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Phenolic variability in fruit from the 'Arbequina' olive cultivar under Mediterranean and Subtropical climatic conditions

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SUMMARY: In the present work, we compared the phenol content and composition of fruit from the 'Arbequina' cultivar in four Mediterranean (in Andalucía, Southern Iberian Peninsula) and two Sub-Tropical (Canary Islands) locations throughout the harvest period. Two Mediterranean and two Sub-Tropical locations were maintained with drip irrigation, while the remaining two Mediterranean locations were in dry farming. Water availability and harvest date seemed to play more important roles than air temperature on the phenolic content and most of the studied components. The variability associated with location was a result of the high values observed in the two Mediterranean locations in dry farming, with respect to the other four maintained with drip irrigation. Few differences were found among the four drip-irrigated locations, despite the fact that two were Mediterranean and the other two Sub-Tropical. In addition, a sharp decrease was observed during the harvest period for phenolic content and most of the phenolic compounds.

KEYWORDS: *Environment; Harvest date; Location; Maturity; Olive fruit*

RESUMEN: ***Variabilidad fenólica del fruto en el cultivo de olivo 'Arbequina' en condiciones climáticas mediterráneas y subtropicales***. En el presente trabajo se compara la variabilidad del contenido y composición en fenoles de la variedad de olivo 'Arbequina' en cuatro localidades con clima Mediterráneo y dos con clima Sub-Tropical. Dos de las localidades mediterráneas y las dos Sub-Tropicales contaban con riego por goteo, mientras que las dos mediterráneas restantes estaban en secano. La disponibilidad de agua y momento de recolección parece ser un factor más importante que la temperatura del aire en el contenido y composición de fenoles del fruto. La mayor parte de la variabilidad asociada a la localidad estuvo causada por los altos valores encontrados en las dos localidades mediterráneas en secano, respecto a las otras cuatro localidades en regadío. Solo pequeñas diferencias se encontraron entre las cuatro localidades en regadío, a pesar de que dos eran mediterráneas y las otras dos sub-tropicales. Además, un descenso acusado del contenido de la mayoría de los fenoles analizados se ha observado conforme avanzaba la fecha de recolección.

PALABRAS CLAVE: *Aceituna; Ambiente; Fecha de recolección; Localidad; Madurez*

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1. INTRODUCTION

Olive oil is one of the main components of the Mediterranean diet due to its nutraceutical properties (Piroddi *et al.,* 2017). Among olive oil constituents, phenols play a very important role in its healthy properties (Serreli and Deiana, 2019). For example, olive phenols reduce chronic inflammation and help in the prevention of some diseases, such as Alzheimer's (Omar *et al.,* 2017) or different types of cancer (Piroddi *et al.,* 2017).

The phenolic composition of olive oil depends, mainly on the phenolic composition of the fruit upon arriving to the mill factory, which is then modified by enzymatic processes occurring during oil extraction (Lukić *et al.,* 2017). This initial fruit phenolic composition could be affected by many factors including genotype, harvest date, tree environmental conditions and management practices (Servili and Montedoro, 2002).

The wide olive germplasm has shown a high genetic variability for both phenolic content and composition in the evaluation of cultivar collections (Miho *et al.,* 2018), breeding progenies (Pérez *et al.,* 2014) and comparative trials (Pérez *et al.,* 2018; El Riachy *et al.,* 2013).

Several studies have also attempted to evaluate the phenolic variability of single cultivars in different environments of their country of origin, such as 'Gemlik' (Ben Ghorbal *et al.,* 2018) in Turkey, 'Picholine Marrocaine' in Morocco (Bajoub *et al.,* 2015), 'Chemlali' (Bouaziz *et al.,* 2004) in Tunis and 'Baladi' in Lebanon (El Riachy *et al.,* 2018). Among the environmental variables, phenol composition is mainly influenced by both abiotic and biotic stresses. Among the first ones, water stress has been reported as one of the main factors influencing phenol content and composition (Dabbou *et al.,* 2015; Gómez-Rico *et al.,* 2006; Gucci *et al.,* 2019). In general, an increase in water stress implies an increase in phenolic content in olive oil (García *et al*., 2020). However, some individual phenols, such as lignans, might show the opposite pattern (Ovar *et al.,* 2002). Biotic stresses such as olive fruit fly (Medjkouh *et al.,* 2018) and Verticillium Wilt (Landa *et al.,* 2019) can also increase oil phenolic content and composition.

The total phenolic content and composition normally decreases during the harvest season, as shown for different olive cultivars (Alowaiesh *et al.,* 2018; Bengana *et al.,* 2013; Bodoira *et al.,* 2015; Dag *et al.,* 2011). This includes 'Arbequina', the most widely planted cultivar in the world (Fernández-Escobar *et al.,* 2013), whose phenolic content has been evaluated in olive oil (Abenoza *et al.,* 2015; Morelló *et al.,* 2004) and fruit (Talhaoui *et al.,* 2015) through maturation in single locations in Spain. On the contrary, some phenols such as lignans and flavones have been reported to increase in oil through maturation (Bengana *et al.,* 2013) as well as verbascoside (Bodoira *et al.,* 2015) and hydroxytyrosol (Bouaziz *et al.,* 2004) in fruits. In other cases, irregular patterns of variation in fruit phenols through maturation have been reported (Ben Ghorbal *et al.,* 2018).

All the mentioned works on olive fruit phenols have been performed in Mediterranean climatic conditions. Few reports exist on olive phenols in other climates as is the case of Argentina (Bodoira *et al.,* 2015). However, olive growing is currently expanding worldwide, in many cases outside the Mediterranean climate. Besides, this expansion is mainly based on few cultivars specially adapted to the new trends of olive growing (Fernández-Escobar *et al.,* 2013), with 'Arbequina' being the clearest example. Therefore, it would be of great interest to compare the influence of very different climate conditions (Mediterranean vs. non-Mediterranean) on variation in an important component of olive oil such as phenolic compounds.

In this sense, Tenerife, in the Canary Islands, is one of the non-Mediterranean locations where olives now have some importance (Medina *et al*., 2018). Its Sub-Tropical climate might be of interest as a natural scenario with climatic conditions similar to those of the typical Mediterranean olive growing area in the likely event of a climate warming (Medina *et al*., 2020).

In the present work, we compared the variation in phenolic content and components of 'Arbequina' fruits through maturation in typical Mediterranean locations from Andalusia in Sothern Iberian Peninsula, with others from the Sub-Tropical climatic conditions in Tenerife, Canary Islands. That comparison was used to compare the test of location, harvest date and their interaction on phenolic content and composition.

2. MATERIALS AND METHODS

2.1. Study sites and plant material

The trees of 'Arbequina' olive cultivar were sampled in four locations of Andalusia, Southern Iberian Peninsula from typical olive growing areas and Mediterranean climate: Antequera (37.17N, -4.64W), Baena (37.60N, -4.23W), La Rambla (37.62N, -4.82W) and Úbeda (37.89N, 3.24W); and two locations in Tenerife, Canary Islands, with Sub-Tropical climate: Los Tomillos (28.13N-16.49W) and El Viso (28.30N, -16,37W, Figure 1). The Mediterranean climate is characterized by colder winters and hotter summers than the Sub-Tropical one, which also has a low intraday temperature range. Antequera and Baena are managed in dry farming while the other four with drip irrigation (year amount of irrigation: La Rambla 250 mm, Úbeda 150 mm, Los Tomillos 372 mm and El Viso 312 mm). In the four Andalusia locations, trees were planted in 2008 at 7 x 6 m distance in a clay-loam soils. In the two Tenerife locations, trees were planted in 2010 at 5 x 5 m distance in a sandy-loam soil.

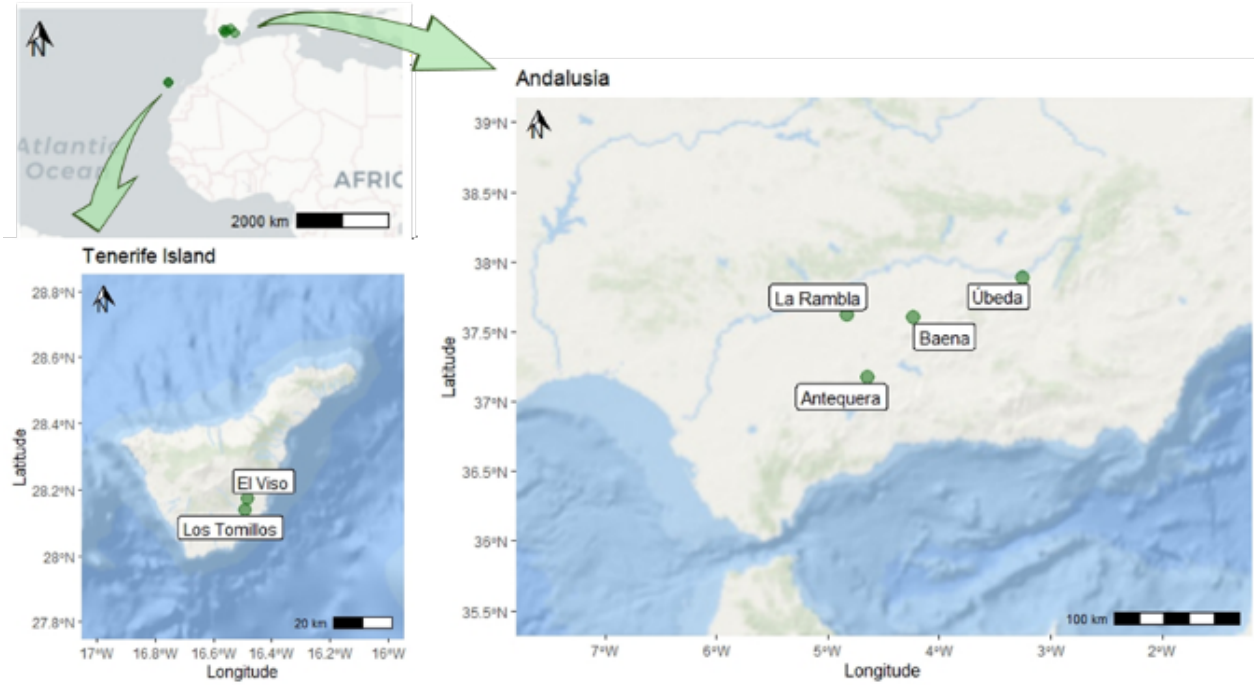

FIGURE 1.- Geografical location of the different tested orchards

Fertilization was applied on the basis of foliar analysis in order to avoid any limitations on growth and yield.

Four sets of three trees per location with similar fruit load (2-3 in a 0-3 visual scale) were sampled on three dates of the olive maturation period in 2017. In the case of Andalusian locations, sampling was performed in mid-September (harvest 1), mid-October (harvest 2) and mid-November (harvest 3). In the case of Tenerife, as the oil accumulation occurs earlier (Figure 2), harvest dates were mid-August (harvest 1), mid-September (harvest 2) and mid-October (harvest 3).

In each harvest date, a sample of 1 kg fruit was randomly hand-picked for each set of trees and location (4 sets x 3 harvest dates x 6 locations) to evaluate phenolic content and composition as well as fruit traits.

2.2. Phenols analysis

Reagents for extraction and other measurements were supplied by Sigma-Aldrich (St. Louis, MO). Phenolic standards were purchased from Extrasynthese (Genay, France).

Fruit phenolic compounds were extracted from each sample according to a previously developed protocol (García-Rodríguez *et al.,* 2011). Longitudinal pieces of mesocarp were cut at least from twenty olives.

For each sample, fruits were finely chopped and used to prepare phenolic extracts. Representative fruits samples (1 g) were kept at 4 °C for 72 h in dimethyl sulphoxide (DMSO, 6 mL) containing siringic acid as internal standard. The extracts were filtered through a 0.45 µm mesh nylon and kept at -20 ºC until HPLC analysis.

The phenolic extracts were analyzed by HPLC on a Beckman Coulter liquid chromatography system equipped with a System Gold 168 detector, solvent module 126, autosampler module 508 and a Waters column heater module following a previously described methodology (Pérez *et al.,* 2014). A Superspher RP 18 column (4.6 mm i.d. x 250 mm, particle size 4 µm: Dr Maisch GmbH, Germany) at a flow rate of 1 mL/ min and a temperature of 35 ºC was used for all the analyses. A total of 9 phenolic compounds were quantified in the phenolic extracts: hydroxytyrosol-4-glucoside, tyrosol-1-glucoside, demethyloleuropein, verbascoside, luteolin-7-glucoside, rutin, oleuropein, comselogoside, and ligustroside.

Phenolic compounds were monitored at two different wavelengths of 280 nm and 335 nm and quantified taking into account the internal standard and specific response factors for each of them (García-Rodríguez *et al.,* 2011).

The tentative identification of compounds by their UV-vis spectra was confirmed by HPLC/ESI-qTOF-HRMS. The liquid chromatography system was Dionex Ultimate 3000 RS U-HPLC liquid chromatography system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a similar Superspher RP 18 column but with 3 µm particle size. Formic acid 1% was used instead of phosphoric acid 0.5% for the mobile phase. A split post-column of 0.4 mL/ min was introduced directly onto the mass spectrometer electrospray ion source. The HPLC/ESI-qTOF operated for mass analysis using a micrOTOF-QII High Resolu-

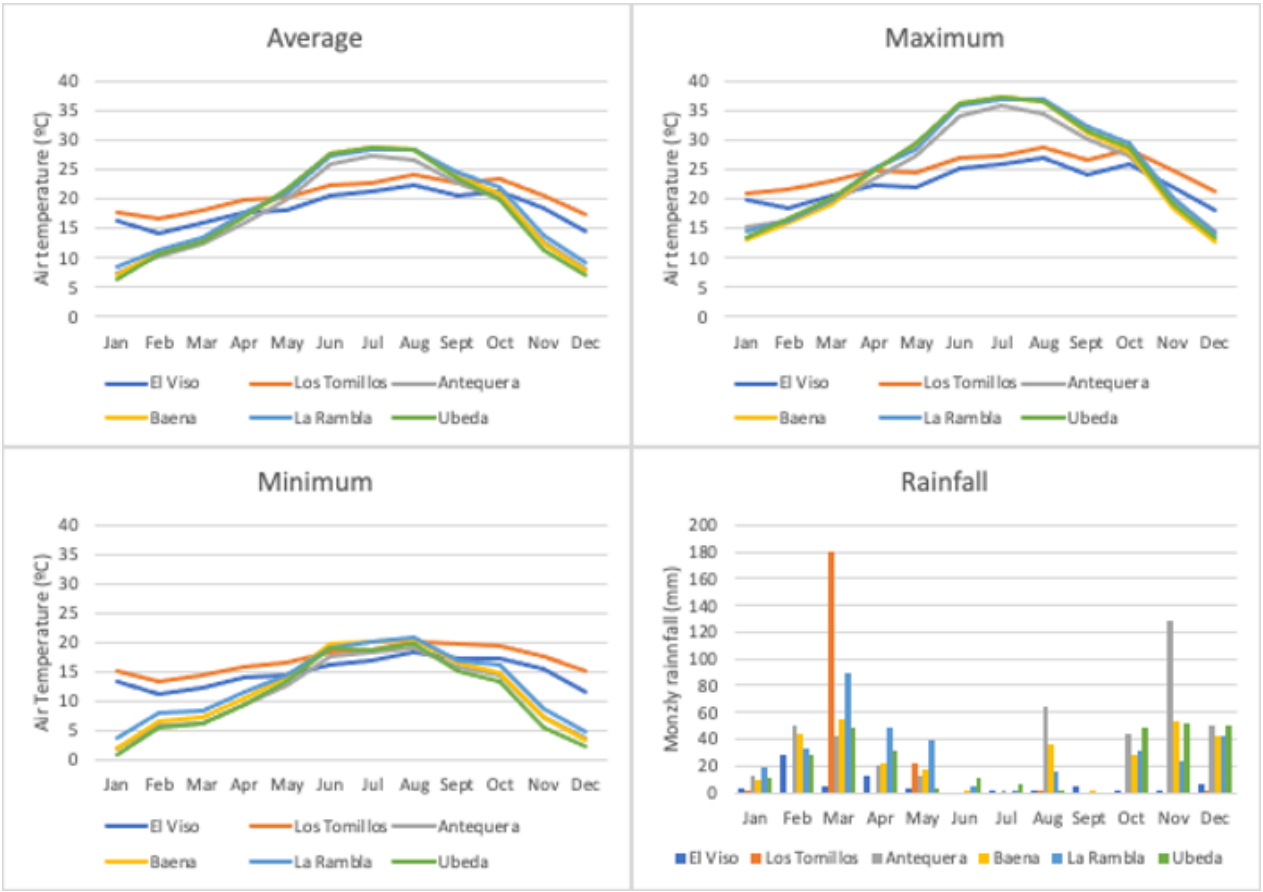

FIGURE 2. Average, maximum and minimum daily temperature (monthly average in ºC) and monthly rainfall (mm/m²) of the six locations considered. Temperature data were recorded hourly to make daily statistics

tion Time-of-Flight mass spectrometer (UHRTOF) with qQ-TOF geometry (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ionization (ESI) interface. Mass spectra were acquired in MS full scan mode and data were processed using Target Analysis 1.2 software (Bruker Daltonics, Bremen, Germany).

2.3. Fruit traits analysis

Ripening index was evaluated according to the procedure described by (Frias *et al.,* 1991). Afterwards, oil content was measured in three random sub-samples of 25 g of each sample. Each sub-sample was oven dried at 105 ºC for 42 h (Río and Romero, 1999) to ensure dehydration, and weighed to measure the percentage of oil content in a NMR fat analyzer.

2.4. Statistics

Analysis of variance was performed to evaluate the relative influence of location, harvest date and their interaction on the variability of phenolic content and composition. Comparison of means (Tukey) was used to test differences among locations, harvest dates and harvest dates-locations, when significant. Previous works have shown that the water availability is the main factor influencing phenol content (Gucci *et al*., 2019). Therefore, in order to test the differences between the Mediterranean and Subtropical conditions of Andalusia and Tenerife regions, a separate analysis was performed with only the four irrigated locations (two in Andalusia and two in Tenerife). Finally, a person correlation was performed to test the correlations among phenol constituents and with fruit traits.

3. RESULTS

The six locations involved in this work showed different climatic conditions during the experimental period, mainly associated with the region level (Figure 2). The two locations in Tenerife, Canary Islands, showed milder temperatures both in summer and winter, with around 7 ºC range of average maximum-minimum temperatures. Temperatures in Los Tomillos (located 200 m.a.s.l.) were 2-3 ºC higher than in El Viso (400 m.a.s.l.). Among the locations in Andalusia, Baena, La Rambla and Úbeda showed quite similar temperature patterns; while only slightly lower temperatures were observed in Antequera. Some differences in

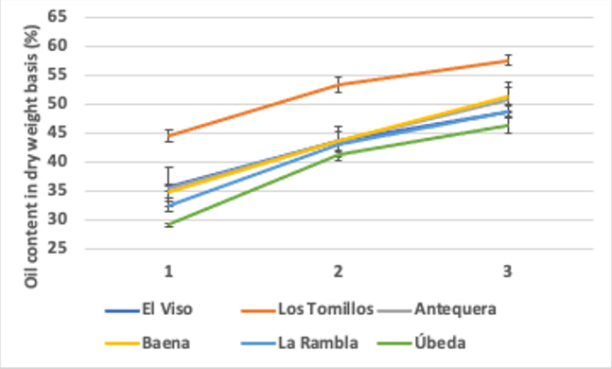

FIGURE 3. Oil content on dry weight basis of 'Arbequina' fruits on three harvest dates in the four locations of Andalusia, Iberian Peninsula (Antequera, Baena, La Rambla and Úbeda) and in two locations of Tenerife, Canary Islands (El Viso and Los Tomillos). In Andalusia, harvests 1, 2 and 3 represent mid- September, mid-October and mid-November at the Iberian Peninsula locations. In Tenerife, oil accumulation occurs one month in advance. For that reason, harvests 1, 2 and 3 represent mid-August, mid- September and mid-October. Three replicates per location and harvest date were averaged.

rainfall distribution among the Andalusian locations were also observed, particularly due to high rainfall in Antequera in the first week of November. Compared to the Peninsula, locations in Tenerife were characterized by limited rainfall throughout the year, with only exceptional rainfall observed in Los Tomillos in March.

The oil accumulation of 'Arbequina' in the six locations sampled showed a similar pattern considering that harvest 1 was done in August for El Viso and Los Tomillos (in Tenerife) and in September for the Andalusian locations (Figure 3).

Among all the identified phenols in 'Arbequina' fruits, oleuropein represented around half of the total phenolic content (Table 1), and together with demethyloleuropein, comseogoside, ligstroside and verbascoside constituted 95% of the total phenolic content. All the phenols showed high variability (high coefficient of variance), being especially high for verbascoside and oleuropein.

This high variability in phenolic content and composition was mainly due to location effect for phenols and most of the components (Table 2) except for oleuropein and ligstroside, for which location and harvest date showed comparable variance and for demethyloleuropein, which showed a double variance for the harvest date effect (41.9) compared to location (21.2) and their interaction (18.0). The interaction between location and harvest date represented the main contributor to total variance only for hydroxytyrosol-4-glucoside. Error variance represented more than half of the total variance for luteolin-7-O-glucoside. In any case, location, harvest date and their interaction showed a significant effect on phenolic content and all its components except for rutin and luteolin-7-O-glucoside, for which location was the only significant factor.

The phenolic components described were found in all locations and harvest dates (Table 3). The high variability due to location for total phenols was mainly due to the high values observed for Antequera and Baena (Table 3, Figure 4). While the decrease in total phenols with harvest date was, on average, more similar between harvest 1 and 2 than between harvest 2 and 3. However, for Antequera and Baena, this decrease was more evident between harvest 2 and harvest 3 -- just the opposite for the rest of the locations. Oleouropein, the major phenol identified, showed a similar pattern of variation to that of total phenols. The same could be said for ligstroside.

Different patterns of variation were observed for the rest of phenolic components (Table 3, Figure 4). Demethyloleuropein showed an increase between harvest 1 and harvest 2 in all locations except for Los Tomillos. In Antequera, this increase was also maintained between harvest 2 and harvest 3, maybe associated to a heavy rainfall at that time. Few variations among harvest dates were observed for comselogoside and verbascoside. Only in

TABLE 1. Descriptive statistics (from a total of 54 samples) of the phenols found in 'Arbequina' olive pulp. Data are presented in µg/g of fresh olive pulp.

Compound	Average	SDb	CVb	Min	Max
Total phenols	23028	11647	51	8180	51767
Oleuropein	11176	10740	96	585	39985
Demethyloleuropein	7103	3722	52	555	14639
Comselogoside	1470	681	46	462	3279
Ligstroside	1103	972	88	171	4251
Verbascoside	916	927	101	30	4009
Hydroxytyrosol-4-glucoside	431	226	52	75	1061
Rutin	408	252	62	49	1046
Luteolin-7-O-glucoside	367	232	63	15	1335
Tyrosol-1-glucoside	53	18	33	35	97

[a]SD=Standard deviation, [b]CV= coefficient of variance

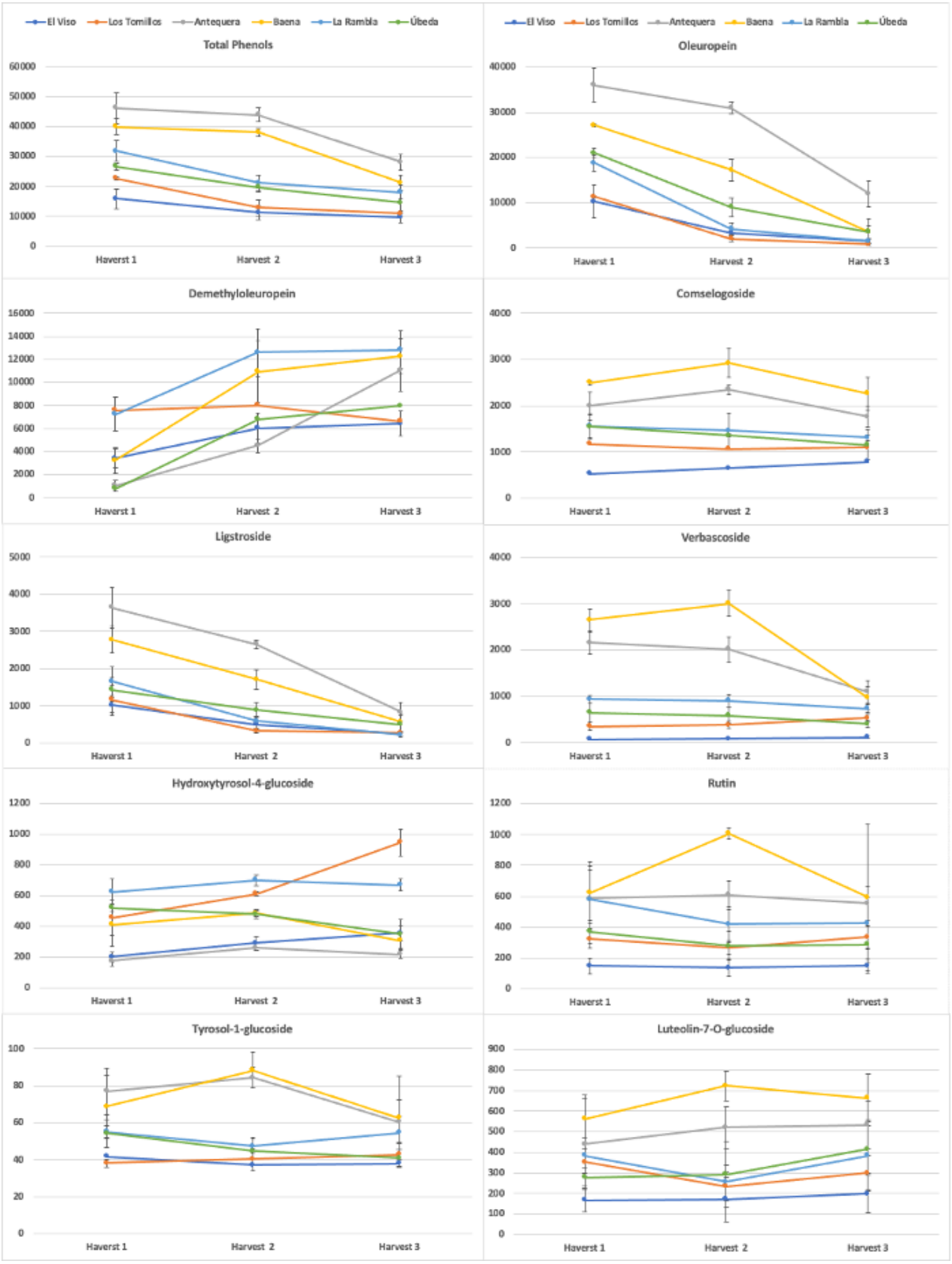

FIGURE 4. Variation in the main phenols found in 'Arbequina' fruits on the three harvest dates of the six locations considered. Data are presented in µg/g of fresh olive pulp. Three replicates per location and harvest date were averaged.

Table 2. Percentage of variance for location, harvest date and their interaction for the phenols found in 'Arbequina' olive pulp by the ANOVA analysis. Values in bold indicate significant differences for this source of variation at p<0.01. The analysis included 6 locations, 3 harvest dates and 3 replicates per location and harvest date.

	Total phenols	**Oleuropein**	**Demethyloleuropein**	**Comselogoside**	**Liustroside**	**Verbascoside**	**Hydroxytyrosol-4-glucoside**	**Rutin**	**Luteolin-7-O-glucoside**	**Tyrosol-1-glucoside**
Location	66.1	43.2	21.2	87.6	38.7	64.1	40.4	64.0	41.5	65.9
Harvest date	25.0	45.6	41.9	1.0	43.4	4.6	0.0	0.0	0.2	1.2
Location * Harvest date	5.0	5.2	18.0	4.1	8.2	10.7	47.4	4.9	0.0	7.1
Error	3.9	6.1	18.9	7.3	9.7	20.6	12.2	31.0	58.3	25.8

Table 3. Total and main phenol means by harvest date, location and harvest* location in 'Arbequina'. Different letters indicate significant differences (p< 0.005) among means within each of the three groups of data (Tukey test). Data are presented in µg/g of fresh olive pulp. Three replicates per location and harvest were averaged.

	El Viso		**Los Tomillos**		**Antequera**		**Baena**		**La Rambla**		**Úbeda**		**Average**	
Total Phenols														
Haverst 1	15834.4	ijk	22650.8	fg	46127.2	a	39922.6	bc	31775.7	d	26668.1	ef	30496.5	a
Harvest 2	11258.0	lm	12827.2	klm	43915.2	ab	38081.9	c	21231.0	gh	19747.0	ghi	24510.1	b
Harverst 3	9783.4	m	10911.0	lm	28063.9	de	21206.2	gh	18091.6	hij	14593.6	jkl	17108.3	c
Average	12292.0	d	15463.0	d	39368.8	a	33070.2	b	23699.4	c	20336.2	c		
Oleuropein														
Haverst 1	10242.6	f	11328.1	f	36031.0	a	27113.0	c	18728.1	de	21014.0	d	20742.8	a
Harvest 2	3360.8	gh	2045.8	gh	30914.5	b	17190.7	e	4235.9	g	9018.4	f	11127.7	b
Harverst 3	1454.5	gh	775.7	h	11946.2	f	3501.4	gh	1453.7	gh	3483.5	gh	3769.1	c
Average	5019.3	e	4716.6	e	26297.2	a	15935.0	b	8139.2	d	11172.0	c		
Demetiloleuropein														
Haverst 1	3429.5	d	7593.4	b	1030.6	ef	3226.2	de	7253.3	b	798.0	f	3888.5	c
Harvest 2	6054.5	bc	7995.4	b	4516.2	cd	10959.2	a	12628.2	a	6808.6	b	8160.3	b
Harverst 3	6439.9	bc	6629.7	bc	11057.3	a	12280.1	a	12852.0	a	7963.1	b	9537.0	a
Average	5308.0	d	7406.2	bc	5534.7	cd	8821.9	b	10911.2	a	5189.9	d		
Cosmelogoside														
Haverst 1	531.3	j	1176.1	gh	2002.0	cd	2501.5	b	1562.6	ef	1550.5	ef	1554.0	ab
Harvest 2	653.5	j	1065.3	hi	2357.2	bc	2922.8	a	1460.0	efg	1362.1	fgh	1636.8	a
Harverst 3	790.3	ij	1096.7	hi	1764.8	de	2269.7	bc	1311.2	fgh	1156.3	gh	1398.2	b
Average	658.4	e	1112.7	d	2041.3	b	2564.7	a	1444.6	c	1356.3	cd		
Ligustroside														
Haverst 1	1013.6	e	1155.5	de	3634.6	a	2774.4	b	1653.1	c	1433.6	cd	1944.1	a
Harvest 2	497.2	fg	322.3	g	2647.3	b	1704.9	c	583.5	fg	882.4	ef	1106.3	b
Harverst 3	245.9	g	262.9	g	833.4	ef	561.9	fg	215.6	g	495.0	fg	435.8	c
Average	585.6	d	580.2	d	2371.8	a	1680.4	b	817.4	cd	937.0	c		
Verbascoside														
Haverst 1	71.9	ef	352.2	def	2154.9	b	2646.2	ab	937.4	d	650.6	cde	1135.5	a
Harvest 2	83.4	f	383.5	def	2011.9	b	3005.0	a	901.4	cd	583.5	cdef	1161.5	a
Harverst 3	114.4	f	525.3	cdef	1099.9	c	969.5	cd	731.1	cd	405.2	def	640.9	b
Average	89.9	d	420.3	cd	1755.6	b	2206.9	a	856.6	c	546.4	cd		
Hydroxytirosol-1-Glucoside-1-														
Haverst 1	187.8	h	331.8	fghi	173.7	hi	407.7	efg	622.3	bcd	519.0	cde	373.7	c
Harvest 2	264.9	ghi	473.7	def	256.4	ghi	481.7	def	700.4	b	476.8	def	442.3	ab
Harverst 3	354.5	efgh	942.6	a	215.8	hi	306.6	fghi	665.5	bc	347.4	efghi	472.1	a
Average	269.1	cd	582.7	a	215.3	d	398.7	bc	662.7	a	447.7	b		

	El Viso		Los Tomillos		Antequera		Baena		La Rambla		Úbeda		Average	
Rutin														
Haverst 1	148.5	fg	323.2	defg	585.0	bc	622.3	b	581.1	bc	370.8	cdef	438.5	ns
Harvest 2	135.5	g	268.0	efg	607.9	bc	1007.0	a	419.3	bcde	277.8	efg	452.6	ns
Harverst 3	148.1	fg	335.0	defg	552.9	bcd	591.0	bc	424.8	bcde	287.7	efg	389.9	ns
Average	144.0	d	308.8	cd	581.9	ab	740.1	a	475.1	bc	312.1	cd		
Luteolin-7-O-glucoside														
Haverst 1	167.6	e	352.4	bcde	438.5	abcde	562.7	abc	383.0	bcde	277.3	cde	363.6	ns
Harvest 2	170.9	e	232.8	cde	519.6	abcd	722.2	a	255.0	cde	292.7	cde	365.5	ns
Harverst 3	197.7	e	300.2	cde	533.1	abcd	663.4	ab	383.2	bcde	414.4	abcde	415.3	ns
Average	178.7	c	295.1	bc	497.0	ab	649.4	a	340.4	bc	328.1	bc		
Glucoside-1-tyrosol														
Haverst 1	41.7	ef	38.2	f	76.9	ab	68.7	bc	55.0	cde	54.2	cde	55.8	ns
Harvest 2	37.1	f	40.3	f	84.3	a	88.3	a	47.4	efg	44.7	ef	57.0	ns
Harverst 3	38.0	f	42.8	ef	60.5	cd	62.7	bc	54.5	cde	40.7	ef	49.9	ns
Average	39.0	c	40.4	bc	73.9	a	73.2	a	52.3	b	46.5	bc		

Table 4. Fruit trait means by harvest date, location and harvest* location in 'Arbequina'. Different letters indicate significant differences ($p< 0.005$) among means within each of thr three groups of data (Tukey test). Three replicates per location and harvest were averaged.

	El Viso		Los Tomillos		Antequera		Baena		Fuencubierta		Úbeda		Average	
Fruit fresh weight (g)														
Haverst 1	0,86	g	1,29	ef	0,93	g	1,44	cde	1,40	cde	1,08	fg	1,17	c
Harvest 2	1,24	ef	1,66	bc	0,86	g	1,37	def	1,75	ab	1,37	def	1,37	b
Harverst 3	1,32	ef	1,85	ab	1,30	ef	1,76	ab	2,02	a	1,60	bcd	1,64	a
Average	1,14	cd	1,60	a	1,03	d	1,52	ab	1,72	a	1,35	bc		
Oil content in dry basis (%)														
Haverst 1	35,6	g	44,6	e	35,5	gh	34,9	gh	32,4	hi	29,1	i	35,4	c
Harvest 2	43,5	ef	53,4	b	43,6	ef	43,6	ef	43,2	ef	41,2	f	44,8	b
Harverst 3	48,8	cd	57,6	a	50,6	bc	51,4	bc	48,7	cd	46,4	de	50,6	a
Average	42,6	b	51,9	a	43,2	b	43,3	b	41,4	bc	38,9	c		
Fruit Moisture (%)														
Haverst 1	59,7	ab	50,9	fg	45,5	ij	50,0	fgh	59,1	abc	56,7	cde	53,7	a
Harvest 2	57,8	abcd	52,1	f	35,6	k	43,9	j	58,1	abcd	55,2	e	50,4	c
Harverst 3	56,3	de	48,7	gh	44,6	j	47,8	hi	60,5	a	56,2	de	52,4	b
Average	57,9	ab	50,6	c	41,9	e	47,2	d	59,2	a	56,1	b		
Maturity index														
Haverst 1	0,8	gh	1,4	ef	m.d.		1,0	fgh	m.d.		1,5	ef	1,5	c
Harvest 2	1,7	de	2,4	c	0,1	i	1,2	fg	m.d.		3,1	b	1,7	b
Harverst 3	2,0	cd	3,2	b	0,8	h	1,2	fg	m.d.		3,7	a	2,0	a
Average	1,5	b	2,3	a	0,4	c	1,2	b	m.d.		2,7	a		

Antequera and Baena, the two locations with the highest values for both components, a significant decrease was observed in harvest 3.

For the four components with lower contents in 'Arbequina' fruits (hydroxytyrosol-4-glucoside, rutin, luteolin-7-O-glucoside and tyrosol-1-glucoside), the highest values were observed in Antequera and Baena samples, probably due to the water stress. The unique exception was hydroxytyrosol-4-glucoside with very high levels in the fruits from La Rambla, and a significantly different accumulation pattern observed in Los Tomillos. This component is probably less influenced by water stress. Few variations with harvest date were observed for rutin, except for the very high values in Baena in harvest 2.

Fruit traits were also evaluated in the six locations and on three harvest dates. Fruit size, moisture and maturity index showed most of the variance due to the location effect. The oil content was mainly influenced by harvest date, as expected (data not shown). Antequera was the location with the smallest fruit size; while Los Tomillos showed higher oil content than the rest of the locations (Table 4). Fruit moisture was much lower in the two dry farming locations (Antequera and Baena), as expected. Maturity index was very delayed in Antequera, and very

Table 5. Percentage of variance of region, harvest date and their interaction for the phenols found in 'Arbequina' olive pulp by the ANOVA analysis. Only data for the four irrigated locations in Andalusia (La Rambla and Úbeda) and Tenerife (Los Tomillos and El Viso) were included. For each location, data of the 3 harvest dates and 3 replicates per location and harvest date were considered. Values in bold indicate significant differences for this source of variation at $p<0.01$.

	Total phenols	**Oleuropein**	**Demethyloleuropein**	**Comselogoside**	**Liustroside**	**Verbascoside**	**Hydroxytyrosol-4-glucoside**	**Rutin**	**Luteolin-7-O-glucoside**	**Tyrosol-1-glucoside**
Region	47,2	19,1	5,9	58,7	13,8	63,1	7,2	45,7	22,3	52,7
Date	38,3	71,0	22,5	3,8	68,1	4,2	8,9	1,8	6,5	4,2
Region * Date	2,4	0,6	11,0	4,1	3,3	4,8	27,3	1,9	7,0	0,3
Error	12,1	9,2	60,5	33,4	14,8	27,9	56,6	50,6	64,2	42,7

Table 6. Pearson correlation coefficients among phenols and fruit characteristics for the data of elementary plots by location and harvest date (18 data points) in 'Arbequina'. Values > 0.7 are highlighted.

	Total phenols	Oleuropein	Demethyloleuropein	Comsegoloside	Ligstroside	Verbascoside	Hydroxytyrosol-4-glucoside	Rutin	Luteolin-7-O-glucoside	Tyrosol-1-glucoside	Fruit fresh weight	Fruit dry weight	Oil content in dry weight	Fruit moisture
Oleuropein	0.93													
Demethyloleuropein	-0.20	-0.55												
Comseogoside	0.82	0.61	0.16											
Ligstroside	0.91	0.96	-0.51	0.61										
Verbascoside	0.81	0.65	0.00	0.83	0.69									
Hydroxytyrosol-4-glucoside	-0.28	-0.38	0.31	-0.07	-0.36	-0.10								
Rutin	0.70	0.48	0.20	0.79	0.50	0.74	0.00							
Luteolin-7-O-glucoside	0.49	0.31	0.18	0.62	0.34	0.51	-0.11	0.87						
Tyrosol-1-glucoside	0.83	0.70	-0.02	0.84	0.70	0.80	-0.17	0.63	0.43					
Fruit fresh weight	-0.39	-0.59	0.61	0.00	-0.54	-0.13	0.63	0.09	0.15	-0.27				
Fruit dry weight	-0.19	-0.42	0.56	0.25	-0.36	0.10	0.54	0.31	0.37	-0.02	0.86			
Oil content in dry weight	-0.50	-0.65	0.53	-0.15	-0.61	-0.25	0.33	-0.10	0.05	-0.26	0.63	0.75		
Fruit moisture	-0.59	-0.50	0.04	-0.65	-0.49	-0.59	0.18	-0.53	-0.47	-0.65	0.22	-0.23	-0.23	
Maturity index	-0.62	-0.63	0.07	-0.41	-0.57	-0.39	0.56	-0.36	-0.25	-0.54	0.59	0.35	0.38	0.39

advanced in Úbeda, although intermediate in the rest of the locations.

A separate analysis of variance was performed for the four irrigated locations to better test differences among the Mediterranean and Subtropical conditions of Andalusia and Tenerife regions (Table 5). Region was the main contributor to the total variance for total phenols, comselogoside, verbascoside, rutin and tyrosol-1-glucoside. This effect was mainly due to the higher values for those components in the two Andalusian locations (La Rambla and Úbeda), especially for the first two harvest dates (Figure 4). While oleuropein and lisgtroside showed higher variance for harvest date mainly due to the sharp decrease in their contents, especially between harvests 2 and 3.

The correlation between phenolic content components and with fruit traits were also studied (Table 6). Total phenolic content showed a high positive correlation with most individual phenolic components except for demethyloleu-

ropein, which increased throughout the harvest season, and hydroxytyrosol-4-glucoside and luteolin-7-O-glucoside which did not show a clear decrease during ripening. Among the phenolic components, the stronger correlation was found between oleuropein and ligstroside. Although comselogoside, verbascoside and rutin seemed to also be highly correlated. Besides, tyrosol-1-glucoside showed very high correlation coefficients with verbascoside and comselogoside. Both have a similar chemical structure in which the glucose is directly linked to the phenolic alcohol moiety, hydroxytyrosol and tyrosol, respectively. No high correlations were found between the total phenolic content or specific phenolic components and fruit traits, with most of them being negative, except for demethyloleuropein and tyrosol-1-glucoside. Ripening index showed low correlations with phenol content and composition and with the fruit traits evaluated. In particular, fruits having a high ripening index (more than 2) showed low phenol contents (less than 20,000 µg/g of fresh olive pulp); while fruits having a ripening index lower than 2 showed a very large range in variation in phenol contents (from 8,180 to 51,767 µg/g of fresh olive pulp).

4. DISCUSSION

The nine major phenolic components identified, in all locations and harvest dates, showed high variability (coefficient of variance higher than 50%, except for comselogoside and tyrosol-1-glucoside). Oleuropein was the main phenol as previously reported for olive fruits of other cultivars (Ben Ghorbal *et al.,* 2018; Valente *et al*., 2020).

The high variability found was mainly due to a location effect for total phenols and for most individual phenolic components. Significant the effect of location was previously reported for phenols in several cultivars. This is the case of 'Gemlik' in Turkey (Ben Ghorbal *et al.,* 2018), 'Arbequina', 'Manzanilla' and 'Arauco' in Argentina (Bodoira *et al.,* 2015) and Italian cultivars from a central region of Italy (Mousavi *et al.,* 2019).

Most variability associated with location was caused by the high values observed for Antequera and Baena compared to the other four locations. The most outstanding difference between Antequera and Baena and the rest of the locations is that olive trees are managed in dry farming, while, in Los Tomillos, El Viso, La Rambla and Úbeda, drip irrigation is used. The great influence of water availability on phenolic content and composition of olive fruits and virgin olive oils was previously reported when olive dry farming and irrigation were compared (Gómez-Rico *et al.,* 2006; Cirilli *et al.,* 2017). It seems that water stress periods caused a higher concentration of phenolic compounds in in the dry farming locations of Antequera and Baena, which remained during most of the maturation period. Water availability in the dry farming locations was especially low at the beginning of the oil accumulation period with respect to the four irrigated locations. Previous reports have suggested that water stress in this period has a strong influence on the phenol content (Gucci *et al*., 2019). This is maybe the reason why rainfall at the beginning of November in Antequera had little influence on phenol content. However, in some other works, a decrease in phenol content with higher stress has been reported (Valente *et al*., 2020). Probably, in the latter case, the combination of water and heat stress gave a different response of the olives.

When considering only the four irrigated locations, two in Andalusia and two in Tenerife, higher content for total phenols and some components was observed in Andalusia. These differences were especially important for the first harvest date. The higher summer temperatures in Andalusia with respect to Tenerife could produce higher stress which could be the cause of those differences.

Previously, the Sub-Tropical temperatures of Tenerife have shown a great influence on the flowering phenology of the olive tree (Medina-Alonso *et al*., 2020). This is important, since the Tenerife climatic conditions could help to predict the influence of climate change in the Mediterranean climate. In our case, it seems that the main factor associated with climate change that would impact phenol content is water availability more than changes in air temperature. However, the higher heat stress in summer, predicted in a climate change scenario, could also increase phenol content although have negative influence on other parameters such as oil content (Navas *et al*., 2019). More experimentation is needed to accurately determine the influence of climate change on phenol content and composition.

Harvest date was also showed to influence the variability in total phenols and phenolic components. As observed in our trials, most of the previous works reported a decrease in total phenolic content with maturation (Abaza *et al.,* 2017; Ferro *et al*., 2020), including 'Arbequina' in North-East Spain (Benito *et al.,* 2013). However, different patterns of variation were observed for individual phenolic components. In fact, the decrease in oleuropein content throughout the ripening process was concomitant with a parallel increase in demethyloleuropein, as previously reported (Gómez-Rico *et al.,* 2008). This was the only component with a significant increase with harvest date. Previous reports have also shown different variation patterns for the different phenolic components in fruit (Ben Ghorbal *et al.,* 2018; Talhaoui *et al.,* 2015; Ferro *et al*., 2020).

ANOVA analysis revealed the comparison of the relative influence of location and harvest date. For total phenols and some components such as comselogoside, tyrosol-1-glucoside, verbascoside and rutin, location had a much greater influence than harvest date. On the contrary, in the case of demethyloleuropein, harvest date

was more important. And for other components such as oleuropein and ligstroside, both factors were equally important. Therefore, the relative influence of harvest date and location very much depend on the phenolic component. No previous study on the comparative variance analysis has been performed for olive phenols, except for the case of 'Baladi' in Lebanon (El Riachy *et al.,* 2018), where a greater influence of location was observed with respect to harvest date for total phenol content determined spectrophotometrically using the Folin-Ciocalteu method. Different influences of location and genotype for the different phenolic components were previously reported in a set of breeding trials (Pérez *et al.,* 2018).

As expected, all phenolic components showing similar behavior across locations and harvest dates were highly correlated. The extremely high correlation found for oleuropein and ligstroside suggest a common biosynthetic pathway for both secoiridoid glucosides. While the negative correlation between oleuropein and demethyloleuropein support the hypothesis on the interconversion of oleuropein into demethyloleuropein (Obied *et al.,* 2008). On the contrary, demethyloleuropein, hydroxytyrosol-4-glucoside and luteolin-7-O-glucoside showed no correlation with the rest as having a different pattern of variation. Different correlations than the ones reported here were found in a previous set of breeding trials (Pérez *et al.,* 2018). This is probably due to the fact that the variability here is only due to environmental factors; while in the previous work, the phenol variability is attributable to different environments but also to different genotypes. The correlation of phenols with oil and moisture contents in the fruit was low and negative, which seems to indicate different metabolic pathways.

Ripening index seems to be also negatively correlated with phenolic content and most of the phenolic components. In fact, ripening index has been proposed as an indicator of fruit composition (Sánchez de Medina *et al.,* 2014). However, the correlation coefficients obtained in this work were not very high and varied greatly across locations. Overall, low phenolic content was found in fruits with high ripening index (2.0 to 4.0). However, at lower ripening indexes (0.8 to 2.0), both fruits with high and low phenolic contents were found. These results could explain some contradictory results reported in previous studies. Indeed, Gomez-Rico *et al.* (2008) and Morelló *et al.* (2004) showed both high correlation and lack of correlation of ripening index with phenolic content and composition in the 'Arbequina' cultivar. Therefore, a general relationship between fruit color and phenolic content should be taken cautiously. A strong association of phenol content with harvest time but not with ripening index could be explained by the fact that there is not a consistent pattern of variation in the ripening index with harvest time in the different locations considered.

5. CONCLUSIONS

In summary, water availability and harvest date seem to play a more important role than air temperature on the phenolic content and composition of 'Arbequina' fruits, even when very different climatic conditions such as Sub-Tropical and Mediterranean are compared. Therefore, in order to obtain olive fruits with high phenolic content, low water availability together with an early harvest should be considered. Taking into account that those two factors would also reduce the oil content in fruit. Among the phenol components studied, comsegoloside seems to be the one with the greatest variance among locations. Therefore, future studies might consider this phenol as a good marker for plant stresses, especially water availability. Further research is needed to determine the relative influence of water availability and harvest date on other cultivars with different phenolic profiles than the 'Arbequina' one here considered.

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