Cultivar influence on variability in olive oil phenolic profiles determined through an extensive germplasm survey

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Resumen

Despite the evident influence of the cultivar on olive oil composition, few studies have been devoted to exploring the variability of phenols in a representative number of monovarietal olive oils. In this study, oil samples from 80 cultivars selected for their impact on worldwide oil production were analyzed to compare their phenolic composition by using a method based on LC–MS/MS. Secoiridoid derivatives were the most concentrated phenols in virgin olive oil, showing high variability that was significantly due to the cultivar. Multivariate analysis allowed discrimination between four groups of cultivars through their phenolic profiles: (i) richer in aglycon isomers of oleuropein and ligstroside; (ii) richer in oleocanthal and oleacein; (iii) richer in flavonoids; and (iv) oils with balanced but reduced phenolic concentrations. Additionally, correlation analysis showed no linkage among aglycon isomers and oleocanthal/oleacein, which can be explained by the enzymatic pathways involved in the metabolism of both oleuropein and ligstroside.

Material and methods

Vegetal material was collected from the World Olive Germplasm Bank of Cordoba (WOGB) (CAP-UCO-IFAPA), specifically in the collection located at the University of Cordoba (Cordoba, Spain, 37°55'56.5" N, 4°43'13.3" W and 173 m a.s.l.). A set of 80 olive cultivars were selected during the 2015–2016 crop season according to their importance for the worldwide olive oil production, their geographical origin, and fruit availability (Table 1). Fruit were independently collected from two olive trees per cultivar. The trees were sampled from October to December with fruits ripening index (RI) equal to 2.0 (yellowish-red color). The virgen olive oil (VOO) were obtained using an Abencor extraction system (30 min at 28 ^{O}C).

Determination of phenolic compounds

Sample preparation—Phenolic compounds were isolated by liquid-liquid extraction , where 1 g of VOO was mixed with 2 mL n-hexane; then, 1 mL of 60:40 (v/v) methanol-water was added and shaken for 2 min, and the hydroalcoholic phase was separated by centrifugation. The extraction was repeated to enhance the extraction efficiency (V. Sánchez de Medina et al., 2017).

LC-MS/MS analysis—Analyses were performed by reversed-phase liquid chromatography followed by electrospray ionization (ESI) in negative mode and tandem mass spectrometry (MS/MS) detection. Ten µL of extract was injected in triplicate into the LC system for chromatographic separation of the target compounds using a C18 Pursuit XRs Ultra (50×2.0 mm i.d., 2.8 µm particle size) from Varian (Walnut Creek, CA, USA). The entire eluate was electrosprayed and monitored by MS/MS in Multiple Reaction Monitoring (MRM) mode for selective transitions from precursor to product ions for each analyte.

Results and Discussion

Evaluation of the phenolic variability in monovarietal VOOs

The phenolic composition of VOO strongly depends on numerous factors, among which the cultivar (genotype) plays a key role (Baiano et al., 2013; El Riachy et al., 2011). We selected 80 olive cultivars according to the following criteria: a) importance in terms of VOO production, b) geographical origin, and c) fruit availability in the WOGB. A huge variability for all the individual phenolic compounds was observed between cultivars. **Figure 1** shows the distribution of the phenolic concentration in the whole sat of samples. The secoiridoid derivatives were the most abundant phenols in all evaluated monovarietal VOOs. The concentration of oleocanthal, one of the most recognized phenols in VOO due to its anti-inflammatory and antioxidant properties, showed an almost 100-fold variation in the cultivar set, ranging from 17 to 1600 mg/kg .

Influence of cultivar on phenolic profile variability of olive oil

An ANOVA test was applied to check the influence of the cultivar (genotype) on the phenolic compound variability. The goodness of fit statistics revealed that the percentage of the variability (R^2) explained by the genotype was highly significant (p-value<0.001) for the nine phenolic compounds and the two consecutive crop seasons **(Table 1).**

Classification of olive cultivars attending to their VOO phenolic profiles

To determine distinctive patterns in the set of cultivars according to their phenolic profiles, a principal component analysis (PCA) was applied using the concentrations of individual phenols determined in the 80 monovarietal oils. The first three principal components (PC1, PC2 and PC3) explained 74.1% of the cumulative variability and allowed clustering of the cultivars into four main groups (G1, G2, G3 and G4), characterized by their distinctive phenolic compositions (**Figure 2**). The G1 group included 18 cultivars characterized by the high concentration of oleuropein and ligstroside aglycon isomers; G2 grouped 16 cultivars with high levels of oleocanthal and oleacein; G3 clustered 10 cultivars with a high concentration of apigenin and luteolin; and finally, G4 included 36 cultivars that showed a balanced composition, with no remarkable concentration in any of the studied phenolic compounds (**Table 2**). **Figure 3** illustrates differences in the concentration of these phenolic compounds in the four groups of cultivars differentiated according to the PCA.

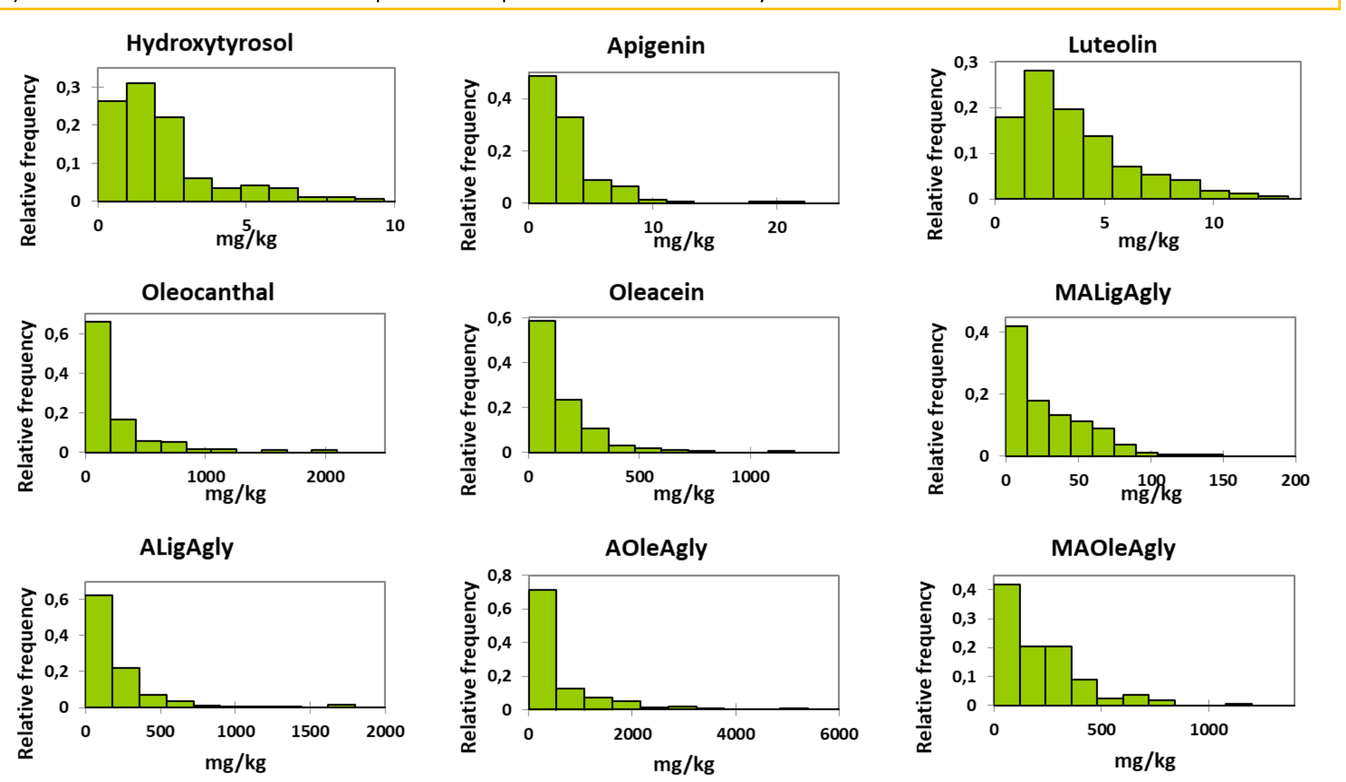

Figure 1. Histograms showing the distribution of the concentration of the nine phenolic compounds evaluated in the 24 monovarietal VOOs (2015/2016 crop season). **AOleAgly** – Aldehydic open forms of Oleuropein Aglycon; **MAOleAgly** – Monoaldehydic closed form of Oleuropein Aglycon.

ALigAgly – Aldehydic open forms of Ligstroside Aglycon; **MALigAgly** – Monoaldehydic closed form of Ligstroside Aglycon.

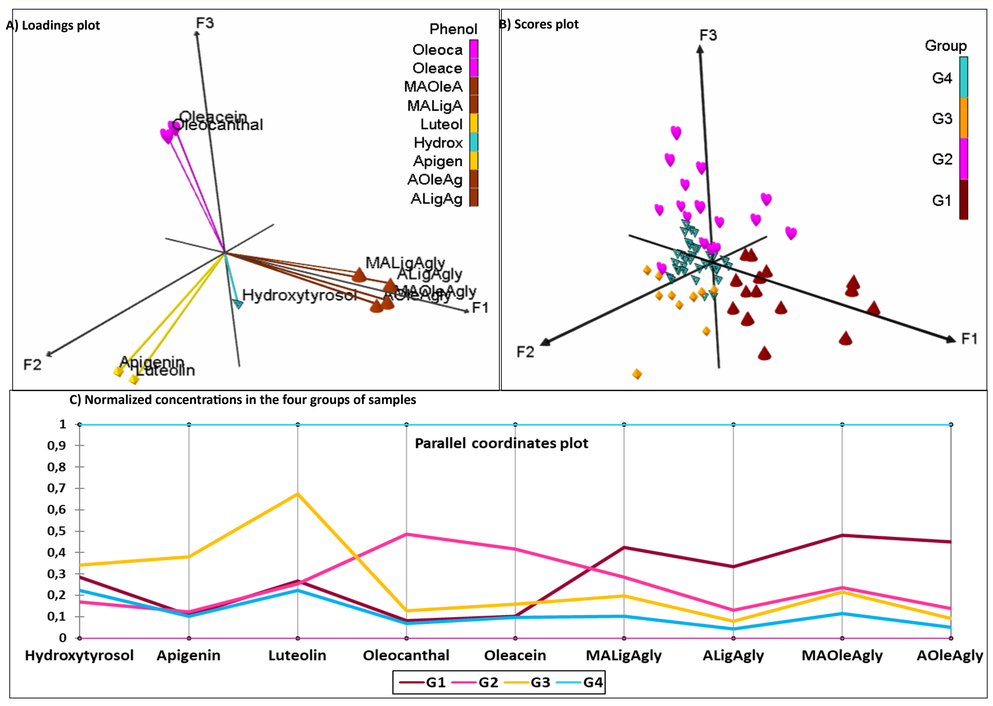

Figure 2. Principal component analysis for the phenolic profiles of the 80 monovarietal VOOs. (A) Loadings plot. (B) Scores plot. (C) Normalized concentration profiles of the four groups of cultivars classified attending to their phenolic profile.

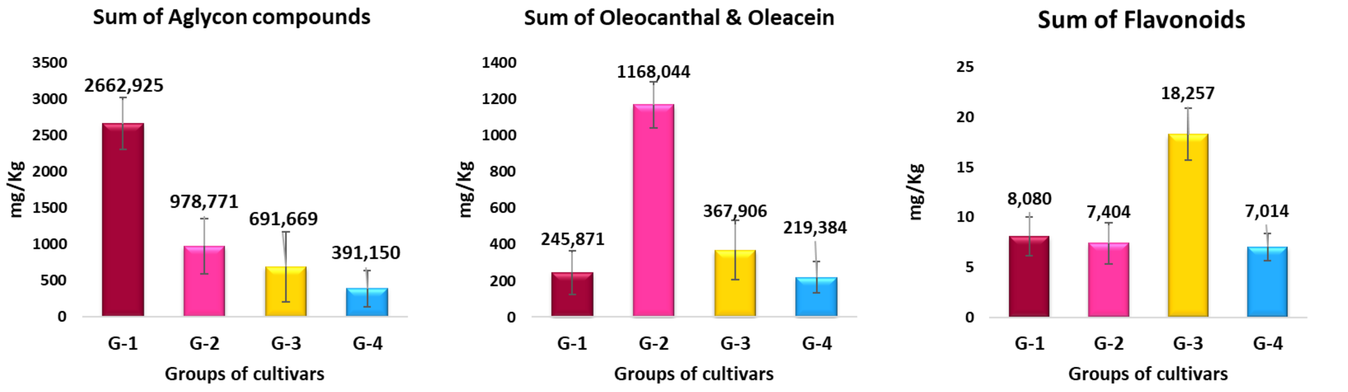

Figure 3. Differences in the concentration of aglycon compounds, olecanthal and oleacein and flavonoids found in the four groups of monovarietal VOO classified according to the PCA.

Table 1. ANOVA test to check the influence of the genotype on the concentration of the nine phenolic compounds (result obtained after logarithmic transformation of the original data).

Phenol	R^2	F	*p*-value
2014/2015 crop season (25 cultivars)			
Hydroxytyrosol	0,916	11,360	< 0,0001
Apigenin	0,965	28,487	< 0,0001
Luteolin	0,962	26,065	< 0,0001
Oleocanthal	0,889	8,370	< 0,0001
Oleacein	0,754	3,185	0,003
MALigAgly	0,960	25,215	< 0,0001
ALigAgly	0,925	12,844	< 0,0001
MAOleAgly	0,894	8,764	< 0,0001
AOleAgly	0,887	8,211	< 0,0001
Phenol	R^2	F	*p*-value
2015/2016 crop season (80 cultivars)			
Hydroxytyrosol	0,831	4,984	< 0,0001
Apigenin	0,948	18,585	< 0,0001
Luteolin	0,887	7,961	< 0,0001
Oleocanthal	0,924	12,270	< 0,0001
Oleacein	0,908	9,943	< 0,0001
MALigAgly	0,930	13,529	< 0,0001
ALigAgly	0,973	36,917	< 0,0001
MAOleAgly	0,957	22,405	< 0,0001
AOleAgly	0,956	21,759	< 0,0001

R^2 (determination coefficient): percentage of variability explained by the genotype in the total variance.
F ratio: variation between samples/variation within the samples.
p-value: significance level.

Table 2. Classification of the 80 olive cultivars into the four groups established by the PCA according to their phenolic profiles.

G1	G2	G3	G4	
Abou Choki	Alfafara	Arbosana	Alameño de Montilla	Manzanilla Cacereña
Barnea	Arbequina	Azapa	Amygdalolia Nana	Manzanilla de Sevilla
Bosana	Blanqueta	Carrasqueño de Elvas	Ascolana Tenera	Mastoidis
Bouteillan	Caballo	Cordovil de Serpa	Bodoquera	Mollar de Cieza
Changlot Real	Cerezuela	Hojiblanca	Carolea	Morisca
Chemlal de Kabilye	Enagua de Arenas	Kusha	Çobrancosa	Ojo de Liebre
Chetoui	Joanenca	Lastovka	Cornicabra de Mérida	Palomar
Coratina	Kalamon	Mission Moojeski	Empeltre	Picual
Cornicabra	Koroneiki	Morona	Farga	Rapasayo
Manzanilla Prieta	Kotruvsi	Picudo	Frantoio	Royal de Cazorla
Mixani	Levantinka		Galega Vulgar	Sabatera
Morrut	Megaritiki		Gemlik	Sandalio
Nasuhi	Moraiolo		Gordal de Granada	Sikitita
Picholine Marocaine	Negrillo de la Carlota		Jabaluna	Tanche
Picual de Almería	Pendolino		Leccino	Ulliri i Bardhe i Tiranes
Royal de Calatayud	Plementa Bjelica		Lechín de Sevilla	Verdale
Villalonga			Loaime	Verde Verdelho
Zaity			Lucio	Verdial de Huévar

Conclusions

In this study, remarkable variability was found for nine phenolic compounds in the largest set of monovarietal VOOs analyzed to date. Genotype was the main factor contributing to this variability for all phenolic compounds with a percentage of total variance between 83% and 97%. The secoiridoid derivatives were the most abundant phenols of all monovarietal VOOs evaluated in this study. Various previously undistinguished olive cultivars were revealed to be very rich, interesting cultivars for certain phenolic compounds.

Multivariate analysis allowed detection of four groups of cultivars (G1, G2, G3 and G4) via their phenolic profile. G1 was characterized by a high concentration of oleuropein and ligstroside aglycon isomers and G2 by a high concentration of oleocanthal and oleacein; G3 was rich in two flavonoids (apigenin and luteolin). The last group, G4, included cultivars for VOOs that did not stand out in terms of the monitored phenols.