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Comparative Study of Virgin Olive Oil Quality from Single Varieties Cultivated in Chile and Spain

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Olive tree varieties that were cultivated only in the Mediterranean basin a few decades ago are now planted in the Southern Hemisphere as well. The chemical composition of the oils produced in countries as far distant as Spain and Chile are affected by differences in latitude and climate. In this work, seven monovarietal virgin olive oils from Chile (Arbequina, Barnea, Frantoio, Koroneiki, Leccino, Manzanilla and Picual) have been characterized by the chemical compounds responsible for taste (phenols) and aroma (volatiles). The oils were produced in five regions of Chile, and the concentration values of some chemical compounds were related to the geographical location of the olive tree orchards. Virgin olive oils from the major cultivars, Arbequina and Picual, were characterized in comparison with the same monovarietal oils produced in Spain. The concentration values of fourteen volatile compounds showed significant differences ($p < 0.05$) between the oils produced in Spain and Chile. Concerning the phenol composition, main differences were found on the secoiridoids derivatives of oleuropein and ligstroside, apigenin and luteolin.

KEYWORDS: Virgin olive oil; volatiles; phenols; *var.* Arbequina; *var.* Picual

INTRODUCTION

Only a few decades ago most of the olive tree orchards were located in the Mediterranean basin and its neighbor countries (e.g., Syria, Portugal). In a dynamically changing olive oil industry—where irrigation, intensive plantation and cultivar are keywords—new orchards are spread today all over the world, not only over the Northern Hemisphere but also in Southern countries such as Argentina, Australia, Chile and New Zealand.

These new producer countries, however, did not have any autochthonous olive trees, but they adapt cultivars imported from ancient producer countries, mostly from Spain, Greece, Italy and Israel. Hence virgin olive oils (VOOs) from autochthonous cultivars today compete, in a successful global market, with those produced in remote countries. This highly competitive global market leads the producers to focus attention on new methods of virgin olive oil authenticity and traceability for geographical identification.

It is well-known that the climate associated with different latitudes and altitudes, where the olive tree orchards are planted, affects the activity of olive enzymes and hence the VOO chemical composition. The olive trees that were brought to America by Spaniards were planted in geographical regions with latitude similar to that of the Mediterranean basin in both the Northern and Southern Hemispheres (Argentina, Chile, Mexico, Peru, Uruguay, and California, USA). On the other hand, it is also well-known that some chemical compounds of VOOs from olive tree orchards located in particular areas of the Southern Hemisphere do not meet IOC—International Olive Council—current trade standards (*1*). Furthermore, new plantations of non-autochthonous cultivars may produce VOOs with unexpected sensory quality even though the agronomic practices and harvesting are carried out under the optimal conditions.

Among the new American producer countries, Chile has an emergent olive oil industry with a promising prospective favored by the climatic conditions and available fields (*2*). Chile is characterized for its shape of strip land, flanked by the Pacific Ocean and The Andes, that is divided into 15 regions, and there are olive tree orchards in five of them (from IV to VII and Metropolitan) along over 700 km (from approximately 29° 57′ S to 35° 26′ S). Thus, Chile covers a wide variety of climates, from arid to Mediterranean, and it is a perfect experimental field to study how the climate affects virgin olive oil chemical composition. The Central Valley, from North to South, is placed between two mountain ranges, and many types of climates and soils can be found. The day temperatures usually reaches over 25 °C during summer and the freeze from the mountains cools orchards resulting in more than 15 °C of difference between day and night. With around 20,000 ha of olive crops, the olive oil industry in Chile is increasing, and it produces around 9 tons per year (*3*).

With respect to the olive cultivars that are currently planted in Chile, they are from several countries of the Mediterranean Basin, such as Greece (Koroneiki), Israel (Barnea), Italy (Frantoio, Leccino) and Spain (Arbequina, Manzanilla, Picual), although the three major varieties are Arbequina (49%), Frantoio (18%) and Picual (8%) (*3*).

As the production of olive oil on an industrial scale in Chile is relatively new, still the oils from Chile are not fully characterized from a chemical viewpoint in comparison with the same monovarietial VOOs produced within the Mediterranean area.

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Another main aspect to be studied is their sensory quality. Although the sensory characteristics of the aforementioned varieties have been extensively described, these descriptions are circumscribed to Mediterranean VOOs, and it is well-known that the chemical compounds responsible for flavor (volatile and phenolic compounds) vary as a function of temperature and latitude (*4*). In consequence, the same variety planted in different geographical zones might be qualified with uneven sensory notes.

This work analyzes the chemical compounds (phenols and volatiles) responsible for VOO sensory quality of the seven more cultivated single varieties (Arbequina, Barnea, Frantoio, Koroneiki, Leccino, Manzanilla, Picual) in Chile, which recently has joined the VOO producer countries with great success. Finally, the importance of Chilean VOOs from Arbequina and Picual cultivars are compared with the same varietal VOOs but produced in Spain; these VOOs are the most known (*var*. Arbequina is cultivated worldwide) and the most commercialized (*var*. Picual means more than 18% of World production) monovarietal oils.

MATERIALS AND METHODS

Plant Material. The Spanish samples were VOOs *var*. Arbequina (6) and Picual (7) produced in Southern Spain during the 2009 crop, and supplied by the producers. The Chilean samples were composed of 26 monovarietal VOOs produced during the 2007−2008 seasons in five regions: Metropolitan (2 samples), IV (5 samples), V (6 samples), VI (4 samples) and VII (9 samples). These samples included 7 cultivars: Arbequina (6), Barnea (3), Frantoio (4), Koroneiki (3), Leccino (3), Manzanilla (3) and Picual (4). Samples were supplied by local producers and ACESUR (Spain). All the orchards were superintensive plantations, olive trees were irrigated to reach an evotranspiration higher than 1.0, and the olives were harvested at a similar level of ripeness, fourth level according to Frías et al. (*5*). Olive oils were collected at the olive mills where olives were processed with two-phase centrifugation systems. The temperature and time of malaxation were within the ranges of 25−35 °C and 75−95 min respectively; values were determined by the foremen in accordance with the characteristics of olives and external climate (humidity and temperature) at any time.

Determination of Phenols. A standard solution (0.5 mL), made with *p*-hydroxyphenylacetic (0.12 mg/mL) and *o*-coumaric (0.01 mg/mL) acids in methanol, was added to a sample of filtered VOO (2.5 g). A rotary evaporator at 40 °C under vacuum was used to evaporate the solvent, and the oily residue was dissolved in 6 mL of hexane.

The diol-bonded phase cartridge was conditioned according to Mateos et al. (*6*). After the sample was loaded, the column was washed with 6 mL of hexane and 3 mL of hexane/ethyl acetate (90:10 v/v). The final residue was extracted with 10 mL of methanol and evaporated at 40 °C under vacuum, and the extract was diluted with 500 μL of methanol/water (1:1, v/v). A filtrated aliquot (20 μL) of the final colorless solution was injected onto the HPLC system (Hitachi LaChrom Elite with a diode array UV detector Hitachi L-2455). The column was a Lichrospher 100RP-18 column (4.0 mm inner diameter × 250 mm; 5 μm particle size) maintained at 30 °C. The gradient elution, at a flow rate of 1.0 mL/min, was achieved using the following mobile phases: a mixture of water/orthophosphoric acid (99.5:0.5, v/v) (solvent A) and methanol/acetonitrile (50:50, v/v) (solvent B). The change of the solvent gradient was programmed as follows: from 95% (A) and 5% (B) to 70% (A) and 30% (B) in 25 min, 62% (A) and 38% (B) in 10 min, 62% (A) and 38% (B) in 5 min, 55% (A) and 45% (B) in 5 min, 47.5% (A) and 52.5% (B) in 5 min, and 100% (B) in 5 min, followed by 5 min of maintenance. The chromatographic signals were obtained at 235, 280, and 335 nm.

The quantifications of flavones and ferulic acid were performed at 335 nm using *o*-coumaric acid as internal standard while the quantification of the rest of the phenols was carried out at 280 nm using *p*-hydroxyphenylacetic acid as internal standard. Their recovery and response factors were those ones described by Mateos et al. (*6*).

Concentration of Volatile Compounds. Olive oil samples (1 g) spiked with 2.6 mg/kg of 4-methyl-2-pentanol (internal standard) were placed in a 20 mL glass vial, tightly capped with polytetrafluoroethylene (PTFE) septum, and left for 10 min at 40 °C to allow for the equilibration of the volatiles in the headspace. After the equilibration time, the septum covering each vial was pierced with a solid-phase microextraction (SPME) needle and the fiber was exposed to the headspace for 40 min. When the process was completed, the fiber was inserted into the injector port of the GC. Temperature and time of the preconcentration step, carried out in a Combipal (CTC Analytics AG, Zwingen, Switzerland), were automatically controlled by the software Workstation version 5.5.2 (Varian, Walnut Creek, CA). The SPME fiber (2 cm length and 50/30 μm film thickness) was purchased from Supelco (Bellefonte PA), and it was endowed with the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fiber was previously conditioned following the instructions of the supplier.

Table 1. Volatile Compounds Identified and Quantified in Chilean VOOs[a]

volatile	concn	volatile	concn
heptane	10 ± 2	3-methyl-1-butanol	27 ± 3
octane	889 ± 219	(*E*)-2-hexenal	7355 ± 1381
methyl acetate	247 ± 122	3-octanone	271 ± 28
(*E*)-2-octene	7 ± 1	hexyl acetate	377 ± 221
ethyl acetate	75 ± 10	2-octanone	35 ± 4
2-methylbutanal	23 ± 7	(*Z*)-3-hexenyl acetate	316 ± 135
3-methylbutanal	21 ± 8	(*E*)-2-heptenal	586 ± 164
ethanol	2628 ± 741	2-heptenol	84 ± 14
ethyl propanoate[b]	30 ± 4	hexanol	1691 ± 351
3-pentanone	346 ± 76	(*E*)-3-hexenol	200 ± 33
pentanal	260 ± 76	(*Z*)-3-hexenol	978 ± 344
4-methyl-2-pentanone	59 ± 7	nonanal	1550 ± 1451
1-penten-3-one	160 ± 35	2,4-hexadienal	161 ± 34
2-butanol	39 ± 6	(*E*)-2-hexenol	2634 ± 884
propanol	6 ± 1	(*Z*)-2-hexenol	120 ± 27
butyl acetate	21 ± 3	(*E*)-2-octenal	403 ± 82
hexanal	1916 ± 391	acetic acid	787 ± 79
ethylbenzene	179 ± 50	propanoic acid	23 ± 3
(*E*)-2-pentenal	45 ± 7	butanoic acid	25 ± 3
(*Z*)-3-hexenal	174 ± 27	pentanoic acid	37 ± 5
butanol	741 ± 182	hexanoic acid	370 ± 37
1-penten-3-ol	425 ± 62	heptanoic acid	395 ± 41
2-methyl-1-butanol	50 ± 8	octanoic acid	730 ± 96

[a] Concentration values [mean ± SEM (standard error of the mean)] are given in μg/kg. [b] Quantified with a tentative recovery factor of 1.

Determination of Volatiles. The volatiles absorbed by the fiber were thermally desorbed in the hot injection port of a GC for 5 min at 260 °C with the purge valve off (splitless mode) and deposited onto a TR-WAX capillary column (60 m × 0.25 mm i.d., 0.25 μm coating; Teknokroma, Barcelona, Spain) of a Varian 3900 gas chromatograph with a flame ionization detector (FID). The carrier gas was hydrogen, at a flow rate of 1.5 mL/min. The oven temperature was held at 40 °C for 10 min and then programmed to rise 3 °C/min to a final temperature of 200 °C, where it was held for 10 min to eliminate the memory effect of the capillary column. The signal was recorded and processed with the WorkStation (version 5.5.2) software. Each sample was analyzed in duplicate.

The identification of the volatile compounds was first carried out by mass spectrometry and later checked with standards (*7*,*8*). The assessment of the aroma notes corresponding to olive oil volatile compounds was already carried out in previous works (*8*, *9*). **Table 1** shows the volatile compounds identified in Chilean VOOs together with their concentrations (mean ± SEM) once the chromatographic values have been corrected with their recovery factors.

Recovery Factors. Volatile compounds described in **Table 1** (Sigma-Aldrich, St. Louis, MO) were added to a fully deodorized olive oil in the range between 0.01 mg/kg and 3.0 mg/kg (0.01, 0.02, 0.03, 0.05, 0.1, 0.2, 0.3, 0.6, 1.0, 1.5, 3.0 mg/kg) although two response factors were calculated, for the ranges from 0.01 to 0.1 mg/kg and from 0.1 to 3.0 mg/kg. The absolute response factors of the volatiles of **Table 1** were calculated as the slopes of the linear regressions obtained from the ratio of total peak area as a function of concentration. Relative response factors were obtained as the ratio of the absolute response factor of each compound to that of the internal standard (4-methyl-2-pentanol). Repeatability of the internal standard (% RSD = 4.5%) was calculated in a previous work (*7*).

Table 2. Monovarietal VOOs Cultivated in Chile: Concentrations (Mean ± SEM[a]) of Volatile Compounds (μg/kg) Clustered inside the Most Remarkable Atom Carbons and Series

	Arbequina	Frantoio	Picual	Koroneiki	Barnea	Leccino	Manzanilla
C5	1580 ± 170	1340 ± 340	1130 ± 270	4400 ± 850	1240 ± 50	1480 ± 30	1550 ± 60
C6	22380 ± 2490	17080 ± 4230	5740 ± 2190	12730 ± 2770	11950 ± 300	6780 ± 580	14730 ± 1140
C6-L	5210 ± 1460	4250 ± 540	1280 ± 260	7500 ± 2360	2240 ± 320	2210 ± 210	1690 ± 90
C6-Ln	17170 ± 2110	12830 ± 4130	4450 ± 1980	5230 ± 1060	9710 ± 670	4570 ± 130	13040 ± 2500
C7	710 ± 170	370 ± 40	1160 ± 820	370 ± 120	410 ± 50	160 ± 30	830 ± 70
C8	790 ± 230	570 ± 70	560 ± 200	1040 ± 160	710 ± 90	260 ± 60	1260 ± 110
aldehydes	21750 ± 4680	11950 ± 3550	4170 ± 2090	3750 ± 860	3020 ± 30	2750 ± 40	3720 ± 40
ketones	650 ± 90	660 ± 100	410 ± 110	1340 ± 220	200 ± 20	200 ± 50	280 ± 40
alcohols	9810 ± 2900	9180 ± 6320	9250 ± 2790	5890 ± 730	15940 ± 4400	8170 ± 2090	17560 ± 580
esters	610 ± 290	550 ± 240	610 ± 250	7560 ± 690	370 ± 150	300 ± 100	2770 ± 900
acids	1670 ± 160	1850 ± 260	1920 ± 940	1610 ± 80	3050 ± 230	2410 ± 220	1310 ± 60
regions	RM, IV−VII	V−VII	IV−VII	RM, VI−VII	IV, V, VII	IV, VI, VII	V, VI, VII

[a] Standard error of the mean.

Sensory Assessment. The sensory evaluation of VOO samples was carried out in accordance with the official method for the olive oil sensory assessment (*10*). A total of 15 mL of each sample was kept in standardized glasses at 29 ± 2 °C for 15 min and then evaluated by five assessors. Assessors were free to qualify VOOs with their own sensory descriptors in addition to those described in the official method (*10*).

Statistical Analysis. The data were analyzed using the analysis of variance (ANOVA) and principal component analysis (PCA). For a univariate statistical analysis, data of individual phenols and volatiles were subjected to ANOVA to test for significant differences between the samples produced in Chile and Spain. The analysis of variance of factorial designs with repeated measures has been used. The Brown−Forsythe test ($p \leq 0.05$) for homogeneity of variances was used to determine the compounds that individually could characterize ($p < 0.05$) the samples from Chile and Spain. The statistical procedure selected for the multivariate analysis of the whole information was principal component analysis (PCA), which is an unsupervised tool oriented toward modeling the variance/covariance structure of the data matrix into a model that represents the significant variations and considers the noise as an error. Thus, PCA was used for interpreting the behavior of the profiles of VOO phenols and volatiles with respect to cultivar and geographical origin. A radar chart was also used to display the result of phenol analysis. Statistica 7.0 (StatSoft, Tulsa, OK) was the software for carrying out all of the statistical analyses.

RESULTS AND DISCUSSION

The quality characterization of the different monovarietal VOOs has been studied by quantifying their volatile and phenolic compounds. The qualitative and quantitative profiles of these compounds are very much related to cultivars and pedoclimatic characteristics, the latter being associated to a given geographical location. Volatile compounds have previously been used to characterize monovarietal VOOs by means of multivariate analysis. Thus, for example, differences in four European monovarietal VOOs have been characterized by using 55 volatile compounds (*11*), and ten C6 volatile compounds to characterize three maturity stages of olives at harvesting time (*12*). **Table 2** shows the characterization of seven monovarietal VOOs produced in Chile by the concentration of the volatiles clustered by the number of their carbon atoms (C5−C8), coming from linoleic and linolenic acids (C6-L and C6-Ln) through the lipoxygenase cascade (LOX) (*13*), and their series of compounds (aldehydes, ketones, alcohols, esters and acids). As all the bunches of olives were harvested at the fourth level of ripeness (*5*) and orchards were irrigated with similar value of crop evapotranspiration (ET_c)—oscillating between 1.3 and 1.4—the differences in concentration seem to be due to the cultivar in addition to possible influence of the climate.

The most remarkable aspect is the high concentration of volatile compounds coming from linoleic acid (C6-L) in comparison with those coming from linolenic acid (C6-Ln). Thus the ratio between these two groups of compounds is 1.43 for Koroneiki VOOs while this value was lower than 1 for all the other Chilean monovarietal VOOs. The C6 compounds coming from linoleic acid through LOX are hexanal, hexanol and hexyl acetate, which are characterized with green-fruity sensory notes (*12*) at the average concentration of VOOs. They contribute to VOO aroma as their concentrations (947 ± 193, 1745 ± 373 and 2470 ± 896 μg/kg, respectively) in *var*. Koroneiki are higher than their odor thresholds (80, 400, and 1040 μg/kg, respectively). Contrary to Koroneiki cultivar, Arbequina and Manzanilla VOOs have the highest concentration values of the compounds coming from linolenic acid, from which only (*Z*)-3-hexenyl acetate (240 ± 43 μg/kg), (*E*)-2-hexenal (11850 ± 1877 μg/kg), (*Z*)-3-hexenal (244 ± 44 μg/kg) and (*Z*)-3-hexenol (1204 ± 471 μg/kg) have concentrations higher than their odor thresholds (200, 420, 3, and 1100 μg/kg respectively). These compounds are responsible for bitter-green, leaf, tomato and grassy sensory perceptions (*8*,*12*) in those two monovarietal VOOs. The other volatile compounds from linolenic acid ((*E*)-3-hexenol, (*Z*)-2-hexenol and (*E*)-2-hexenol) have too high an odor threshold (1150, 1000, and 5000 μg/kg) in comparison with the concentration (248 ± 62, 150 ± 57 and 2827 ± 1190 μg/kg) and they do not contribute to the aroma of VOOs analyzed in this study.

Following with the concentrations of volatiles in terms of their number of carbon atoms, Koroneiki VOOs have the highest concentrations of C5, compounds that are associated with a sweet-green sensory perception (*13*). No significant difference concerning C7 compounds was observed, with the exception of VOOs from *var*. Leccino, which showed the lowest concentrations while VOOs *var*. Manzanilla show high values. On the other hand, the highest concentrations of C8 compounds corresponded to VOOs from Koroneiki and Manzanilla cultivars while the lowest were determined in *var*. Leccino. Some of these compounds are related to sensory defects as 2-octanone and 1-octen-3-one, which contribute to mold and mushroom odors (*9*). The low concentrations of individual C8 compounds in VOOs of *var*. Koroneiki and Manzanilla (2-octanone, 40 ± 12; 1-octen-3-one, traces; (*E*)-2-octene, 10 ± 1; 3-octanone, 246 ± 27; octane, 950 ± 236; all in μg/kg) with respect to their odor thresholds (510, 10, 100, 8000, and 940 μg/kg respectively) point out absence of sensory defects in VOOs and also that they were extracted under optimum conditions. On the contrary, (*E*)-2-octenal, whose concentration (400 ± 80 μg/kg) is much higher than its odor threshold (4 μg/kg), contributes to the herbaceous sensory perception.

Analyzing the monovarietal VOOs from the perspective of main series of volatile compounds, Koroneiki VOOs are characterized by the highest concentration of esters, which contribute to their fragrant odor and a sweet-fruity taste (*14*). The oils from

Table 3. Volatiles That Distinguish ($p < 0.05$) Spanish from Chilean VOOs, *var.* Arbequina and Picual[a]

volatile	Spain	Chile	*p*	odor descriptors	OT
		Arbequina			
(*E*)-2-octane	47 ± 12	7 ± 2	0.002	earthy, green leaf, fresh	8750
ethyl acetate	403 ± 124	61 ± 14	0.000	sweet, pleasant	940
4-methyl-2-pentanone	15 ± 5	85 ± 19	0.026	fruity, strawberry,	300
1-penten-3-one	25 ± 4	74 ± 33	0.000	pungent, mustard	1
ethylbenzene	8 ± 2	301 ± 137	0.000	glue	14000
1-penten-3-ol	94 ± 56	513 ± 163	0.049	butter, slight green aroma	400
3-methyl-1-butanol	1 ± 1	29 ± 11	0.003	undesirable, burnt	100
(*E*)-2-hexenal	1964 ± 1000	11396 ± 1973	0.001	almond, bitter, green	420
hexyl acetate	135 ± 50	415 ± 335	0.001	green, sweet, apple, fruity	1040
(*Z*)-3-hexenyl acetate	8671 ± 3343	242 ± 57	0.000	fruity, green leaves	200
hexanol	237 ± 187	1447 ± 561	0.046	banana, fruity	400
		Picual			
2-methyl-1-butanal	31 ± 7	4 ± 2	0.006	dry fruit, cheese	24
3-methyl-1-butanal	25 ± 6	5 ± 2	0.005	ripen fruit	80
ethylbenzene	9 ± 3	38 ± 15	0.008	glue	14000
1-penten-3-ol	88 ± 25	294 ± 119	0.013	butter, slight green aroma	400
3-methyl-1-butanol	4 ± 2	27 ± 11	0.001	undesirable, burnt	100
hexanol	41 ± 27	408 ± 231	0.000	banana, fruity	400
(*E*)-3-hexenol	38 ± 14	205 ± 81	0.001	green, bitter almond	1150

[a] Concentration [mean ± SEM (standard error of the mean)] and odor threshold (OT) values are given in μg/kg.

Barnea and Manzanilla cultivars are characterized by a high concentration of alcohols, such as hexanol, (*Z*)-3-hexenol, (*E*)-3-hexenol and (*E*)-2-hexenol, which are synthesized from the aldehydes *via* alcohol dehydrogenase (*15*). A high concentration of alcohols is not usually correlated with good sensory perceptions but with a harsh odor and, sometimes, with a strong retronasal astringent taste although the latter perception depends on the concentrations of some individual alcohols (*16*). Comparing the concentrations of these volatiles in these two monovarietal VOOs versus their odor thresholds (hexanol, 1797 ± 269 vs 400; (*Z*)-3-hexenol, 4312 ± 2186 vs 1100; (*E*)-3-hexenol, 380 ± 105 vs 1500; (*E*)-2-hexenol, 5054 ± 518 vs 5000; all in μg/kg) at least two of those compounds contribute to a harsh bitter-green sensory perception, which was checked later when oils were tasted by the panelists.

Table 2 also shows that VOOs from *var.* Arbequina and Frantoio are characterized by the highest concentration of aldehydes, which means that their aroma is reminiscent of cut green lawn with an appreciated slight bitter taste (*12*, *13*). The description of green and bitter oils for Arbequina VOOs disagrees with the sensory attributes that usually qualified the oils from this cultivar in the past. In studies carried out only a decade ago, VOOs from this cultivar were characterized with ripe fruity aroma, slight oily odor, sweet taste and low levels of astringency and bitterness (*17*). However, as a result of the consumer demand for greener and bitterer oils, Arbequina oils are obtained today from olives in earlier ripeness stages and, in consequence, their sensory descriptors have turned into cut green lawn aroma, tomato odor and medium bitter taste (*18*).

The next classification criterion in the study was the latitude of the orchards regardless all the cultivars. The results from the Brown−Forsythe test showed that VOOs from orchards located in the VII region are distinguished from orchards situated in more arid regions (IV and V region) by three compounds: 2-methyl-1-butanol (30 ± 12 vs 53 ± 7 μg/kg; odor threshold, 480 μg/kg), ethylbenzene (79±18 vs 193±77 μg/kg; odor threshold, 14000 μg/kg) and pentanal (509 ± 120 vs 45 ± 27 μg/kg; odor threshold, 240 μg/kg). These differences mean that pentanal, characterized by a wood odor and an almond flavor, can contribute with these sensory descriptors to the sensory quality of VOOs from the VII region as its odor threshold is much lower than its mean concentration in the oils of this region. Differences among VOOs produced in regions with very different climate (semi arid vs Mediterranean) are smaller than expected (*8*) due to the compensatory effect of the high level of irrigation of the orchards, which harmonizes the volatile composition of the oils (*18*).

In a further step, the study was focused on the geographical characterization of Chilean VOOs though only with Arbequina and Picual cultivars (6 and 4 samples respectively). Those VOOs were clustered into two great groups. One of the groups was constituted by the oils produced inlands (3 + 2 samples), while the other group was made up of oils from zones near the coast (2 + 2 samples). The Brown−Forsythe test showed that three compounds—ethyl acetate, 2-octanone and (*Z*)-2-hexenol—were affected by differences in the environment. The concentrations of two of them—ethyl acetate and 2-octanone—are higher in VOOs from inland orchards (122 ± 26 vs 46 ± 6 and 44 ± 6 vs 28 ± 6 μg/kg respectively), whichever the cultivar, although they do not contribute to aroma because of their high odor threshold (940 and 510 μg/kg respectively). On the other hand, the highest concentration of (*Z*)-2-hexenol (179 ± 54 vs 86 ± 17 μg/kg) was determined in VOOs from olive tree orchards nearer the coast (i.e., Los Vilos and La Ligua), although these concentrations were lower than the odor threshold for this compound (1 mg/kg).

Once the differences between monovarietal VOOs produced in Chile had been analyzed, a study focused on the comparison of Arbequina and Picual VOOs produced in Chile and Spain was carried out in terms of their volatile profiles. **Table 3** shows the concentrations of volatiles selected by applying the Brown−Forsythe algorithm. Although most of these compounds have odor thresholds much higher than their concentrations in the samples, and hence they do not contribute to VOO aroma, we can deduce that the Chilean VOOs *var.* Arbequina are qualified by higher intensity of sensory descriptors as butter, almond, bitter, banana and green aroma, while VOOs *var.* Picual cultivated in Chile are not distinguished from VOOs cultivated in Spain except for a slightly higher banana perception in American oils. Besides the sensory relevance of the selected compounds, they still can be

used as markers for chemical traceability. Approximately the number of volatile compounds selected for distinguishing Spanish and Chilean oils was double for Arbequina in comparison with Picual VOOs (**Table 3**). The compounds ethylbenzene, 1-penten-3-ol, 3-methyl-1-butanol and hexanol were selected for characterizing both varietal oils. The concentrations of many of the selected compounds were higher in Chilean VOOs (e.g., the concentration of ethylbenzene is 37 times higher than that in Spanish oils) though the concentrations of (*E*)-2-octane, ethyl acetate, (*Z*)-3-hexenyl acetate, 2-methyl-1-butanal and 3-methyl-1-butanal are lower in Chilean VOOs.

A PCA carried out with 43 volatiles (**Figure 1**) showed that the highest similarities in the aroma of Chilean and Spanish VOOs were found for Picual cultivar, while Arbequina oils produced in the two countries were clearly different. Thus, factor 1, with 37% of explained variance, allowed for the classification of Arbequina oils by country with no misclassified samples.

With respect to the concentrations of the chemical compounds responsible for taste, the phenols (**Table 4**) are also largely dependent on olive tree variety (*19*). Other factors, like the olive ripeness (*20*) and the irrigation regimes of the orchards (*18*, *21*), have strong influence on the individual and total concentrations of phenols as well. Esti et al. (*19*) characterized olive maturity stages by the increase of hydroxytyrosol although its concentration levels vary from one to another varietal VOO. This fact explains differences found in its concentration among the monovarietal VOOs whose olives were harvested at similar stages of ripeness. **Table 4** shows that Arbequina, Manzanilla and Leccino VOOs were characterized by lower concentrations of hydroxytyrosol. Furthermore, also the concentration of tyrosol was significantly low in Arbequina oils. Arbequina and Barnea VOOs might be distinguished from the rest of varieties by their concentration of elenoic acid, a compound that has already been suggested as marker of varietal VOOs (*19*).

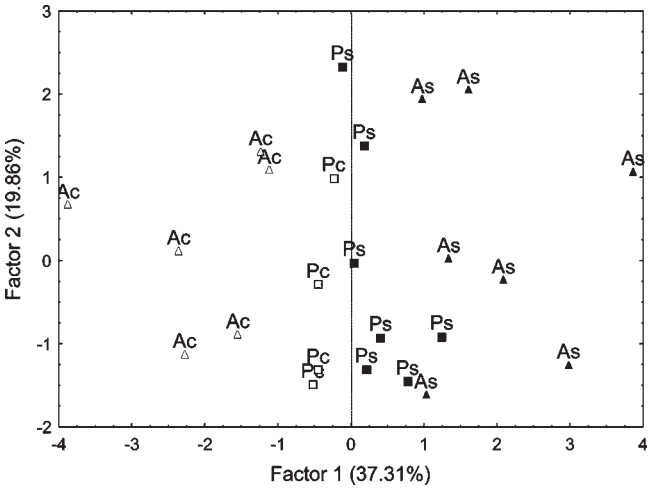

Figure 1. Principal component analysis carried out with 43 volatile compounds quantified in VOOs *var.* Arbequina (A) and Picual (P) from Chile (c) and Spain (s).

Analyzing the concentration of other individual phenols described in **Table 4**, we can find that the concentration of *p*-HPEA-EDA—a compound correlated with pungent sensory perception—is higher in Frantoio—followed by Picual, Arbequina and Koroneiki—VOOs while the highest concentration of 3,4-DHPEA-EDA, related to the combined sensory perceptions of bitterness and pungency (*22*), was quantified in Arbequina VOOs and the lowest in Koroneiki oils.

With respect to lignans (pinoresinol and 1-acetoxypinoresinol), their concentrations are not different enough among cultivars though the highest concentrations of 1-acetoxypinoresinol were quantified in Frantoio VOOs and pinoresinol in Barnea oils. Finally, concerning the flavonoids, the concentrations of apigenin are similar among the cultivars while the highest concentrations of luteolin were quantified in Arbequina VOOs and the lowest in Koroneiki oils.

Focused on the two most remarkable cultivars (Picual and Arbequina), **Table 5** shows the average concentration of individual and total phenols in three Spanish VOOs of each one of these cultivars. The main differences are not only in the concentration of the total phenols (slightly higher in Picual VOOs) but also in some individual phenols. The differences in individual phenols are more noticeable in Arbequina VOOs whose secoiridoid derivatives of oleuropein and ligstroside (3,4-DHPEA-EDA, *p*-HPEA-EDA, 3,4-DHPEA-EA and *p*-HPEA-EA) are at higher concentrations in Spanish VOOs, while the concentrations of apigenin, luteolin and elenoic acid seem to be lower in Spanish Arbequina VOOs. The differences among olive oils from *var.* Picual from diverse hemispheres are focused on the latter compounds whose concentrations of luteolin and apigenin seem to be higher in Spanish oils though the Spanish oils have lower concentrations in 3,4-DHPEA-EA and *p*-HPEA-EA.

Table 4. Concentration (Mean ± SEM[a]) of Individual Phenols (mg/kg) Quantified in the Main Chilean Monovarietal VOOs

	Arbequina	Frantoio	Picual	Koroneiki	Barnea	Leccino	Manzanilla
hydroxytyrosol	3.44 ± 0.88	7.45 ± 4.34	6.00 ± 0.59	7.05 ± 0.57	6.54 ± 0.62	5.35 ± 0.46	4.18 ± 0.46
tyrosol	2.34 ± 0.44	8.19 ± 3.14	6.77 ± 1.51	4.55 ± 0.38	7.40 ± 0.82	4.69 ± 0.39	11.62 ± 2.17
vanillic acid	0.28 ± 0.05	tr	0.37 ± 0.07	tr	tr	0.56 ± 0.42	0.40 ± 0.17
ferulic acid	1.22 ± 0.16	0.65 ± 0.24	3.61 ± 1.67	tr	2.35 ± 0.98	0.03 ± 0.00	4.83 ± 0.42
hydroxytyrosol acetate	9.52 ± 1.58	1.74 ± 0.87	1.40 ± 1.10	1.33 ± 0.67	tr	0.44 ± 0.02	0.08 ± 0.00
3,4-DHPEA-EDA	56.67 ± 11.78	38.41 ± 12.46	36.76 ± 7.63	14.70 ± 6.17	35.42 ± 7.12	38.93 ± 11.00	34.25 ± 9.12
p-HPEA-EDA	18.26 ± 4.08	41.41 ± 9.85	24.77 ± 0.87	16.31 ± 2.45	14.11 ± 3.11	10.91 ± 0.89	12.77 ± 0.96
pinoresinol	3.02 ± 0.14	3.07 ± 0.64	2.86 ± 0.75	2.58 ± 0.98	5.59 ± 1.96	3.42 ± 0.79	2.99 ± 0.46
1-acetoxypinoresinol	27.46 ± 1.74	36.23 ± 6.21	21.73 ± 11.98	21.47 ± 6.87	20.20 ± 6.12	28.39 ± 5.14	30.82 ± 2.19
3,4-DHPEA-EA	25.35 ± 6.62	56.19 ± 12.84	93.10 ± 18.81	57.93 ± 9.12	52.16 ± 8.76	43.57 ± 6.13	45.86 ± 5.27
p-HPEA-EA	15.35 ± 1.56	17.48 ± 8.09	33.45 ± 9.23	22.49 ± 4.12	29.67 ± 4.59	29.51 ± 5.28	24.36 ± 4.17
elenoic acid	133.60 ± 19.70	176.83 ± 11.44	162.49 ± 9.55	164.66 ± 39.21	129.35 ± 9.90	182.59 ± 45.25	199.93 ± 44.72
luteolin	10.14 ± 1.46	6.51 ± 1.02	5.73 ± 2.23	3.09 ± 0.61	4.51 ± 0.44	5.72 ± 0.52	6.27 ± 0.32
apigenin	3.34 ± 0.45	2.10 ± 0.29	2.99 ± 1.78	2.31 ± 1.61	2.48 ± 1.10	3.20 ± 0.21	3.70 ± 0.30
total phenols	309.98 ± 36.12	386.78 ± 26.50	402.03 ± 36.59	318.47 ± 13.81	309.78 ± 17.84	357.31 ± 19.37	382.06 ± 20.75
regions	RM, IV−VII	V−VII	IV−VII	RM, VI−VII	IV, V, VII	IV, VI, VII	V, VI, VII

[a] Standard error of the mean.

Table 5. Concentration (Mean ± SEM[a]) of Individual Phenols (mg/kg) Quantified in Spanish VOOs *var.* Arbequina and Picual

phenol	Arbequina	Picual	phenol	Arbequina	Picual
hydroxytyrosol	3.04 ± 0.77	6.58 ± 0.59	pinoresinol	2.71 ± 0.10	2.11 ± 0.65
tyrosol	2.85 ± 0.96	8.28 ± 1.51	1-acetoxypinoresinol	34.41 ± 3.40	33.70 ± 11.98
vanillic acid	0.00	0.73 ± 0.31	3,4-DHPEA-EA	79.48 ± 61.30	74.29 ± 15.01
ferulic acid	1.68 ± 0.50	5.28 ± 1.67	*p*-HPEA-EA	18.68 ± 13.32	33.45 ± 6.68
hydroxytyrosol acetate	7.75 ± 5.58	2.80 ± 1.23	elenoic acid	94.23 ± 31.64	154.49 ± 3.35
3,4-DHPEA-EDA	96.16 ± 48.74	29.13 ± 6.04	luteolin	2.77 ± 0.82	7.96 ± 2.23
p-HPEA-EDA	28.26 ± 9.23	23.89 ± 0.87	apigenin	1.06 ± 0.07	4.76 ± 1.78
DH oleuropein aglycon	4.69 ± 0.05	5.36 ± 2.07	total phenols	363.07 ± 40.70	392.85 ± 15.23

[a] Standard error of the mean.

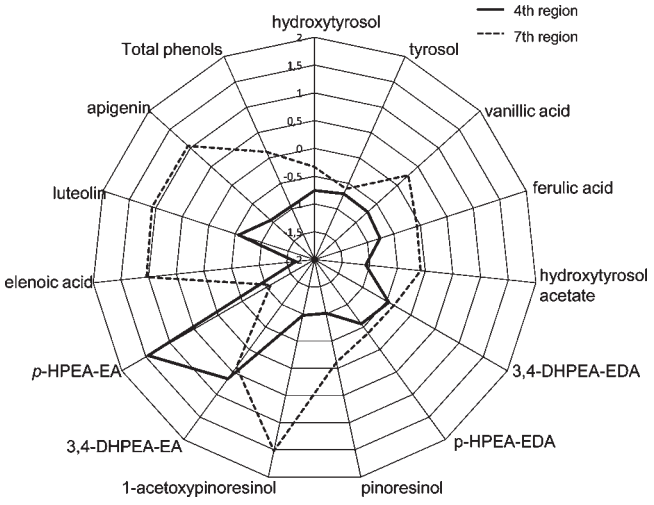

Figure 2. Radar plot of the average concentrations of individual phenols quantified in Chilean *var.* Arbequina VOOs from the IV and VII regions.

Concerning the relationship between the concentration of phenols and the latitude of the Chilean orchards, the study was carried out with *var*. Arbequina only since it is the most spread in the Chilean agricultural regions. **Figure 2** displays the results in a radar chart of individual phenols (plus total phenols) whose concentrations were previously normalized (column standardization) as they oscillate between ppb and ppm. Although the climate varies from semiarid (IV region, rainfall 78.5 mm per year) to Mediterranean (VII region, rainfall 701.9 mm) (750 km distance among regions), great differences in the concentrations of individual phenols were not detected due to the irrigation of the orchards to compensate evotranspiration. Thus, differences concern higher concentrations of 1-acetoxypinoresinol, luteolin, apigenin and elenoic acid in VOOs from VII region while the oils from the most arid region (IV region) are characterized by higher concentration of *p*-HPEA-EA, which is a good predictor of bitterness and pungency (*22*).

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