Research Article

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Influence of agroclimatic parameters on phenolic and volatile compounds of Chilean virgin olive oils and characterization based on geographical origin, cultivar and ripening stage

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Abstract

BACKGROUND: This study involved two commercial orchards located in Limarí Valley and Molina from two important Chilean production zones of extra virgin olive oil (EVOO). The investigation evaluated the effects of climate, soil composition, agricultural practices (fertilization and irrigation) and variety (considering two harvests) on the compounds responsible for the flavor of EVOO (volatiles and phenols) and how these compounds can explain the differences in chemical profiles by geographical origin, cultivar and fruit ripeness stage.

RESULTS: Varieties from the Limarí Valley presented the highest content of phenolic compounds. A significant relationship ($P < 0.05$) between volatile compounds and climate indicated that the compounds produced via the lipoxygenase cascade were affected by the maximum temperature and, to a lesser extent, by evapo-transpiration and irrigation. The selection of different individual phenolic and volatile compounds independently allowed the significant differentiation of EVOOs, principally by geographical origin, crop season, fruit ripeness stage and, in a few cases, by cultivar.

CONCLUSION: Soil and climate of the Chilean regions have much more influence than cultivars on the concentration of sensory quality compounds. Difference in latitude between orchards increases the importance of the geographical origin on the virgin olive oil chemical composition while full irrigation decreases the impact of the cultivar.

Keywords: *Olea europaea* L; virgin olive oil; phenols and volatiles; climate; soil composition; principal component analysis

INTRODUCTION

The production of olive oil is slowly moving beyond Mediterranean countries, and olive trees (*Olea europaea* L.) are being planted in countries such as Chile and New Zealand. This expansion, which is primarily due to new agricultural practices devised by farmers to increase olive oil yield per hectare without a loss of sensory and nutritional properties, is based on the adaptation of cultivars to climates associated with latitudes and altitudes different from their autochthonous regions. For example, environments may affect the activity of olive enzymes and, subsequently, the chemical composition of extra-virgin olive oil (EVOO). Reports of variation in the levels of C_6 aldehyde and alcohol volatile compounds for oil samples from different regions implies that environmental growth conditions may influence the activity of the enzyme alcohol dehydrogenase.[1,2]

It is known that the composition and quality of EVOO is affected by factors such as cultivar, fruit ripeness and agroclimatic conditions.[3] Tura *et al.*[4] recently showed that the flavor profile of oils is mainly affected by the geographical provenance, and by cultivar and ripening stage to a lesser extent.

The agronomic practice of fertilization is of particular interest in olive production because fertilization programs of both macro- and micro-nutrients influence the sources of assimilation for oil

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formation in olive trees.[5] Some of these nutrients are involved in photosynthesis, nitrogen fixation, respiration, changes in the oil content[6] and the profile of phenols.[7] In fact, some studies have reported that foliar fertilization can modify the quality and chemical composition of olives[8] and influence the quality of virgin olive oil (VOO).[9] Soil elements also contribute to changes in the concentration of phenols[10] and volatiles.[11]

To our knowledge, there have been no multidisciplinary studies evaluating the agroclimatic parameters and the chemical compounds responsible for the sensory properties of EVOO from various olive tree varieties and orchards with different climates and agricultural practices.

Chile, which presents a wide variety of climates, is a perfect place as experimental field to investigate the effects of climate on the quality of different EVOO cultivars that are native to Spain.[12] Uneven sensory notes have explained the differences between transnational EVOOs, primarily because the chemical compounds responsible for flavor (volatiles and phenols) vary as a function of temperature and latitude.[11] However, a multivariate investigation of the influences of climate, soil composition, agronomic practices, variety and ripening stage on the objective quality parameters of EVOOs from different geographical regions in Chile have not been reported, despite the reputed quality of Chilean olive oils and their increasing presence in the international market.

This study evaluated the effects of climate, soil composition, agricultural practices (fertilization and irrigation) and variety (considering two harvests) on the compounds responsible for the flavor of EVOO (volatiles and phenols) and how these compounds can explain the differences in chemical profiles by geographical origin, cultivar and fruit ripeness stage.

Most olive tree varieties are traditionally cultivated under rain-fed conditions in Europe due to their drought tolerance; however, in Chile, they are fully irrigated, which influences the transpiration efficiency of the leaves and subsequently affects photosynthesis and EVOO chemical composition. Thus, the climatic factors (e.g. maximum temperature, accumulated degree-days and humidity) that influence biological functions, such as leaf evapo-transpiration (ET_o), were evaluated in this study. The individual concentrations of the compounds were used to explain differences between cultivars and their geographical origins.

MATERIALS AND METHODS

Reagents

All reagents were analytical or HPLC grade (Merck, Darmstadt, Germany). Volatile standards [hexanal, hexan-1-ol, hexyl acetate, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-3-hexen-1-ol, 1-penten-3-ol, pentan-3-one, pentanal, 1-penten-3-one, ethyl acetate, 4-methyl-pentan-2-one, ethanol, 2-methyl-butan-1-ol, heptane, and 4-methyl-2-pentanol (internal standard)] were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The phenol standards [3-hydroxytyrosol, 2-(4-hydroxyphenyl) ethanol (tyrosol), *p*-coumaric acid, vanillic acid, vanillin, luteolin, apigenin, pinoresinol, *p*-hydroxyphenylacetic (internal standard 1), *o*-coumaric acid (internal standard 2) and oleuropein] were also obtained from Sigma–Aldrich. All standards had a purity of 98% or higher.

Orchard characteristics and agricultural aspects

This study involved two commercial orchards located in two Chilean production areas. The Limarí Valley production area (Coquimbo, IV region; latitude 30° 30′ S, longitude 71° 29′ W) is characterized by a Marino Subtropical Desert climate with a mean annual rainfall of 22 mm. The second production area, Molina (El Maule, VII region; latitude 35° 07′ S, longitude 71° 16′ W), is characterized by a Mediterranean climate with a mean annual rainfall of 735 mm (June is the month with the most rainfall). The super-high density (SHD) hedgerow olive orchards consisted of juvenile olive trees (6 years old in 2011) at a spacing of 5×1.5 m (1333 trees ha^{-1}) in a north–south orientation. Weeds under the trees were controlled with non-residual herbicides. Fertigation was applied by drip irrigation in the weeks prior to ripening. The commercially managed orchards of the Limarí Valley and Molina had mean irrigations of 4943 and 6323 $m^3\ ha^{-1}$, respectively, in the 2011–2012 season and 3728 and 4628 $m^3\ ha^{-1}$, respectively, in the 2012–2013 season. The orchards in the Limarí Valley and Molina were fertilized with 98, 20, 80 and 128, 92, 324 units (kg $ha^{-1}\ year^{-1}$) of N, P_2O_5 and K_2O, respectively, in the 2011–2012 season and 70, 25, 65 and 58, 22, 145 units of N, P_2O_5 and K_2O, respectively, in the 2012–2013 season. In order to know the real situation of orchard management and how this affects oil quality, the fertilization and the irrigation were managed by the farmers; only the effects of these variables were studied and correlated with chemical composition.

Automatic weather stations near the orchards recorded the temperature, rainfall and humidity that were used to determine the potential evapo-transpiration (ET_o).[13]

Soil characterization was conducted by collecting samples from pits at different depths (20, 40 and 60 cm) near the olive trees of each cultivar. Samples were collected in June 2012 and 2013 (winter season). Each sample was characterized by the variables described in Table 1. The methodologies for determining the variables were based on the methods of analysis suggested for Chilean soils[14] and the standard methods for examining water and wastewater.[15]

Plant material

In both orchards, three random rows of trees (approx. 100 per row) per cultivar (Arbequina, Arbosana and Koroneiki) were selected. Trees of similar vigor were selected according to age, present and historical production, soil type and agronomicmanagement. The study spanned two harvest seasons; the orchards were harvested on five dates between April 23 and June 12 in the 2011–2012 season and between March 22 and June 07 in 2012–2013 season, with levels of ripeness between 2 and 6.[16] At each harvest, 10 kg of olives (experimental unit) were handpicked from the middle portion of three trees randomly selected; the olives were mixed prior to extracting the oil. Each ripening stage was harvested in triplicate. Only healthy fruits, without any sign of infection or physical damage, were used. In total, 90 samples of EVOO were processed in each season.

Olive oil extraction

Olive oils were collected at olive mills where olives were processed using the Frantoino model Monoblock extraction equipment (Toscana Enologica Mori, Firenze, Italy) with a two-phase centrifugation system. The fresh olives (10 kg) were crushed and then slowly mixed for 30 min at 26 ± 2 °C. The resulting paste was centrifuged at $1027 \times g$ for 5 min to separate the oil. All samples were subsequently filtered through hydrophilic cotton, placed in amber glass bottles and stored in the dark at 4 °C until analysis (within 1 month).

Table 1. Values of climate and soil physical–chemical parameters determined in the Limarí Valley and Molina orchards

Variable	Limarí Valley		Molina	
	2011–2012	2012–2013	2011–2012	2012–2013
Climate				
Maximum temperature (°C)	20.72 ± 2.45	20.80 ± 3.37	16.00 ± 2.42	14.12 ± 4.69
Minimum temperature (°C)	8.42 ± 2.82	7.92 ± 2.16	5.90 ± 4.34	2.29 ± 3.79
Amplitude temperature (°C)	12.30 ± 4.43	12.88 ± 4.74	10.10 ± 4.99	11.83 ± 4.89
Humidity (%)	74.04 ± 5.30	76.44 ± 4.83	90.82 ± 4.25	87.65 ± 6.84
Evapo-transpiration (ET_o) (mm)	3.41 ± 0.38	2.81 ± 0.35	0.95 ± 0.32	1.11 ± 3.05
Agricultural practices				
Irrigation (m^3 ha^{-1})	4943.00 ± 183.14	3727.00 ± 76.60	6313.87 ± 26.04	4613.8 ± 72.43
Soil fertility				
pH	7.37 ± 0.71	6.74 ± 0.96	6.26 ± 0.17	6.33 ± 0.53
EC (dS m^{-1})	15.11 ± 6.42	11.50 ± 3.27	0.46 ± 0.14	0.37 ± 0.09
Organic material (%)	0.80 ± 0.38	1.33 ± 0.50	3.09 ± 1.78	6.23 ± 0.84
Total N (%)	0.04 ± 0.02	0.03 ± 0.02	0.13 ± 0.12	0.19 ± 0.05
Availability of N, P and K (mg kg^{-1})				
N	27.31 ± 10.89	26.70 ± 1.49	52.68 ± 9.92	39.91 ± 8.65
P	9.62 ± 6.53	21.31 ± 6.05	7.25 ± 2.18	9.17 ± 3.69
K	138.08 ± 46.93	150.87 ± 44.39	161.63 ± 73.58	203.38 ± 34.84
Cation exchange (meq 100 g^{-1})				
Ca	8.46 ± 2.75	6.78 ± 2.17	5.30 ± 2.16	5.51 ± 2.06
Mg	5.27 ± 2.47	4.22 ± 0.37	0.78 ± 0.34	0.76 ± 0.16
K	0.32 ± 0.11	0.37 ± 0.12	0.41 ± 0.19	0.54 ± 0.08
Na	4.09 ± 1.11	2.90 ± 0.49	0.23 ± 0.05	0.19 ± 0.04
Sum of bases*	18.47 ± 5.87	13.33 ± 2.40	6.67 ± 2.61	6.99 ± 1.07
CEC†	17.79 ± 5.77	14.93 ± 3.92	11.00 ± 2.64	15.16 ± 2.22
Availability of nutrients (mg kg^{-1})				
Fe	9.24 ± 3.77	16.91 ± 3.21	40.17 ± 11.66	31.76 ± 10.42
Mg	8.97 ± 6.70	21.48 ± 9.79	10.20 ± 4.85	10.19 ± 5.19
Zn	0.70 ± 0.46	1.51 ± 0.87	0.66 ± 0.72	0.86 ± 0.29
Cu	2.67 ± 0.63	3.29 ± 0.64	1.04 ± 0.74	0.99 ± 0.30
B	2.94 ± 1.18	1.95 ± 0.77	0.76 ± 0.42	2.46 ± 0.72

Values are mean ± SD ($n = 9$).
*Sum of bases: (Ca + Mg + K + Na).
†CEC, cation exchange capacity.

The samples were analyzed in triplicate using the chemical analytical methods described below. All of the Chilean olive oils were extra virgin according to official analytical methods and limits (free acidity ≤ 0.8% in oleic acid, K232 ≤ 2.50, K270 ≤ 0.22, ΔK ≤ 0.01).[17]

Determination of phenolic compounds

A standard solution (0.5 mL) of *p*-hydroxyphenylacetic (0.12 mg m L^{-1}) and *o*-coumaric (0.01 mg mL^{-1}) acids in methanol was added to EVOO (2.5 g). The phenolic compounds were isolated by solid-phase extraction and analyzed by reverse-phase high-performance liquid chromatography (HPLC)[18] using a Waters HPLC (Milford, MA, USA) system equipped with a binary pump (model 1525), a diode array UV detector (model 2998), an autosampler (model 2707) and a Waters Spherisorb ODS RP-18 column (4.6 mm i.d. × 250 mm; 5 µm particle size). Phenols were identified using Sigma standards and an HPLC-MS Agilent 1100 (Agilent Technologies Inc., Santa Clara, CA, USA) system coupled to a mass spectrometer electrospray Esquire 4000 ion trap ESI-IT (Bruker Daltonik GmbH, Bremen, Germany) and by comparing absorbance spectra to those in the literature.[18,19] The quantification of phenolic compounds other than flavones and ferulic acid was carried out at 280 nm using *p*-hydroxyphenylacetic acid as an internal standard, while flavones (luteolin and apigenin) and ferulic acid were quantified at 335 nm using *o*-coumaric acid as an internal standard. The recovery and response factors were obtained from a previous study.[18] The results were expressed in mg kg^{-1}.

Concentration of volatile compounds

The EVOO samples (1 g), spiked with 4-methyl-2-pentanol (2.6 mg kg^{-1} internal standard), were placed in a 20 mL glass vial, tightly capped with a polytetrafluoroethylene (PTFE) septum, and held 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. The temperature and time of the pre-concentration step, performed in an HT280T (HTA s.r.l, Brescia, Italy), were automatically controlled by the software HT-COMSOFT (HTA s.r.l). The SPME fiber (2 cm length and 50/30 µm film thickness) was from Supelco (Bellefonte, PA,

USA) and consisted of a stable flex stationary phase divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), selected for its sensitivity, repeatability and linearity of response,[20] and conditioned following the instructions given by the supplier.

Determination of volatile compounds

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 were then injected onto a TR-WAX capillary column (60 m × 0.25 mm i.d., 0.25 µm coating; Teknokroma, Barcelona, Spain) of a Shimadzu GC-2010 Plus gas chromatograph with a flame ionization detector (FID) (Shimadzu, Kyoto, Japan). The carrier gas was hydrogen with a flow rate of 1.5 mL min^{-1}. The oven temperature was held at 40 °C for 10 min and then programmed to increase by 3 °C min^{-1} to a final temperature of 200 °C, where it was held for 10 min. The data were recorded and processed with GC solution Ver. 2 Workstation Software (Shimadzu). Each sample was analyzed in triplicate.

The identification of the volatile compounds was performed by mass spectrometry and was subsequently verified with standards.[12]

Statistical analysis

Initially, the Duncan multiple comparison and Kruskal–Wallis tests were performed to detect significant differences between samples.[21] All datasets were processed using Statgraphics Centurion 16 (Statpoint Inc., Warrenton, VA, USA). The initial analyses were followed by the multivariate unsupervised procedure of principal component analysis (PCA)[22] on selected variables to provide a basic explanation of the phenolic and volatile compounds with the information of climate and soils. All of these analyses were processed considering $\alpha = 0.05$, using SIMCA-P+ software (version 12; Umetrics AB, Umeå, Sweden).

A step-wise linear discriminant analysis (*F*-to enter = 5)[23] was used to determinate the influence of olive maturity on the composition of phenolic and volatile compounds in EVOOs using Statistica release 6.0 software (StatSoft, Tulsa, OK, USA).

Finally, canonical correlation was also used to have a basic explanation of the variance of volatile compounds and phenols with the information of climate and soils. Variables of canonical correlation were obtained from the principal components of PCAs applied to datasets of volatiles, phenols and soils and climate independently.

RESULTS AND DISCUSSION

Table 1 shows the values of climate and soil physical–chemical parameters determined for the Limarí Valley and Molina orchards. The humidity was higher in Molina than in the Limarí Valley during harvest time; however, the maximum temperature was higher in the latter, which explains the higher evapo-transpiration (three-fold) in the leaves of Limarí Valley olive trees. Due to the high amount of water added by irrigation, which was 30% higher in Molina, the average annual rainfall data were not of interest in this study.

The analysis of the orchard soils showed that the sum of the bases (Ca + Mg + K + Na) was doubled in the Limarí Valley samples compared with the Molina samples, regardless of cultivar. This indicates that the Limarí Valley soil contained a higher concentration of nutrients, with the exception of K, that are accessible through the roots. The high nutrient content in the Limarí Valley soil is partially explained by higher content of clay compared with the Molina soil. The soil pH is slightly more alkaline in the Limarí Valley whereas the Molina soil is slightly more acidic. The Limarí Valley soils have a sandy clay loam texture (data not shown), which is characteristic of arid zones and, thus, they have better chemical fertilization compared to the Molina soils, which receive more rainfall. The Fe and Mg contents are higher in the Molina soils, while the available boron content is more than twice as high in the Limarí Valley soils.

Differences were also observed in the soil salinity between the Limarí Valley and Molina soils, with substantially higher concentrations of Ca, Mg, K and Na found in the Limarí Valley soils (data not shown). In addition, the ET_o and electrical conductivity (EC) were higher in the Limarí Valley (Table 1).

The climatic and irrigation differences, in conjunction with the soil composition, between the two locations were expected to affect the chemical composition of phenolic and volatile compounds of the extracted olive oil. Researchers have reported that VOOs obtained from traditional rain-fed orchards have higher phenol contents, particularly hydroxytyrosol derivatives and secoiridoid compounds, than do irrigated orchards.[24] This is because the hydric stress of olive trees influences the activity of phenylalanine ammonia lyase (PAL).[25] In addition, the high content of water in the olives from irrigated orchards affects the solubilization of phenolic compounds and alters the release of polysaccharide-linked phenolic compounds in the cell wall during the milling and malaxation steps of the olive oil production process.[26]

The fertilization treatments and element availability in the soil decreases total phenols and *o*-diphenol contents of the oils. Thus, the level of boron, an essential micronutrient, alters biochemical functions through its effect on enzyme activities, such as PAL, polyphenol oxidase (PPO) and peroxidase (POD).[27] Camacho-Cristobal *et al.*[28] demonstrated that boron deficiency increases phenol production. Furthermore, the combined action of boron and zinc enhances the quantity of phenolic compounds in olive fruits, particularly in semi-arid areas.[29]

Nitrogen nutrition is related to PAL activity because a high availability of nitrogen likely induces protein synthesis rather than the synthesis of phenylpropanoids via PAL,[30] thereby decreasing the *o*-diphenol contents in VOOs and, consequently, antioxidants. Furthermore, high concentrations of nitrogen and potassium have a negative effect because they enhance the PPO activity, which catalyzes the oxidation of *o*-diphenols to produce quinones.[10]

Table 2 shows the means and standard deviations of the quantified phenolic compounds from the EVOOs produced in the orchards. The total and individual concentrations of phenolic compounds were higher in the orchards located in the Limarí Valley than in Molina (473 vs. 326 mg kg^{-1}, respectively, in the 2011–2012 season and 493 vs. 208 mg kg^{-1}, respectively in the 2012–2013 season). This may be a result of the higher evapo-transpiration and lower irrigation in the Limarí Valley orchards compared with the Molina orchards. The availability of elements in the orchards of the Limarí Valley did not contribute to increasiing the production of phenolic compounds; however, the higher contents of nitrogen in the orchards of Molina contributed to a decrease in the *o*-diphenol content. Thus, Rosati *et al.*[31] reported a lower level of phenolic compounds in olives cultivated with conventional treatments versus organic treatments. This was most likely related to greater soil nitrogen availability in the conventional treatments (derived directly from N fertilization). The effect of these parameters on phenolic compound production was further examined using statistical procedures.

Table 2. Concentrations of phenolic compounds (mg kg^{-1}) quantified in EVOOs by geogra-phical origin, whichever their cultivar and olive ripeness

	Limarí Valley		Molina	
Compound	2011–2012	2012–2013	2011–2012	2012–2013
Hydroxytyrosol	8.72 ± 5.15	11.19 ± 8.55	3.66 ± 3.39	5.79 ± 4.98
Tyrosol	6.81 ± 4.07	10.09 ± 9.87	4.95 ± 4.16	9.21 ± 7.84
Vanillic acid	0.65 ± 0.35	0.38 ± 0.16	0.40 ± 0.20	0.45 ± 0.20
p-Coumaric acid	0.31 ± 0.22	0.84 ± 0.68	0.19 ± 0.17	0.29 ± 0.23
Elenolic acid	121.66 ± 27.84	137.63 ± 65.25	137.31 ± 45.81	100.78 ± 32.85
Pinoresinol[a]	16.33 ± 3.63	15.66 ± 3.22	14.10 ± 4.21	13.91 ± 3.42
3,4-DHPEA-EDA, oxidized	0.88 ± 0.64	4.80 ± 3.94	0.64 ± 0.29	1.05 ± 2.06
3,4-DHPEA-EDA[b]	112.48 ± 82.87	72.00 ± 54.17	48.78 ± 66.55	13.43 ± 12.73
3,4-DHPEA-EDA-DOA[c]	61.69 ± 62.39	48.96 ± 37.14	25.88 ± 37.12	11.98 ± 26.33
p-HPEA-EDA[d]	39.69 ± 19.68	72.17 ± 40.03	30.73 ± 17.43	20.59 ± 11.37
p-HPEA-EDA-DLA[e]	3.76 ± 3.69	3.25 ± 4.42	2.38 ± 1.68	2.49 ± 1.31
Luteoline	3.63 ± 0.37	4.16 ± 1.85	1.94 ± 0.92	2.24 ± 1.15
3,4-DHPEA-EA[f]	93.99 ± 57.16	108.42 ± 71.90	53.35 ± 50.85	22.81 ± 30.37
Apigenine	1.89 ± 1.38	2.92 ± 2.01	1.76 ± 2.19	2.03 ± 1.19
Methyl luteoline	0.48 ± 0.59	0.78 ± 0.21	0.33 ± 0.25	0.71 ± 0.15
Total phenols	472.97 ± 58.11	493.25 ± 68.96	326.43 ± 68.36	208.76 ± 60.23

Values are mean ± SD ($n = 45$).
[a] Mixed with 1-acetoxy-pinoresinol.
[b] 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl oleuropein aglycon.
[c] 3,4-DHPEA-EDA-DOA, dialdehydic form of oleouropein aglycon.
[d] *p*-HPEA-EDA, dialdehydic form of decarboxymethyl ligstroside aglycon.
[e] *p*-HPEA-EDA-DLA, dialdehydic form of ligstroside aglycon.
[f] 3,4-DHPEA-EA, oleuropein aglycon.

The first step was to have global vision by applying the statistical procedure of canonical correlation. The number of samples, however, is much lower than the number of variables (chemical compounds and soil parameters) to use canonical correlation with the whole dataset. Thus, the solution was found by applying PCA to the set of variables described in Table 1 (climate and information from soils), Table 2 (phenols) and Table 3 (volatiles) independently; the factors of each set were used as new variables for applying canonical correlation.

Thus, the climate and soil variables explained 31.4% of the variance of all the phenolic compounds, indicating that the soil composition and climate had some effect on the phenolic profiles. This result was, however, contrary to the expected influence of these variables. Furthermore, the correlation coefficient between the individual phenolic compound concentrations and the irrigation, climate and soil variables were not ever higher than 0.39, which cannot explain their effect on the chemical compounds responsible for olive oil composition. It is possible that synergistic and antagonistic activities between the nutrients do not contribute from a mathematical perspective. The role of temperature on polyphenol contents is controversial, and depends on genotype and environmental conditions.[3] For example, in a study carried out by Ripa *et al.*[32] in different Italian regions, a negative relationship between accumulated degree-days and total polyphenol contents in the oils was observed. In contrast, Tura *et al.*[33] found that the total polyphenol content increased with accumulated degree-days in 'Casaliva', but not in 'Leccino' varieties, in the cool area of northern Italy.

Table 3 shows the volatile compounds identified in EVOOs together with their concentrations (mean ± SD) after the correction of the chromatographic values with recovery factor.[12] The coefficient of correlation (0.68) between the volatile compounds and climate ($P < 0.05$) indicates that the compounds produced via the lipoxygenase cascade [i.e. hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol and pentanal] seem to be affected by the maximum temperature and, to a lesser extent, by ET_o and irrigation. According to Inglese *et al.*,[3] the effects of temperature on the composition of volatile compounds in olive oils are not unambiguous and depend on environment and its interaction with the genotype. In the northern and relatively rainy areas of Italy, positive relationships have been reported between the accumulated degree-days and the total volatile compounds in oils of the Casaliva cultivar, whereas no significant effects of temperature in oils of the cultivar Leccino were observed.[33] In addition, it was observed that the altitude, which has a relationship with the thermal regimen, affected the volatile compounds. Oils of Biancolilla, Carpellese, and Racioppella varieties produced in a warm coastal area were less fruity, bitter, and pungent and sweeter than those produced in the fresh hilly areas where these cultivars are traditionally grown.[3] The response of volatile compound concentrations to irrigation is complex and variable; it has been shown to be dependent on cultivar and environmental conditions. This may explain some discrepancies existing in the literature.[34] Gomez-Rico *et al.*[35] reported an increase of (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol and hexan-1-ol concentrations with increasing water applied.

Although the changes in the individual and total concentrations of phenols and volatiles were not fully explained by the climate and soil parameters, statistical procedures were applied to evaluate the geographical traceability of the EVOOs. Thus, the Kruskal–Wallis test was used to select particular compounds that characterized the geographical origin independently of the cultivar type. Although the individual phenol concentrations from the Limarí Valley are higher and there are several volatile

Table 3. Concentrations[a] and odor threshold (OT) values of volatiles[b], in mg kg^{-1}, quantified in EVOOs by geographical origin whatever the cultivar and olive ripeness

	Limari Valley		Molina		
Compound	2011–2012	2012–2013	2011–2012	2012–2013	OT
Octane	0.195 ± 0.212	0.056 ± 0.045	0.096 ± 0.046	0.044 ± 0.048	940
Ethyl acetate	0.607 ± 0.576	0.467 ± 0.212	0.417 ± 0.228	0.412 ± 0.253	940
2-Methyl butanal	0.123 ± 0.076	0.141 ± 0.078	0.077 ± 0.036	0.096 ± 0.029	–
3-Methyl butanal	0.098 ± 0.073	0.109 ± 0.068	0.045 ± 0.022	0.062 ± 0.023	5.4
Ethanol	0.258 ± 0.332	0.675 ± 0.647	0.639 ± 0.852	1.133 ± 1.179	3104
Ethyl propionate	0.0192 ± 0.016	0.044 ± 0.014	0.019 ± 0.014	0.033 ± 0.013	100
3-Pentanone	0.017 ± 0.0159	0.036 ± 0.011	0.009 ± 0.013	0.028 ± 0.014	–
Pentanal	0.470 ± 0.219	0.444 ± 0.133	0.215 ± 0.119	0.361 ± 0.282	240
4-Methyl-2-pentanone	0.118 ± 0.055	0.127 ± 0.039	0.084 ± 0.043	0.114 ± 0.039	300
1-Penten-3-one	0.047 ± 0.044	0.117 ± 0.035	0.027 ± 0.030	0.118 ± 0.041	0.7
Butyl acetate	0.038 ± 0.017	0.055 ± 0.017	0.031 ± 0.019	0.052 ± 0.019	–
Hexanal	2.889 ± 0.929	2.759 ± 1.166	3.883 ± 1.274	4.004 ± 1.530	800
(*E*)-2-Pentenal	0.008 ± 0.005	0.021 ± 0.010	0.012 ± 0.009	0.017 ± 0.007	300
(*Z*)-3-Hexenal	0.009 ± 0.007	0.005 ± 0.003	0.007 ± 0.009	0.009 ± 0.004	–
1-Penten-3-ol	0.037 ± 0.017	0.041 ± 0.025	0.053 ± 0.033	0.096 ± 0.066	–
2-Methyl-1-butanol	0.034 ± 0.013	0.043 ± 0.031	0.059 ± 0.028	0.073 ± 0.044	480
3-Methyl-1-butanol	0.037 ± 0.032	0.023 ± 0.021	0.032 ± 0.026	0.029 ± 0.022	100
(*E*)-2-hexenal	2.428 ± 1.279	3.671 ± 2.989	5.037 ± 3.224	5.239 ± 3.495	420
3-Octanone	0.158 ± 0.049	0.161 ± 0.064	0.129 ± 0.038	0.131 ± 0.031	–
Hexyl acetate	0.035 ± 0.063	0.034 ± 0.023	0.053 ± 0.067	0.039 ± 0.017	1040
2-Octanone	0.019 ± 0.060	0.090 ± 0.175	0.009 ± 0.013	0.099 ± 0.106	500
(*E*)-2-heptenal	0.030 ± 0.016	0.053 ± 0.021	0.020 ± 0.024	0.047 ± 0.015	0.5
Hexanol	1.449 ± 0.609	1.086 ± 0.459	1.815 ± 0.648	2.465 ± 1.307	400
(*E*)-3-Hexen-1-ol	0.026 ± 0.016	0.026 ± 0.012	0.036 ± 0.016	0.076 ± 0.042	1000
(*Z*)-3-Hexen-1-ol	0.057 ± 0.048	0.083 ± 0.053	0.093 ± 0.059	0.249 ± 0.149	1100
(*E*)-2-Hexen-1-ol	0.129 ± 0.196	0.102 ± 0.091	0.175 ± 0.106	0.472 ± 0.494	5000
(*Z*)-2-Hexen-1-ol	0.004 ± 0.004	0.006 ± 0.004	0.006 ± 0.004	0.022 ± 0.015	–
(*E*)-2-Octenal	0.001 ± 0.003	0.003 ± 0.002	0.002 ± 0.003	0.010 ± 0.006	–
Acetic acid	–	0.283 ± 0.156	0.0005 ± 0.002	0.376 ± 0.316	500
1-Octanol	0.017 ± 0.014	0.028 ± 0.019	0.044 ± 0.036	0.036 ± 0.013	–
Butanoic acid	0.069 ± 0.085	0.012 ± 0.009	0.081 ± 0.101	0.009 ± 0.010	650
Pentanoic acid	0.014 ± 0.007	0.018 ± 0.012	0.020 ± 0.013	0.012 ± 0.008	600
Hexanoic acid	0.079 ± 0.031	0.087 ± 0.033	0.114 ± 0.048	0.098 ± 0.039	700
Heptanoic acid	0.017 ± 0.008	0.040 ± 0.020	0.015 ± 0.008	0.035 ± 0.016	100
Octanoic acid	0.004 ± 0.005	0.015 ± 0.023	0.018 ± 0.008	0.029 ± 0.022	3000
Nonanoic acid	0.019 ± 0.006	0.016 ± 0.018	0.023 ± 0.008	0.032 ± 0.029	–
Total volatiles	9.559 ± 2.636	10.975 ± 4.196	13.396 ± 4.824	16.157 ± 6.559	–

Values are mean ± SD ($n = 45$).
[a] Values of concentrations were adjusted with their recovery factors, except (*Z*)-3-hexen-1-ol and ethyl propionate, which were adjusted with a tentative value of 1.
[b] Expressed as milligrams of internal standard (4-methyl-2 pentanol) per kg of oil.
OT values are in g kg^{-1}.

compounds that distinguish EVOOs by their geographical provenance [i.e. 2-methyl butanal, 2-methyl-1-butanol, (*E*)-2-hexenal and 1-octanol] (Table 4), the geographical traceability was not good enough after applying PCA to the selected compounds. PCA fitted the 59.5% of the explained variance ($R^2_{cumulative}$).[36]

Figure 1A shows that there was not a clear separation of EVOO samples by their geographical origin. For example, EVOOs from Limarí Valley were classified with EVOOs from Molina. The changes in concentration of some selected volatiles cannot be explained exclusively by the geographical origin because the cultivar and ripeness also influence the volatile compound concentration,[4] i.e. (*E*)-2-hexenal, 2-methyl-1-butanol and hexanal.

Based on data from the literature correlating the concentrations of phenolic compounds in EVOO (Table 2) with the perception of the sensory attributes bitter and pungent,[37] the oils resulting from the Limarí Valley location are most likely more bitter and slightly pungent (Table 4). The volatile compounds, which have concentrations higher than their odor thresholds (Table 3), OAV > 1,[38] indicate that the Molina EVOOs are greener and have a smell similar to almonds (Table 4). The higher contents of (*E*)-2-hexenal in Molina could be explained by the higher amount of water applied in this zone.[35] A difficulty in distinguishing EVOOs by their geographical origin is that the time of harvest of each cultivar can modulate the intrinsic values of chemical compounds, up to the point

Table 4. Concentration of phenolic and volatile compounds together with their sensory descriptors (*) and their PCA loadings** (F1: PCA 1 and F2: PCA 2) for Fig. 1: selection of chemical compounds, by the Kruskal–Wallis test, for distinguishing EVOOs from orchards located in the Limarí Valley and Molina ($P < 0.05$)

Compound	Taste/odor*	F1**	F2**
Hydroxytyrosol	Bitter	−0.22	0.32
Pinoresinol	Bitter	0.19	0.23
3,4-DHPEA-EDA	Bitter, slightly pungent	−0.08	0.34
3,4-DHPEA-EDA-DOA	Bitter	−0.30	0.25
2-Methyl butanal	Dry fruit, cheese	0.28	0.29
3-Methyl butanal	Ripened fruit	0.26	0.29
Luteoline	–	0.18	0.34
Pentanal	Woody, bitter oily	0.13	0.35
Hexanal	Green strong	0.42	0.05
1-Penten-3-ol	Butter, slight green aroma	0.07	−0.08
2-Methyl-1-butanol	Spicy, winey	0.40	0.09
(*E*)-2-Hexenal	Bitter almond, green	0.41	0.11
1-Octanol	Metal	0.02	0.11
Hexanoic acid	Pungent, rancid	0.03	0.12

Values are mean ± SD ($n = 60$).
*Sensory descriptors are based on the literature (Servili *et al.*[37] and Luna *et al.*[38]).
Abbreviations are as given in Table 2.

of homogenizing the chemical composition of the resulting olive oils regardless of the cultivar and the geographical location of the orchards.

Because of the influence of cultivar on the concentration of the volatiles,[11] the next portion of this study was the characterization of the EVOOs. In Fig. 1B, the three cultivars are distinguished according to eight compounds, i.e. five phenols and three volatiles. The concentrations of each are listed in Table 5. The chemical compounds were selected by Duncan's test. None of the selected compounds, with the exception of 3,4-DHPEA-EDA (the dialdehydic form of the decarboxymethyl ligstroside aglycon), were selected for distinguishing EVOOs according to their geographical origin. The selection of 3,4-DHPEA-EDA is due to the lower concentration of this compound in the variety *Koroneiki* in comparison with the other two. In general, 3,4-DHPEA-EDA was present at high levels when the harvest occurred earlier and its content diminished abruptly with increasing olive maturity, particularly in the Molina orchards. This behavior was similar to that reported by Franco *et al.*[39]

The concentration of the compounds, in terms of their contribution to taste and odor, seems to indicate that samples of the variety *Koroneki* are less fruity and slightly bitter, samples of the variety *Arbosana* are the most fruity and bitter, and samples of the variety *Arbequina* present an intermediate taste and odor.

The selected chemical compounds differentiated the cultivars, including the harvests 2011–2012 and 2012–2013 (Fig. 1B), when the non-supervised PCA statistical procedure was applied.

The PCA model retained five principal components, which explained 77.3% of the total variability of the data. As seen in Fig. 1B, factor 1 (39.4% explained variance) arranges the samples according to the geographical origins (Molina and Limarí Valley); while factor 2 separates the samples by harvest (24.9% explained variance). A clear differentiation between varieties was not observed, possibly due to the effects of fruit ripening stage on the composition of oils, as previously mentioned. It was found that the samples collected at more advanced levels of ripeness are distributed in the border of each quadrant, indicating the importance of the olive maturity when harvesting. Significantly, it is possible to observe a clear differentiation by geographical area and by crop season. Tura *et al.*[4] clearly demonstrated that the flavor profiles of oils depend more on their geographical origin (environmental influence), than on cultivar (genetic influence) or fruit maturation at harvest (influence of the ripening stage). In addition, Caruso *et al.*[34] reported that the influence of harvest year on composition of volatile compounds was more important than the soil water availability. The effect of crop seasons was observed in total polyphenol compounds and *o*-diphenol contents in the VOO with PDO 'Les Garrigues' because of environmental factors.[40] Franco *et al.*[39] reported an interaction between crop season and variety.

Finally, the influence of olive maturity on the composition of phenolic and volatile compounds in EVOOs was evaluated. Olives were harvested at five different ripeness stages in the Limarí Valley and Molina. To simplify the analysis, the five ripeness stages were grouped into three levels.

In this case, the linear discriminant analysis method with stepwise selection (*F*-to enter = 5) allowed us to classify the EVOOs according to fruit ripeness stage, based on four volatile and two phenolic compounds [ethyl acetate, (*E*)-2-hexenal, 3-octanone, (*Z*)-3-hexen-1-ol, 3,4-DHPEA-EA (oleuropein aglycon) and *p*-HPEA-EDA (dialdehydic form of ligstroside aglycon)] (Fig. 2). This demonstrates the difference in EVOOs from mature (Level 3 with 57 samples) and less mature olives (Level 1 with 56 samples), with some exceptions, while the intermediate level of maturity (Level 2 with 67 samples) was in the middle.

(*E*)-2-Hexenal, the major component of C_6 volatile compounds from the lipoxygenase (LOX) pathway reached the highest levels of concentration (mg kg^{-1}) in L2 and then decreased as a result of alcohol dehydrogenase (ADH) activity (L1: 2.53 ± 2.50; L2: 5.35 ± 3.09; L3: 3.99 ± 2.89), which increased the (*Z*)-3-hexen-1-ol concentration[1] (L1: 0.07 ± 0.05; L2: 0.12 ± 0.10; L3: 0.16 ± 0.16).

The behavior of (*E*)-2-hexenal was different to that reported by Gomez-Rico *et al.*[35] for the Cornicabra variety. The other volatiles, ethyl acetate (L1: 0.67 ± 0.48; L2: 0.40 ± 0.20; L3: 0.35 ± 0.17) and 3-octanone (L1: 0.19 ± 0.05; L2: 0.14 ± 0.04; L3: 0.12 ± 0.03) diminished with olive maturity to approximately half of their initial concentrations.

For the phenolic compounds, their behaviors agreed with the fact that the concentration of phenols decreases with the increasing olive maturity;[35] 3,4-DHPEA-EA diminished approximately 70% (L1: 136.10 ± 69.35; L2: 56.59 ± 46.23; L3: 41.97 ± 41.36) and *p*-HPEA-EDA diminished approximately 65% (L1: 72.26 ± 42.89; L2: 38.17 ± 20.91; L3: 25.53 ± 17.10). EVOO variety *Koroneiki* exhibited high contents of 3,4-DHPEA-EA from less mature olives, especially in the Limarí Valley. The values for these phenolic compounds were comparable to those reported by other authors.[37] The decrease in these compounds with fruit maturity, especially for *p*-HPEA-EDA, could diminish the pungent sensation of EVOOs.[37]

CONCLUSIONS

This work explains that the soil and the climate of the Chilean regions have much more influence than cultivars on the concentration of sensory quality compounds, which was unknown. The difference in latitude between the orchards increases the importance of the geographical origin on the virgin olive oil chemical composition, while at the same time, full irrigation decreases the

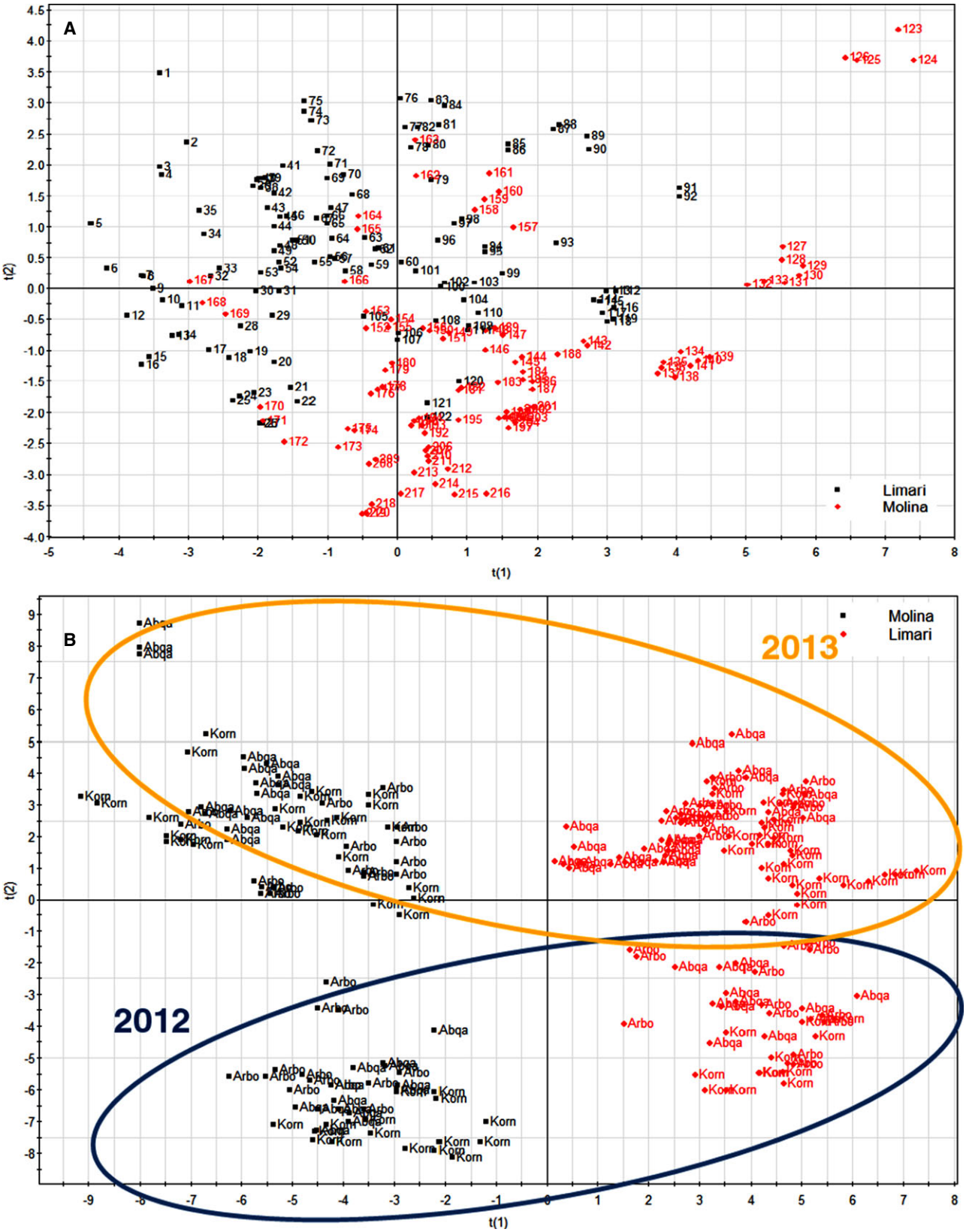

Figure 1. Results of applying principal component analysis (PCA) to compounds of Table 4 and Table 5. Notes: Abqa, var. *Arbequina*; Arbo, var. *Arbosana*; Korn, var. *Koroneiki*.

Table 5. Concentration of phenolic and volatile compounds together with their sensory descriptors (*) and their PCA loadings** (F1: PCA 1 and F2: PCA 2) for Fig. 1: selection of chemical compounds, by the Kruskal–Wallis test, for distinguishing EVOOs from their cultivars ($P < 0.05$)

Compound	*Koroneiki*	*Arbequina*	*Arbosana*	Taste/odor*	F1**	F2**
Tyrosol (mg kg^{-1})	12.07 ± 2.82	5.31 ± 3.67	5.61 ± 1.07	Bitter	0.09	0.29
p-Coumaric acid (mg kg^{-1})	0.31 ± 0.06	0.71 ± 0.47	0.26 ± 0.21	Bitter	−0.43	0.22
3,4-DHPEA-EDA (mg kg^{-1})	34.73 ± 2.41	72.79 ± 27.73	87.73 ± 36.67	Bitter, slightly pungent	−0.51	−0.16
p-HPEA-EDA-DLA (mg kg^{-1})	1.50 ± 0.10	2.13 ± 0.55	5.58 ± 0.64	Bitter	−0.57	0.07
Apigenine (mg kg^{-1})	1.89 ± 0.53	0.90 ± 0.30	4.01 ± 1.06	–	0.10	−0.56
Octane (μg kg^{-1})	160.18 ± 145.91	55.21 ± 44.95	82.92 ± 7.10	Alkane	0.12	0.17
Hexanol (μg kg^{-1})	1077.21 ± 141.12	2023.24 ± 394.72	2246.19 ± 147.67	Fruity, banana	0.28	−0.19
Nonanoic acid (μg kg^{-1})	19.57 ± 4.11	17.36 ± 10.86	30.80 ± 13.06	Acid, unpleasant	0.32	−0.30

Values are mean ± SD ($n = 60$).
*Sensory descriptors are based on the literature (Servili *et al.*[37] and Luna *et al.*[38]).
Abbreviations are as given in Table 2.

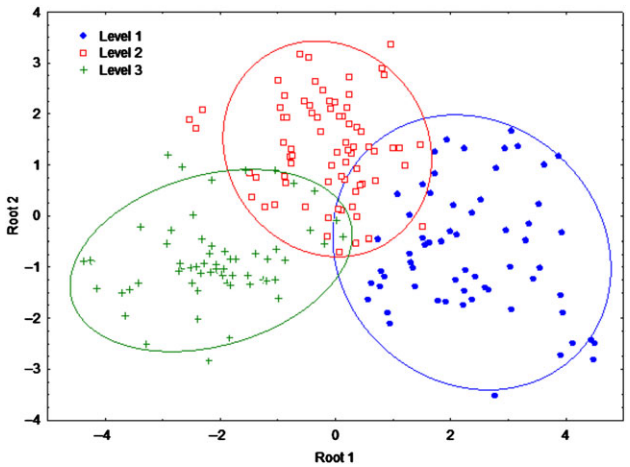

Figure 2. Step-wise linear discriminant analysis based on six compounds [ethyl acetate, (*E*)-2-hexenal, 3-octanone, (*Z*)-3-hexen-1-ol, 3,4-DHPEA-EA and p-HPEA-EDA]. The plot shows the characterization of EVOOs by three levels of olive ripeness. Level 1, EVOOs from less mature olives; Level 2, EVOOs from medium mature olives; level 3, EVOOs from mature olives.

impact of the cultivar. It is just the opposite of VOOs produced in the Mediterranean basin where the cultivars and not the geographical provenance explain the great range of its VOOs.

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