Effect of Growing Area on Pigment and Phenolic Fractions of Virgin Olive Oils of the Arbequina Variety in Spain

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ABSTRACT: The purpose of this investigation was to study differences in the chlorophyll, carotenoid, and phenolic fractions of virgin olive oils from the Arbequina variety cultivated in different olive growing areas of Spain. Virgin olive oil from Lleida was less heavily pigmented, and these oils showed more negative values for the ordinate a* (of the CIELAB colorimetric system). Pheophytin *a* was the major chlorophyll pigment, and lutein was the major component of the carotenoid fraction in all oils analyzed. The chlorophyll *a* concentration in virgin olive oils from Lleida was 700 μg kg^{-1}, but was 175 μg kg^{-1} in oils from Jaén, and 200 μg kg^{-1} in oils from Tarragona. Finally, the chlorophyll *a*/chlorophyll *b* ratio was 9 in oils from Lleida and around 0.6 in the other two Arbequina olive oils. In relation to the phenolic fraction, the hydroxytyrosol and tyrosol contents were significantly higher in olive oils from Jaén (grown at higher altitude and precipitation rates). The secoiridoid derivatives showed a significantly higher concentration in olive oils from Tarragona, probably due to the low altitude where they grow, and finally the ratio of (dialdehydic form of elenolic acid linked to tyrosol)/lignans had a value of 1.4 in olive oils from Lleida, whereas this value was around 0.7 in the other Arbequina olive oils.

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KEY WORDS: Arbequina cultivar, carotenoids, chlorophylls, phenols, virgin olive oil.

Virgin olive oil is obtained from the fruit of the olive tree (*Olea europaea* L.) by mechanical or other means under conditions, particularly thermal, that do not lead to alteration of the oil. In the Mediterranean diet, where virgin olive oil is the major source of fat, rates of coronary heart disease are relatively low compared with northern European societies and the United States. Extra virgin olive oil has been proposed as an important factor in the favorable health effects of the Mediterranean diet.

It is estimated that there are 165 million olive trees in Andalusia, Spain. Of these, 50% are in Jaén, and 30% are in Córdoba. The remaining 20% of Spanish olive cultivars are distributed throughout different areas of the Mediterranean basin, with variations in climate and soil giving the oils their specific characteristics. The range of varieties of olives that are produced in Spain combine with the widely differing microclimates around the country to produce a wide variety of oils.

One of the main Spanish olive varieties, which is well known in the international oil market for its excellent taste and flavor, is the Arbequina variety. This cultivar owes its name to the municipal district of Arbeca (Lleida, Catalonia, Spain) where it was first grown. It is characterized by frost resistance, low vigor, small-sized fruit, and high productivity. The oval-shaped olive has a low flesh-to-stone ratio. Because of its small size, an average of 1.9 g, it is difficult to harvest mechanically, but it is very highly regarded because the trees produce a large amount of fruit and with a relatively high oil yield of 20.5%. These oils are dense and fluid, tasting of orchard fruit. Although they are highly regarded oils, production tends to suffer due to the variations in climate in the growing areas, especially during periods of drought.

It has long been known that the chemical composition of virgin olive oil is influenced by genetic (cultivar) and environmental factors (edaphologic characteristics and climatological conditions), so that the olive production area is greatly responsible for the specific characteristics of olive oil. In the last few years, there has been increasing interest in the geographical classification of virgin olive oil, as a reliable criterion for its authentication and quality, as a foodstuff with an identifiable growing area. Many studies have been carried out to characterize virgin olive oils from different geographical origins according to different chemical parameters, such as FA composition (1), hydrocarbon fraction (2), terpenes (3), pigment composition (4), and phenolic fraction (5).

Pigments (chlorophyll and carotenoids) are responsible for the color of virgin olive oil, ranging from yellow-green to greenish gold. Color is one of the major attributes that affects the consumer perception of quality. In addition, chlorophylls and carotenoids play an important role in oxidative stability owing to their antioxidant nature in the dark and pro-oxidant activity in the light. The apparent color (measured from the chromatic coordinates L*, a*, and b*) has been correlated with the pigment composition in virgin olive oil (6). Differences have been observed in chlorophyll and carotenoid composition depending on the olive variety, the growing region, the ripeness of the fruit, the extraction process, and the storage conditions of the oil (7–9). Virgin olive oils from the Arbequina cultivar are characterized by low pigment content and the presence of pigments unique to this variety: mono- and di-esterified xanthophylls and pheophorbide *a*. This fact could be used as a chemotaxonomic differentiator of the oils

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of this variety. The presence of xanthophylls esterified with FA in the Arbequina variety plays an important role in the stability of these carotenoids (10), stabilizing the color of oils and ensuring a sufficient level of substances with antioxidant activity (11). On the other hand, the esterification of the carotenoid pigments helps to improve their bioavailability when these micronutrients are ingested (12). Finally, the fact that pheophorbide *a* is present only in oils from the Arbequina variety, whose extraction process is similar to that of the other varieties, suggests that the fruit has a much higher chlorophyllase content than other varieties (4).

The phenolic fraction extracted from Arbequina virgin olive oil is characterized by a low content of simple phenols, such as hydroxytyrosol, tyrosol, vanillic, vanillin, and 4-(acetoxyethyl)-1,2-dihydroxybenzene (3,4-DHPEA-AC) and a high percentage of secoiridoid derivatives (the dialdehydic form of elenolic acid linked to hydroxytyrosol, 3,4-DHPEA-EDA; the dialdehydic form of elenolic acid linked to tyrosol, *p*-HPEA-EDA; and oleuropein aglycon, 3,4-DHPEA-EA), which are closely related to the oxidative stability and bitter taste of the oils (13,14). Although the interest in phenolic compounds is related primarily to their antioxidant activity, they also show important biological activity *in vivo* and may be beneficial in combatting diseases resulting from oxygen radical formation that exceeds the defensive capacity of the antioxidants in the human body.

The aim of this study was to analyze the qualitative and quantitative differences of the minor fractions, pigments, and phenols of commercial virgin olive oils from the Arbequina variety cultivated in different olive growing regions in Spain, namely, Lleida and Tarragona located in the northeast and Jaén in the south.

MATERIALS AND METHODS

Oil samples. The study was carried out on commercial virgin olive oil of the Arbequina variety from three producing regions in Spain—Lleida (Catalonia), Tarragona (Catalonia), and Jaén (Andalusia)—with different edaphological characteristics and climatological conditions. Four virgin olive oil samples from different mills of each region were obtained at the end of December 2002. At sampling time, olive drupes from Jaén had a maturation index of 6–7, whereas olive drupes from Lleida and Tarragona had a maturation index of 4–5. Oils were extracted using metal hammer crushers, mixed at 35°C for 45 min, and finally separated by a dual-phase decanter. The oils were taken directly from the production line and stored in the dark at −20°C.

Climatological data were obtained during 2002 from meteorological stations at La Granadella (Lleida), Vila-rodona (Tarragona), and Pozo Alcón (Jaén) (Fig. 1).

The climate of the Lleida area is Mediterranean with a continental influence, and the average altitude varies between 400 and 450 m above sea level. The daily and annual temperature variations are greater than in a pure Mediterranean climate, with its maritime influence. The winters are much colder and the summers drier and hotter. The average temperature ranges from a minimum of −2°C to a maximum of 24°C. Rainfall in general is scarce, mostly falling in spring. Annual rainfall varies from 350 to 450 mm, with an average relative humidity of 66%.

The olive-growing area of Tarragona runs perpendicular to the Mediterranean coast. The climate is pure Mediterranean, with a maritime influence. The average temperature ranges from a minimum of −3°C to a maximum of 40°C, and the annual rainfall varies from 400 to 500 mm.

The climate in Jaén is extreme, with temperatures varying from a high of 35°C to a low of −8°C. Average annual rainfall varies from 500 to 700 mm, and the average altitude of the production area is 899 m above sea level.

In reference to edaphological data (15), the Lleida area belongs to the Calcisol-Solonchak-Cambisol region and is characterized by a parental material composed of sedimentary deposits from Oligocene and Miocene (loam) and quaternary fluvial deposits. The olive-growing area of Tarragona belongs to the Cambisol-Leptosol region, and the parental material is characterized by sedimentary deposits from Triassic and Tertiary (sand, loam, and limestone). The Jaén area is located in a Leptosol-Cambisol region, which is composed of sedimentary rocks from the Mesozoic (limestone, loam, and sand) and metamorphic rocks from the Paleozoic.

Pigment extraction. The method used to obtain the pigment extract is based on a selective separation of pigments with *N,N*-dimethylformamide (DMF) and hexane. Lipids and the carotene fraction appeared in the hexane phase while the DMF phase retained chlorophylls and xanthophylls (9). The sample of virgin olive oil (15 g) was dissolved in 150 mL of DMF saturated with $MgCO_3$ and treated with five successive 50-mL portions of hexane.

The DMF phase was transferred to a 1000-mL separatory funnel containing 400 mL of 2% sodium sulfate solution at approximately 0°C. A 70-mL portion of hexane and another 70-mL portion of ethyl ether were added, and the mixture was shaken and then held until the phases had separated (about 20 min). The ether phase with the chloroplast pigment solution was washed three times with an aqueous solution of Na_2SO_4 (2%) at 0°C. This solution was concentrated in a rotary evaporator. The dry residue was dissolved in 1 mL of acetone.

The hexane phases were mixed in a 500-mL separatory funnel containing 100 mL of ether, saponified with 100 mL of 15.6% KOH in methanol, and strongly shaken to hydrolyze the lipids and purify the possible carotenoids. After 1 h, distilled water was added, the solution was shaken for 1 min, and the mixture was held until the phases had separated. The ether phase with the carotene fraction was washed three times with water to neutrality and another three times with an aqueous solution of Na_2SO_4. It was concentrated by drying in a vacuum. The final residue was dissolved in 1 mL of acetone.

The chlorophyll and carotene solutions were stored in the dark in a freezer at −30°C awaiting the HPLC analysis. All extractions were performed in duplicate under a green light to prevent pigment alteration.

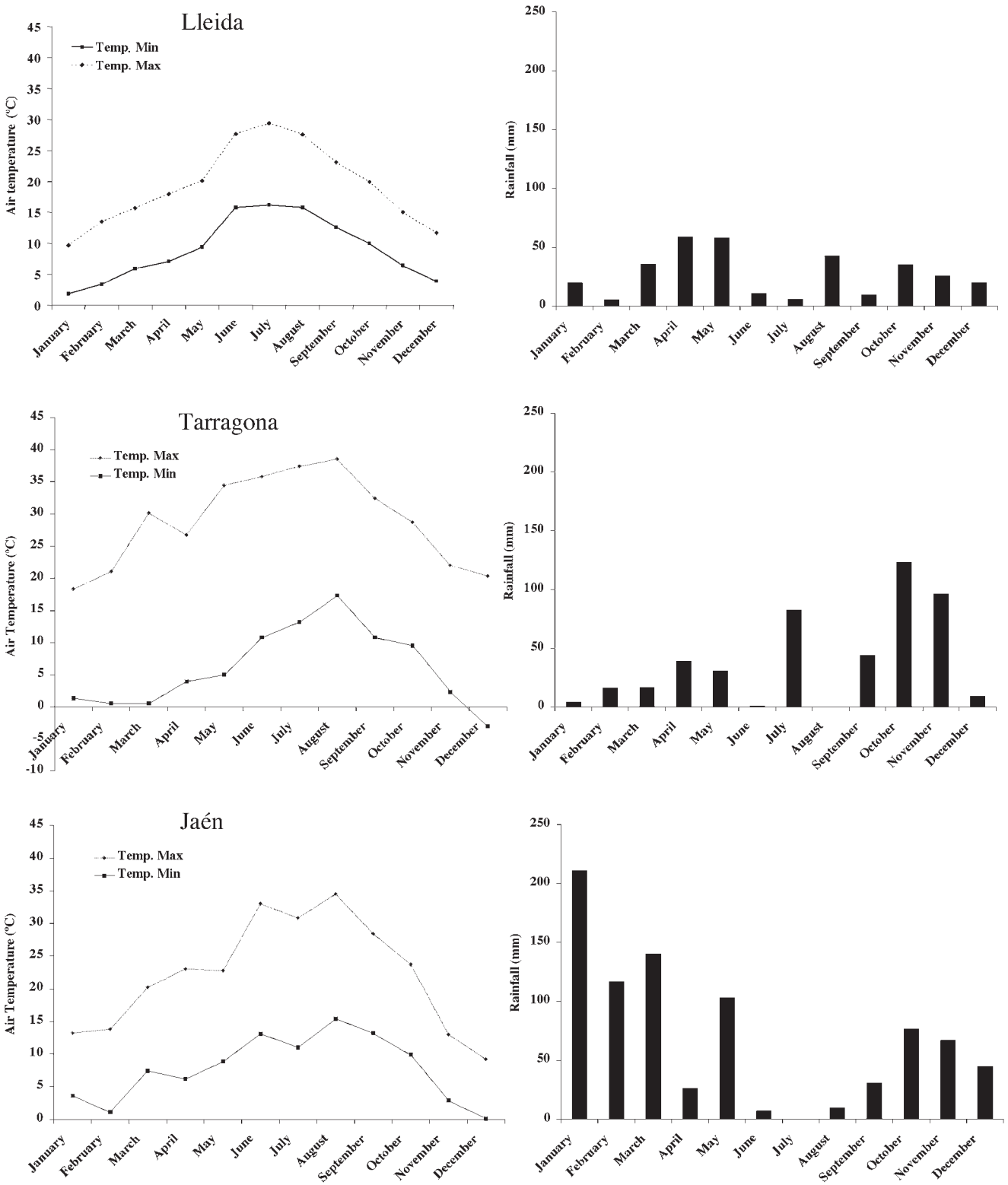

FIG. 1. Annual patterns for year 2002 of air temperature and rainfall in the Spanish olive-growing areas of Lleida, Tarragona, and Jaén.

Phenolic extraction. Phenols were extracted from virgin olive oil following the procedure of Montedoro *et al*. (16). Two × 20 mL of methanol/water (80:20 vol/vol) were added to 45 g of virgin olive oil and homogenized for 2 min with a Polytron homogenizer (Lutau, Switzerland). The two phases were separated by centrifuging at 704 × *g* for 10 min. The aqueous alcoholic extracts were then combined and concentrated in a vacuum at temperatures below 35°C until a syrupy

consistency was reached. Acetonitrile (5 mL) was added to the extract, and the solution was washed with 3 × 20 mL of hexane. The apolar phases were also washed with 5 mL of acetonitrile. The resulting acetonitrile solution was evaporated under vacuum, and the residue was dissolved in 5 mL of acetonitrile. Finally, an aliquot of 2 mL was evaporated under a stream of nitrogen.

HPLC analysis of pigments and phenolic compounds. The HPLC system was made up of a Waters 717 plus Autosampler, a Waters 600 pump, a Waters column heater module, and a Waters 996 photodiode array detector managed by Empower software (Waters Inc., Milford, MA).

Pigments. The column was a stainless steel Spherisorb ODS-2 column (5 µm, 25 cm × 4.6 mm i.d.; Technokroma, Barcelona, Spain) equipped with a Spherisorb S5 ODS-2 (5 µm, 1 cm × 4.6 mm i.d., Technokroma) precolumn. HPLC analysis was performed following the same procedure as Minguez-Mosquera *et al*. (9). The pigment solution in acetone was centrifuged at 6000 × *g* (EBA 12 Hettich Zentrifugen) prior to its injection into the chromatograph (40 µL). Separation was performed using an elution gradient (flow rate = 2 mL min^{-1}) with mobile phases (A) water/ion pair reagent/methanol (1:1:8 by vol) and (B) methanol/acetone (1:1). The ion pair reagent was 0.05 M tetrabutylammonium acetate and 1 M ammonium acetate in water. Detection was performed simultaneously at 410 nm to measure pheophytin *a* and pheophorbide *a*, 430 nm to measure chlorophyll *a*, 435 nm to measure pheophytin *b*, 440 nm to measure neoxanthin, violaxanthin, and their corresponding isomers, 445 nm to measure antheraxanthin, 446 nm to measure lutein, 453 nm to measure β-carotene, and 466 nm to measure chlorophyll *b*. All peaks were identified by their chromatographic and spectroscopic characteristics.

External standard calibration was used for quantification. Chlorophyll *a* (No. C-6144 from algae), chlorophyll *b* (No. C-5878 from spinach), and β-carotene (No. C-4582) were supplied by Sigma (St. Louis, MO). Pheophytins *a* and *b* were obtained from the respective solutions of chlorophylls by shaking the ether solution with 2 or 3 drops of 13% HCl until the green chlorophyll color changed to the grayish pheophytin color (17,18). Pheophorbide *a* was formed by enzymatic deesterification of pheophytin *a* (19). The enzymatic extract of chlorophyllase was obtained from *Ailanthus altissima* leaves (20). Lutein, antheraxanthin, violaxanthin, and neoxanthin were obtained from a pigment extract of fresh spinach and separated by TLC on silica gel GF_{254} (0.2 mm) on 20 × 20 cm plates using petroleum ether (boiling point 65–95°C)/acetone/diethylamine (10:4:1) (9). The results are expressed in µg of pigment per kg of oil.

Phenolic compounds. The extracted phenolic fraction was dissolved in 1 mL of methanol and analyzed by HPLC. The column was an Inertsil ODS-3 (5 µm, 15 cm × 4.6 mm i.d.; GL Sciences Inc., Tokyo, Japan) equipped with a Spherisorb S5 ODS-2 (5 µm, 1 cm × 4.6 mm i.d.; Technokroma) precolumn. HPLC analysis was performed following the same procedure as Montedoro *et al*. (16). The eluents were 0.2% aqueous acetic acid solution (pH 3.1) and methanol, the flow rate was 1.5 mL/min, and the injection volume 20 µL. The total run time was 60 min, the initial composition was 95% aqueous acetic acid solution (0.2%), and 5% methanol, and the gradient changed as follows. The concentration of methanol was maintained for 2 min, then it was increased to 25% for 8 min, and finally the methanol percentage was increased to 40, 50, and 100% for 10-min periods. It was maintained at 100% for 5 min. Initial conditions were reached in 15 min. Chromatograms were obtained at 280 and 339 nm.

Reference compounds were used for quantification. Tyrosol and *p*-coumaric acid were obtained from Extrasynthèse Co. (Genay, France). Vanillic acid, vanillin, and ferulic acid were obtained from Fluka Co. (Buchs, Switzerland). Hydroxytyrosol was kindly donated by Professor Montedoro (University of Perugia, Italy). The rest of the phenolic compounds were obtained using a semipreparative HPLC column Spherisorb ODS-2 (5 µm, 25 cm × 10 mm i.d.; Technokroma) and a flow rate of 4 mL/min. The mobile phases and gradient were described by Tovar *et al.* (13). Sample extracts were analyzed using a ZMD mass spectrophotometer (Waters Inc.) equipped with an electrospray ionization ion source (ESI). The ion spray mass spectra in the negative-ion mode were obtained under the following conditions: capillary voltage, 2.5 kV; cone voltage, 10 V; desolvation temperature, 400°C; and source temperature, 120°C.

Individual phenols were quantified by a four-point regression curve on the basis of the standards obtained from commercial suppliers or from preparative HPLC as described above. Quantification of the phenolic compounds was carried out at 280 nm. The results are expressed as mg of phenol per kg of oil.

Oil color. A colorimeter (chromometer type Color-Eye 3000, Macbeth) was used to assess the oil color with the Optiview 1.1 computer program, and the CIELAB colorimetric system was applied. The oil color is expressed as chromatic ordinates L* (lightness), a* (redness), b* (yellowness).

Total pigment content. The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each virgin olive oil sample (7.5 g) dissolved in cyclohexane (25 mL) (6). The chlorophyll and carotenoid contents are expressed as mg of major pigment, pheophytin *a,* and lutein per kg of oil, respectively.

Total phenol content. The total phenol content was analyzed using the modified isolation method described by Vázquez Roncero *et al*. (21) with triple extraction of an oil-in-hexane solution with a 60% (vol/vol) water/methanol mixture. The concentration of total polyphenols was estimated with Folin–Ciocalteau reagent at 725 nm. The results were expressed as mg of caffeic acid per kg of oil.

Bitter index. The bitter index (K_{225}) was evaluated by the extraction of the bitter components of a sample of 1.0 ± 0.01 g oil dissolved in 4 mL hexane passed over a C18 column (Waters Sep-Pack Cartridges), previously activated with methanol (6 mL) and washed with hexane (6 mL). After elution, 10 mL of hexane was passed through to eliminate the fat, and the retained compounds were then eluted to 25 mL with methanol/

water (1:1). The absorbancy of the extract was measured at 225 nm against methanol/water (1/1) in a 1-cm cuvette (22).

Oxidative stability. Oxidative stability of virgin olive oil is expressed as the oxidation induction time (hours) measured with a Rancimat 679 apparatus (Metrohm Co., Herisau, Switzerland) using a 3-g oil sample heated to 120°C, and 20 L h^{-1} air flow. The time taken to reach a fixed level of conductivity was measured (23).

Statistical analysis. The data was subjected to an ANOVA using SAS, version 8.02 (SAS Institute Inc., Cary, NC). Separation of the means was obtained using the least square means test, and significant difference was defined as $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the values corresponding to the concentrations of the chlorophyll and carotenoid pigments and chromatic ordinates of Arbequina oils from different olive growing areas in Spain. Significant differences ($P < 0.001$) were observed in the total content of pigments between oils from Lleida and those coming from two other producing regions. From the results obtained, the behavior of the virgin olive oils from Lleida is totally different in spite of the geographical proximity to the Tarragona region. Virgin olive oil from Lleida showed the least pigmentation. This may be a consequence of the minimum temperatures reached in the former area in November and December, implying heavy frosts that could have led to a deterioration of the olive fruit and degradation of the pigment, mainly in the chlorophyll fraction.

The color measurement by tristimulus ordinates b*, a*, and L* of the CIELAB chromatic space showed significant differences ($P < 0.001$) between growing regions and environmental conditions (Table 1). It was observed that lightness (L*) was negatively correlated with the total pigment content of the oils, and the oils from Lleida showed more negative values of a*. There was a clear relationship between the values of b* and the carotenoid concentration. The oils from Tarragona and Jaén had higher chlorophyll and carotenoid contents. Significant differences ($P < 0.001$) in the total content of pigments and color were observed between Lleida oils and olive oils coming from other geographical origins.

The concentrations of individual chlorophylls and carotenoids in virgin olive oil of the Arbequina variety from the three different growing areas of Spain are shown in Table 2. The qualitative study of virgin olive pigments demonstrated a common pattern that is not dependent on growing region or environmental conditions. The qualitative pigment profile of the virgin olive oil encompasses chlorophyll *a*, chlorophyll *b*, and derivative pigments associated with the acidic medium of the oil extraction process—pheophytin *a*, pheophytin *b*—and the carotenoids lutein, β-carotene, and the epoxide xanthophylls, neoxanthin, violaxanthin, and antheraxanthin (Figs. 2 and 3). Besides these, mono- and di-esterified xanthophylls and pheophorbide *a*, characteristic pigments of Arbequina oils, were detected. This fact could be used as a chemotaxonomic differentiating parameter for Arbequina variety oils. However, in this study of virgin olive oils coming from Tarragona, esterified xanthophylls, which are pigments characteristic of Arbequina oils, were not found. On the other hand, pheophytin *b* was not observed in oils from Lleida.

The same table shows there were significant differences in the ratio of chlorophyll *a*/chlorophyll *b* between oils from Tarragona and Jaén and oils from Lleida. The value was 0.6 in the oils from Tarragona and Jaén, and 9 in oils from Lleida. The chlorophyll *a*/*b* ratio is an indirect measurement of the reaction center/antenna complex distribution in the thylakoid (24). Consequently, this result could be due to differences in the photosynthetic apparatus of olive fruit grown in different areas.

With respect to chlorophyll content, pheophytin *a* was the major chlorophyll pigment in all the oils (54 to 88% of the total chlorophyll). The main difference in the chlorophyll fraction between oils from Lleida and oils coming from the other two growing areas was the different quantitative contribution of chlorophyll *a*. Chlorophyll *a* was 39% of the total chlorophyll fraction in virgin olive oils from Lleida, but the percentage fell to 3% of the total chlorophyll content in oils from Tarragona and Jaén.

TABLE 1
Chlorophyll and Carotenoid Contents, Chromatic Ordinates, and Stability Indices of Virgin Olive Oils Obtained from the Arbequina Cultivar in Three Different Growing Areas in Spain (*n* = 8)

	Growing area[b]		
Parameter[a]	Lleida	Tarragona	Jaén
Chlorophyll content (mg/kg oil)	5.14 ± 0.00[a]	14.47 ± 0.38[b]	11.65 ± 0.03[b]
Carotenoid content (mg/kg oil)	4.74 ± 0.14[a]	7.43 ± 0.10[b]	7.01 ± 0.03[b]
Chromatic ordinates			
L*	92.07 ± 0.03[a]	86.21 ± 0.29[b]	85.83 ± 0.10[b]
a*	–5.22 ± 0.00[a]	–1.64 ± 0.00[b]	–1.80 ± 0.01[b]
b*	77.90 ± 0.04[a]	102.20 ± 37.00[b]	98.15 ± 0.12[b]
Stability (h)	7.93 ± 0.08[a]	8.80 ± 0.04[b]	7.88 ± 0.21[a]
Total phenol content (mg/kg oil)	100 ± 3.94[a]	111 ± 2.49[b]	92 ± 2.19[a]
Bitter index	0.138 ± 0.007[a]	0.158 ± 0.011[b]	0.126 ± 0.006[a]

[a]Means of duplicate analyses.
[b]Different superscript roman letters in the same row indicate a significant difference ($P < 0.001$).

TABLE 2
Concentrations (μg kg^{-1}) of Individual Chlorophylls, Carotenoids, and Ratios Between Pigments in Virgin Olive Oil Obtained from the Arbequina Cultivar in Three Different Growing Areas in Spain (*n* = 8)

		Growing area[a]			
Peak	Pigment	Lleida	Tarragona	Jaén	Difference
1	Pheophorbide *a*	83 ± 16[a]	437 ± 45[b]	565 ± 35[b]	**
6	Chlorophyll *b*	78 ± 25[a]	324 ± 36[b]	270 ± 40[b]	*
7	Chlorophyll *a*	705 ± 12[a]	203 ± 18[b]	175 ± 25[b]	***
9	Pheophytin *b*	ND	104 ± 12[a]	120 ± 20[a]	*
12	Pheophytin *a*	983 ± 37[a]	6283 ± 386[b]	4590 ± 610[b]	**
	Total chlorophylls	1849 ± 88[a]	7351 ± 497[b]	5720 ± 730[b]	*
2	Neoxanthin	121 ± 4[a]	302 ± 22[b]	240 ± 40[b]	*
3,3′	Violaxanthin and violaxanthin isomer	313 ± 52[a]	713 ± 47[b]	615 ± 85[b]	*
4	Antheraxanthin	165 ± 19[a]	371 ± 27[b]	275 ± 45a[b]	*
5	Lutein	1540 ± 69[a]	2380 ± 135[b]	2625 ± 45[b]	*
8,10	Esterified xanthophylls[b]	56 ± 6[a]	ND	12 ± 7[b]	**
11	All-*trans*-β–carotene	123 ± 39[a]	461 ± 7[b]	490 ± 60[b]	*
	Total carotenoids	2318 ± 185[a]	4227 ± 236[b]	4257 ± 282[b]	*
	Ratios				
	Chlorophyll *a*/chlorophyll *b*	9.04 ± 3.05[a]	0.63 ± 0.01[b]	0.65 ± 0.00[b]	***
	Chlorophylls/carotenoids	0.8 ± 0.03 [a]	1.74 ± 0.02 [b]	1.34 ± 0.08 [b]	**
	Lutein/β-carotene	12.52 ± 3.50 [a]	5.16 ± 0.50[b]	5.36 ± 0.22[b]	*

[a]Different superscript roman letters in the same row indicate a significant difference. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[b]Violaxanthin monoesterified, neoxanthin esterified. ND, not detected. Detection limits for pigments were 1 μg/kg in olive oil.

Although lutein was the major compound of the carotenoid fraction in all the oils analyzed (from 56 to 66% of total carotenoids), the lutein/β-carotene ratio ranged between 5 in oils from Tarragona and Jaén and 12 in oils from Lleida. It was observed that the major xanthophyll epoxide was violaxanthin in all the oils studied.

The ratio between the chlorophyll and the carotenoid fractions was maintained at around one unit in oils from Lleida, showing that the green and yellow fractions were balanced. However, in oils from Tarragona and Jaén, this ratio indicated a higher content of chlorophyll than of carotenoids.

No qualitative differences were observed in the HPLC phenolic fraction profile (Fig. 4) between virgin olive oils from different growing regions. However, significant quantitative differences ($P < 0.01$) were observed in a wide number of phenolic compounds (Table 3). As a means of explaining those differences, the phenolic fraction was divided into four main groups (simple phenols, secoiridoid derivatives, flavonoids, and the

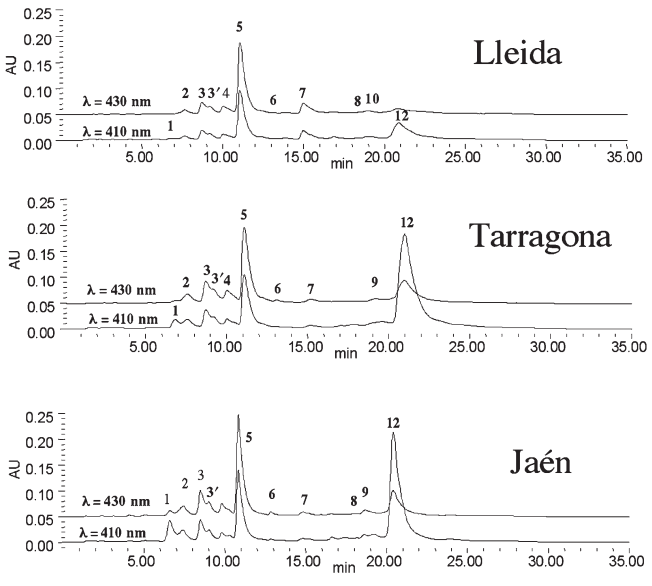

FIG. 2. HPLC chromatograms (detector at 410 and 430 nm) of chlorophyll extracts of Arbequina olive oil from different geographical origins. See Materials and Methods section for chromatographic conditions, and Table 2 for identification.

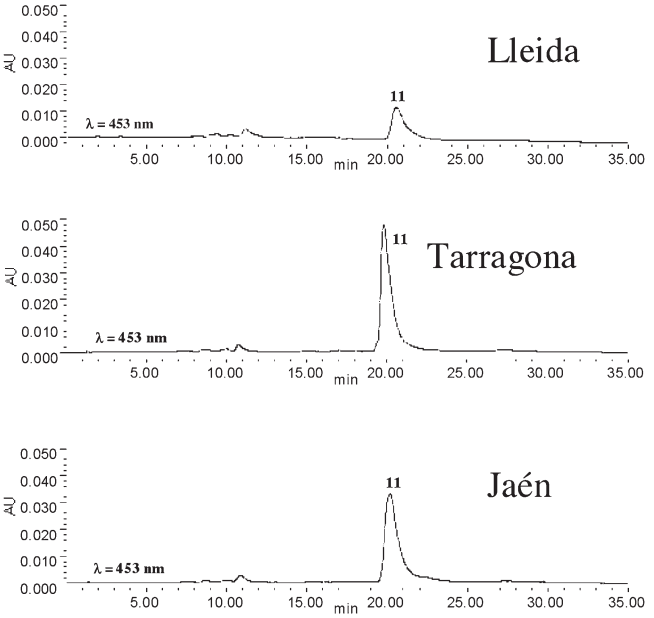

FIG. 3. HPLC chromatogram (detector set at 453 nm) of the all-*trans*-β-carotene (Table 2, peak 11) of Arbequina olive oil from three different geographical origins.

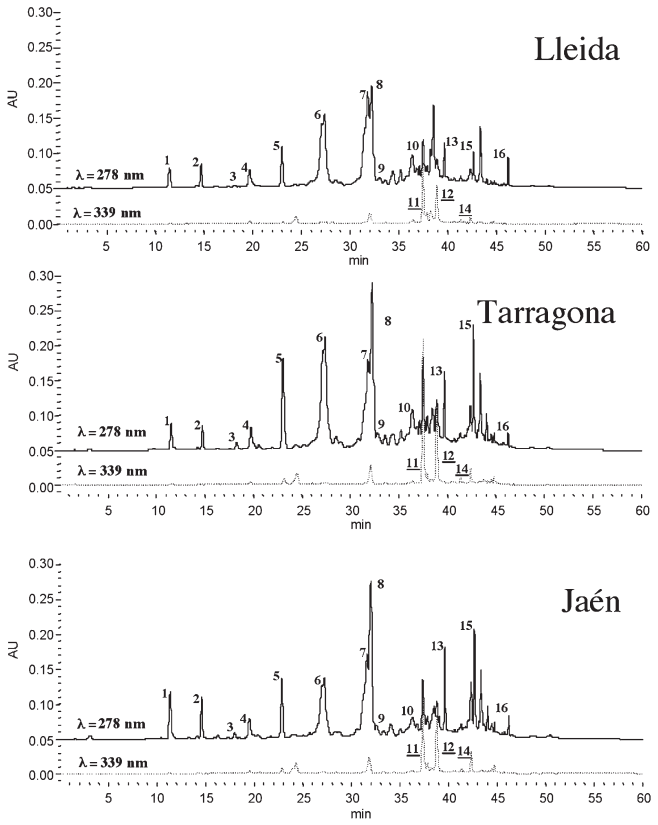

FIG. 4. HPLC chromatograms (detector set at 278 and 339 nm) of phenolic extracts of Arbequina olive oil from three different geographical origins. See Materials and Methods section for chromatographic conditions, and Table 3 for identification.

latter part of the chromatogram, which is composed of unknown phenolic compounds that have UV and mass spectra similar to secoiridoid compounds except for a flavonoid compound identified at RT 42.3 min).

In relation to simple phenols, higher concentrations of hydroxytyrosol and tyrosol were observed in virgin olive oils coming from Jaén. These higher levels could be related to the more advanced maturation indexes of olive drupes in this area.

Higher concentrations of DHPEA-EDA and DHPEA-EA were observed in virgin olive oils from Tarragona. This is closely related to the total phenol content, stability, and bitter index (see Table 1) as reported in Tovar *et al*. (13). The total phenol content varied between 92 and 111 mg kg^{-1}, with oils from Tarragona having the highest values together with the highest oxidative stability (8.80 h) and the highest bitter values (0.158) of all the oils analyzed. The higher phenol content in oil from Tarragona could be due to the altitude of the growing region as proposed by Mousa *et al*. (5), who observed that lower altitudes produced higher phenol contents. The main difference between Lleida and olive oils from the other geographical origins is the *p*-HPEA-EDA/lignans ratio, which is 1.4 in Lleida olive oils whereas it shows values of 0.7 in oils from Jaén and Tarragona.

Low flavonoid levels represented by luteolin and apigenin, were observed in all the olive oils analyzed, with concentrations that varied from 1.24 to 2.91 and 1.26 to 2.37 mg kg^{-1}, respectively, being the most representative compounds of these phenolic compounds. In spite of their low concentrations, flavonoids showed significant differences between olive oils from the three different growing areas.

TABLE 3
Phenolic Compounds (mg kg^{-1}) of Virgin Olive Oil Obtained from Arbequina Cultivar in Three Different Growing Areas of Spain (n = 8)

		Growing area[b]		
Peak	Phenolic compounds[a]	Lleida	Tarragona	Jaén
1	3,4-DHPEA	1.83 ± 0.07[a]	2.53 ± 0.08[b]	4.71 ± 0.23[c]
2	*p*-HPEA	2.65 ± 0.05[a]	2.80 ± 0.13[a]	4.75 ± 0.22[b]
3	Vanillic acid	0.11 ± 0.01[a]	0.28 ± 0.01[b]	0.26 ± 0.00[c]
4	Vanillin	0.39 ± 0.02[a]	0.50 ± 0.02[b]	0.44 ± 0.01[c]
5	3,4-DHPEA-AC	33 ± 4[a]	79 ± 1[b]	48 ± 2[c]
6	3,4-DHPEA-EDA	130 ± 6[a]	214 ± 15[b]	112 ± 8[c]
7	*p*-HPEA-EDA	54 ± 2[a]	44 ± 3[b]	43 ± 2[b]
8	Lignans	38 ± 1[a]	65 ± 5[b]	61 ± 4[b]
9	Peak 9, RT 32.65	3.65 ± 1.08[a]	7.23 ± 0.38[b]	5.26 ± 0.26[c]
10	3,4-DHPEA-EA	137 ± 5[a]	205 ± 34[b]	103 ± 20[a]
11	Luteolin	1.24 ± 0.01[a]	2.91 ± 0.18[b]	1.98 ± 0.16[c]
12	Apigenin	1.26 ± 0.09[a]	2.37 ± 0.13[b]	1.99 ± 20[c]
12	Peak 12, RT 39.65	49 ± 15[a]	73 ± 20[a,b]	91 ± 13[b]
13	Peak 13, RT 42.33	0.07 ± 0.00[a]	0.20 ± 0.00[b]	0.30 ± 0.02[c]
14	Peak 14, RT 42.65	0.91 ± 0.11[a]	8.55 ± 0.11[b]	5.24 ± 0.57[c]
15	Peak 15, RT 46.18	0.89 ± 0.28[a]	0.86 ± 0.20[b]	1.81 ± 0.23[c]

[a]3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycon; RT, retention time (in minutes).
[b]Different superscript roman letters in the same row indicate a significant difference ($P < 0.01$).

The latter part of the chromatogram is made up of several peaks eluting at retention times (RT) from 39 to 47 min; the role of these compounds in oxidative stability and structure is being studied. At present, only their UV spectra and M.W. have been elucidated. The characterization of the molecular structures of these compounds by means of NMR is in progress. Those unidentified compounds are mainly present in olive oils from Tarragona and Jaén, with lower contents in oils from Lleida.

Qualitative study of the minor fractions of virgin olive oil demonstrated a common pattern that is not dependent on growing region and environmental conditions; but significant quantitative differences were observed in the composition of the pigment and phenol fractions. The main differences between oils from Lleida and oils from the other two growing areas in Spain were the different quantitative contributions of chlorophyll *a*, the chlorophyll *a*/chlorophyll *b* ratio, and the lutein/β-carotene ratio. In relation to the phenol fraction, the main differences were in the (i) hydroxytyrosol and tyrosol contents, whose values are higher in oils from Jaén, and (ii) secoiridoid derivatives, whose values are higher in oils from Tarragona, probably owing to the low altitude of the growing region. The *p*-HPEA-EDA/lignans ratio must also be taken into account. This ratio was 1.4 in Arbequina olive oils from Lleida but around 0.7 in the other Arbequina olive oils.

These results could be used to characterize virgin olive oils of the Arbequina variety, using the quantitative and qualitative parameters of pigments and phenols as differentiators of the growing areas. This study should be confirmed by other trials in the next harvest in order to eliminate the crop season effect.

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REFERENCES

1. Alonso, V., and R. Aparicio, Characterization of European Virgin Oils Using Fatty Acids, *Grasas Aceites 44*:18–24 (1993).
2. Bortolomeazzi, R., P. Berno, L. Pizzale, and L.S. Conte, Sesquiterpene, Alkene and Alkane Hydrocarbons in Virgin Olive Oils of Different Varieties and Geographical Origins, *J. Agric. Food Chem. 49*:3278–3283 (2001).
3. Salvador, M.D., F. Aranda, S. Gomez-Alonso, and G. Fregapane, Influence of Extraction System, Production Year and Area on Cornicabra Virgin Olive Oil: A Study of Five Crop Seasons, *Food Chem. 80*:359–366 (2003).
4. Gandul-Rojas, B., and M.I. Minguez-Mosquera, Chlorophyll and Carotenoid Composition in Virgin Olive Oils from Various Spanish Olive Varieties, *J. Sci. Food Agric. 72*:31–39 (1996).
5. Mousa, Y.M., D. Gerosopoulos, I. Metzidakis, and A. Kiritsakis, Effect of Altitude on Fruit and Oil Quality Characteristics of "Mastoides" Olives, *Ibid. 71*:345–350 (1996).
6. Mínguez-Mosquera, M.I., L. Rejano-Navarro, B. Gandul-Rojas, A.H. Sánchez-Gómez, and J. Garrido-Fernández, Color-Pigment Correlation in Virgin Olive Oil, *J. Am. Oil Chem. Soc. 68*:332–336 (1991).
7. Serani, A., and D. Piacenti, Kinetics of Pheophytin *a* Photodecomposition in Extra Virgin Olive Oil, *Ibid. 69*:469–470 (1992).
8. Mínguez-Mosquera, M.I., B. Gandul-Rojas, J. Garrido-Fernández, and L. Gallardo-Guerrero, Pigments Present in Virgin Olive Oil, *Ibid. 67*:192–196 (1990).
9. Mínguez-Mosquera, M.I., B. Gandul-Rojas, and L. Gallardo-Guerrero, Rapid Method of Quantification of Chlorophylls and Carotenoids in Virgin Olive Oil by High-Performance Liquid Chromatography, *J. Agric. Food Chem. 40*:60–63 (1992).
10. Gross, J., *Pigments in Vegetables: Chlorophylls and Carotenoids*, Van Nostrand Reinhold, New York, 1991, pp. 75–254.
11. Krinsky, N.I., Antioxidant Functions of Carotenoids, *Free Radical Biol. Med. 7*:617–635 (1989).
12. Parker, R.S., J.E. Swanson, C.S. You, A.J. Edwards, and T. Huang, Bioavailability of Carotenoids in Human Subjects, *Proc. Nutr. Soc. 58*:155–162 (1999).
13. Tovar, M.J., M.J. Motilva, and M.P. Romero, Changes in the Phenolic Composition of Virgin Olive Oil from Young Trees (*Olea europaea* L. cv. Arbequina) Grown Under Linear Irrigation Strategies, *J. Agric. Food Chem. 49*:5502–5508 (2001).
14. Morelló, J.R., M.J. Motilva, T. Ramo, and M.P. Romero, Effect of Freeze Injuries in Olive Fruit on Virgin Olive Oil Composition, *Food Chem. 81*:547–553 (2003).
15. Finke, P., R. Hartwich, R. Dudal, J. Ibáñez, M. Jamagne, D. King, L. Montanarella, and N. Yassoglou, *EUR 18092—Una Base de Datos de Suelos Georeferenciada para Europa, Manual de procedimientos Versión 1.1,* European Communities, Rome, 1999, pp. 152–160.
16. Montedoro, G., M. Servili, M. Baldioli, and E. Miniati, Simple and Hydrolyzable Phenolic Compounds in Virgin Olive Oil. 1. Their Extraction, Separation and Quantitative and Semiquantitative Evaluation by HPLC, *J. Agric. Food Chem. 40*:1571–1576 (1992).
17. Holden, M., Analytical Methods—Chlorophylls, in *Chemistry and Biochemistry of Plant Pigments Vol. 2*, edited by T.W. Goodwin, Academic Press, New York, 1976, pp. 2–32.
18. Sievers, G., and P.H. Hynninen, Thin-Layer Chromatography of Chlorophylls and Their Derivatives on Cellulose Layers, *J. Chromatogr. 134*:359–364 (1977).
19. Hynninen, P.H., IV Preparation and Purification of Some Derivatives of Chlorophylls a and b, *Acta Chem. Scand. 27*:1771–1780 (1973).
20. Terpstra, W., and J.W.J. Lambers, Interactions Between Chlorophyllase, Chlorophyll a, Plants Lipids and Mg^{2+}, *Biochim. Biophys. Acta 746*:23–31 (1983).
21. Vázquez Roncero, A., C. Janer del Valle, and M.L. Janer del Valle, Determinación de los Polifenoles Totales en Aceite de Oliva, *Grasas Aceites 24*:350–357 (1973).
22. Gutiérrez, F., S. Perdiguero, R. Gutiérrez, and J.M. Olías, Evaluation of the Bitter Taste in Virgin Olive Oil, *J. Am. Oil Chem. Soc. 69*:394–395 (1992).
23. Laübli, M., and P.A. Bruttel, Determination of the Oxidative Stability of Fats and Oils: Comparison Between the Active Oxygen Method and the Rancimat Method, *Ibid. 63*:792–795 (1986).
24. Anderson, J.M., W.S. Chow, and D.J. Goodchild, Thylakoid Membrane Organization in Sun/Shade Acclimation, *Aust. J. Plant Physiol. 15*:11–26 (1988).

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