than those found for the progeny of the cross Picual x Arbequina (Pérez et al., 2014), either for the mean value (557 µg/g oil) or for the content range (18-1666 µg/g oil). These findings are of interest for the olive breeding programs since they demonstrate that new olive cultivars with improved phenolics profiles may arise from single crosses of olive cultivars. Contrary to the tyrosol derivatives, the lignan variability range for the WOGC cultivar subset is higher than that of the progeny genotypes (Figure 1). Lignans are the second main group of phenolics in the oils, although they represented on average around 5% of the total phenolics, which is about 20 times less content than the tyrosol derivatives group. The third major class of VOO phenolics was the flavonoids. The mean content of these compounds in the oils was 6.82 µg/g oil and the range of variability from 0.88 to 27.29 µg/g oil. It is very similar to those found for the flavonoids in the oils of the Picual x Arbequina cross progeny, as displayed in Figure 1, which suggests that the synthetic machinery for flavonoid synthesis might be highly conserved in the olive species. Finally, there is a group of simple phenolics, mostly phenolic acids, which also contributes, although at a very low level, to the total phenolic pool of the oils. The average content found for the WOGC cultivar subset was 1.95 µg/g oil, which is higher than that found in the oils obtained from the progeny of the Picual x Arbequina cross (1.52 µg/g oil). However, the content range (0.21-6.25 µg/g oil) was very similar to that found in the cross oils as shown in Figure 1.

Aroma volatile profiles

The WOGC cultivar subset showed a high degree of variability in terms of the content of volatile compounds (Figure 2). Contrary to what found for the phenolics, this variability is larger than that found for the progeny of the Picual x Arbequina cross for all the major classes of volatile compounds. Most of the volatiles were produced by the sequential action of enzymes included in the so-called LOX pathway (Olías et al., 1993), and they may be clustered into different groups according to their chain length (C6 and C5 volatiles). After peroxidation of polyunsaturated fatty acids by LOX activity, the hydroperoxide lyase enzymatic activity produces C6 volatiles. C5 volatiles seem to be generated through an additional branch of the LOX pathway involving the production and scission of a 1,3-pentene allylic radical. In addition, esters and a group of compounds that featured a branched-chain (BC volatiles) chemical structure derived from amino acid metabolism also contributed significantly to the volatile fraction of the oils and are very relevant to the aroma of olive oil (Pérez et al., 2016).

Figure 1. Variability of the major classes of phenolic compounds in the oils from the WOGC cultivar subset (solid lines). For comparison purposes, the variability in the oils from the Picual x Arbequina progeny is included (dashed lines).

The main group of volatile compounds in the oils was the C6 compounds, whose contents were on average 4-300 times higher than those of the rest of the groups of volatile compounds in the oils. The range of variability for the content of C6 compounds was 5985-74836 ng/g oil. This range exceeded that found by Luna et al. (2006) for oils produced from 39 cultivars obtained from trees cultivated in the same orchard under the same edaphoclimatic conditions, 2520-18110 ng/g oil, and what found for the progeny of the Picual x Arbequina cross, 3132-46712 ng/g oil (Pérez et al, 2016) (Figure 2). Among the C6 compounds, those coming from the linolenic acid were the most abundant, (E)-hex-2-enal being the main contributor (90% of total C6 compounds on average) in good agreement with the average value found for the volatile fractions of the progeny of the Picual x Arbequina cross (Pérez et al., 2016). Only the C5 group of volatile compounds displayed comparable contents to those of the C6 volatiles. Among them, pentene dimers represented on average 80% of the C5 compounds content, although their sensory contribution to the VOO aroma seems to be quite low or negligible given the estimated odor thresholds for these compounds (Pérez et al., 2016). Although displaying comparatively lower contents

than pentene dimers, the rest of C5 compounds seem to have a notable involvement in the VOO aroma according to their low odor thresholds. Pent-1-en-3-one and the pent-2-en-1-ols are particularly notable in good agreement with earlier findings (Aparicio et al., 1996).

The content of esters in the oils of the WOGC cultivar subset, mainly synthesized through the LOX pathway, ranged 57-19485 ng/g oil and showed an average value of 2054 ng/g oil, which were much higher than what found for the progeny of the Picual x Arbequina cross (range 86-6282 ng/g oil; average value 987 ng/g oil) (Figure 2). Esters presence in the oils is very important from an oil commercial grading and a consumer perspective, as they are responsible for the fruity odor notes in this product (Pérez et al., 2016). Finally, the BC compounds were found at low concentrations in the oils, showing a mean value of 100 ng/g oil and a variability range of 20-740 ng/g oil. As displayed in Figure 2, the variability profile is quite similar to that of the Picual x Arbequina cross oils. Despite the low contents in the oils, they seem to play also a very relevant role in the aroma of the oils. Among them, the 2 and 3-methyl-butanals seems to contribute largely to the VOO aroma due to their very low odor thresholds (Pérez et al., 2016).

Figure 2. Variability of the major classes of volatile compounds in the oils from the WOGC cultivar subset (solid lines). For comparison purposes, the variability in the oils from the Picual x Arbequina progeny is included (dashed lines).

CONCLUSIONS

Data obtained for the composition of the main phenolics and volatile compounds of the cultivars from the WOGC shows a high level of variability in terms of the deduced nutritional and sensory quality of the oils. This variability suggests that the WOGC may be very useful for the choice of optimal parents for olive breeding programs aiming at obtaining new cultivars with improved oil quality. Moreover, data comparison with those from the oils from the Picual x Arbequina cross suggests that it might be more effective to consider a greater number of individuals within the same cross than the use of different crosses with a smaller number of individuals.

ACKNOWLEDGEMENTS

Funding for this research came from the OLEAGEN project of Genoma España and AGL2011-24442 project from the Programa Nacional de Recursos y Tecnologías Agroalimentarias, both financed by the Spanish Government.

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