Effect of Crop Season on the Composition of Virgin Olive Oil with Protected Designation of Origin "Les Garrigues"

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ABSTRACT: In recent years a growing demand for agricultural produce with an identifiable geographical origin has developed. The aim of this work was to study differences in quality and composition of virgin olive oils produced over four consecutive crop seasons in the region of the protected designation of origin "Les Garrigues" (Catalonia, Spain), taking the harvesting period and the climatic conditions of the year into consideration. The results obtained in this study indicate that virgin olive oil composition is greatly influenced by climatic conditions, mainly the cumulative rainfall in the case of FA composition and phenolic compounds, and the minimum temperatures during harvest period in the case of chlorophyll, carotenoid pigments, and α-tocopherol content. The harvest period influenced most of the parameters analyzed, apart from the PV and FFA content. Prediction models for carotenoid pigment content, oxidative stability, and bitter index were found.

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KEY WORDS: Fatty acids, oxidative stability, phenolics, pigments, protected designation of origin, α-tocopherol, virgin olive oil.

In recent years consumers have tended to attach greater importance to the quality rather than the quantity of foodstuffs. This quest for specific products generates a growing demand for agricultural produce or foodstuffs with an identifiable geographical origin. Moreover, the promotion of products with certain specific characteristics can be of considerable benefit to the rural economy, particularly to less-favored areas, by raising farmers' incomes and thus retaining the rural population in these areas.

Virgin olive oils with designation of origin are regulated by administrative norms that define several requirements. These include the existence of an olive-growing area with particular climatic and soil characteristics where oils with similar features have traditionally been obtained; defined agronomic practices and the use of some olive cultivars for oil production; and uniform methodologies for highest-quality virgin olive oil extraction. All this leads to obtaining oil that corresponds to the distinctive sensory quality of the area.

Olive tree cultivation for extra virgin olive oil production is spread over more than two-thirds of the Spanish territory. In Spain, eight regions produce extra virgin olive oils with designation of origin; two of them ("Les Garrigues" and "Siurana") are located in Catalonia. Most of the oil produced in the southern part of Lleida (Catalonia, Spain) is included in the protected designation of origin (PDO) "Les Garrigues," recognized by the Spanish Ministerial Orders of October 28, 1975, and January 22, 1994; the European Community recognition corresponds to EEC Regulation 1107/96 (1).

The PDO "Les Garrigues" is located in the southern part of the Spanish province of Lleida (Catalonia, Spain), and the climate of the area is the continental Mediterranean, characterized by hot, dry summers and long, cold winters. The important thermal difference between winter and summer, with annual minimum temperatures of −8°C and maximum temperatures above 40°C, distinguishes this area from other olive-growing areas with a more temperate climate due to their proximity to the Mediterranean sea. Rainfall in the area is scarce and very irregular, being more abundant in the spring and lowest in summer (July) and winter (January and February). The rainfall pattern differs from one year to another, a feature of the Mediterranean climate.

The native olive tree cultivar in this area is Arbequina, characterized by frost resistance, low vigor, small fruit size, and high productivity. Arbequina olive oil is well known in the international oil market for its excellent taste and flavor. Traditionally, in the area of the PDO "Les Garrigues," olives are hand-picked, and the extraction of oil immediately follows the harvesting of the fruits. The oils are classified as extra virgin and have a distinctive sensory quality as a result of the olive cultivar (Arbequina cv.) and the careful extraction of the oil (2).

Olive oil quality is affected by genetic, agronomic, and environmental factors (3). The altitude and temperature at which the olive trees are grown affect the olive oil composition (4). Seasonal aspects (temperature and rainfall) are part of the agronomic factors and influence the physiology of the plant. Some studies have shown that the climatic conditions, particularly rainfall during the growing and ripening of the olive fruit, influence the quality of the olive oil (5). The stage of ripening of olives and their health (whether they are infested by pests) mainly affect the sensory quality of oils. Olives harvested relatively early yield oil with a fruity flavor, lower acidity, and greener color than olives harvested late in the season (6).

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The aim of this work was to study the differences in quality and composition of virgin olive oils produced over four consecutive crop seasons in the producing region of the PDO "Les Garrigues" (Catalonia, Spain), taking the harvesting period and the climatic conditions of the year into consideration.

EXPERIMENTAL PROCEDURES

Oil samples. Virgin olive oil samples (160) from various olive oil mills from all over the region of "Les Garrigues" were obtained in four successive crop seasons, corresponding to 1996/97, 1997/98, 1998/99, and 1999/00. Forty oil samples were collected from different oil mills located around the region between November 1 and January 31 in each crop season. Oils collected from November 1 to December 15 were classified as the first of two harvest periods (20 samples × crop season), and oils collected between December 15 and January 31 were classified as the second harvest period (20 samples × crop season). Oils were taken directly from the production line on the basis of a protocol established by the Regulatory Organism of the PDO "Les Garrigues."

Climatic data (temperatures and rainfall) were obtained daily during the four experimental years (1996–1999) by the meteorological station situated at La Granadella in the geographical center of the "Les Garrigues" region. The average values of the maximum and minimum temperatures and the amount of precipitation for each week have been used (Figs. 1, 2) for discussion of the results. In this region, autumn frosts are very common, and the harvesting period covers the 3 mon from November to January.

Oil quality parameters. Oil quality parameters, such as the FFA content, PV, and UV absorption characteristics (K_{270}), were determined according to the analytical methods described in European Union Commission Regulations EEC/2568/91 (7). The results are expressed as percentage of oleic acid (% oleic acid), milliequivalents of active oxygen per kilogram of oil (meq O_2/kg), and absorbance at 270 nm, respectively.

FA composition. The FA composition of the oils was determined by GC as FAME. FAME were prepared by saponification/methylation with sodium methylate according to European Union Commission modified Regulation EEC 2568/91 (8). Chromatographic analysis was performed in an HP 5890 Series II gas chromatograph equipped with an FID (Hewlett-Packard, Palo Alto, CA), using an SP 2330 capillary column (30 m, 0.25 mm i.d., 0.20 µm film thickness; Supelco Inc., Bellefonte, PA). The column temperature was isothermal at 190°C, and the injector and detector temperatures were 220°C. FA were identified by comparing retention times with standard compounds, which were obtained from Sigma

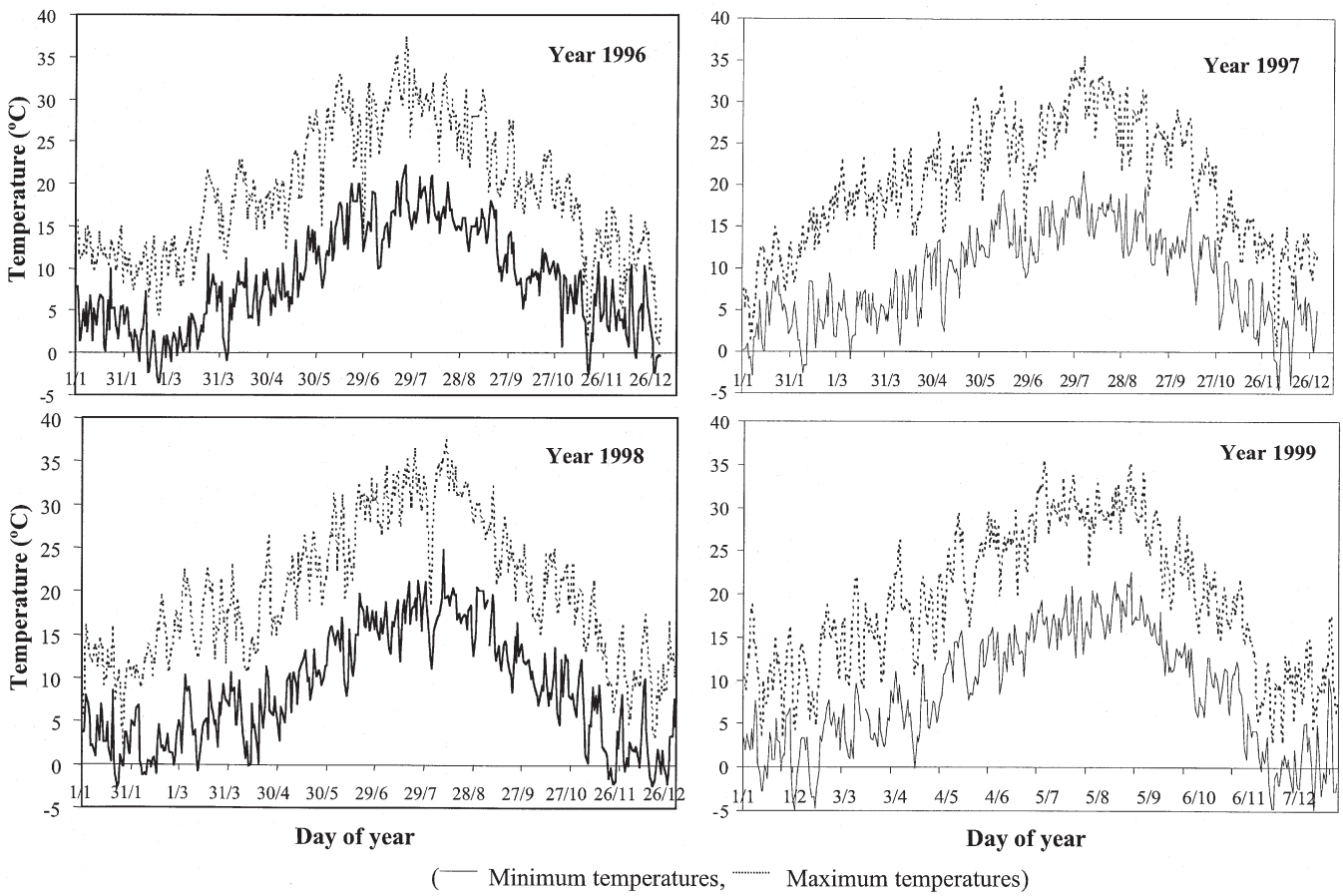

FIG. 1. Annual patterns of air temperature for 1996, 1997, 1998, and 1999.

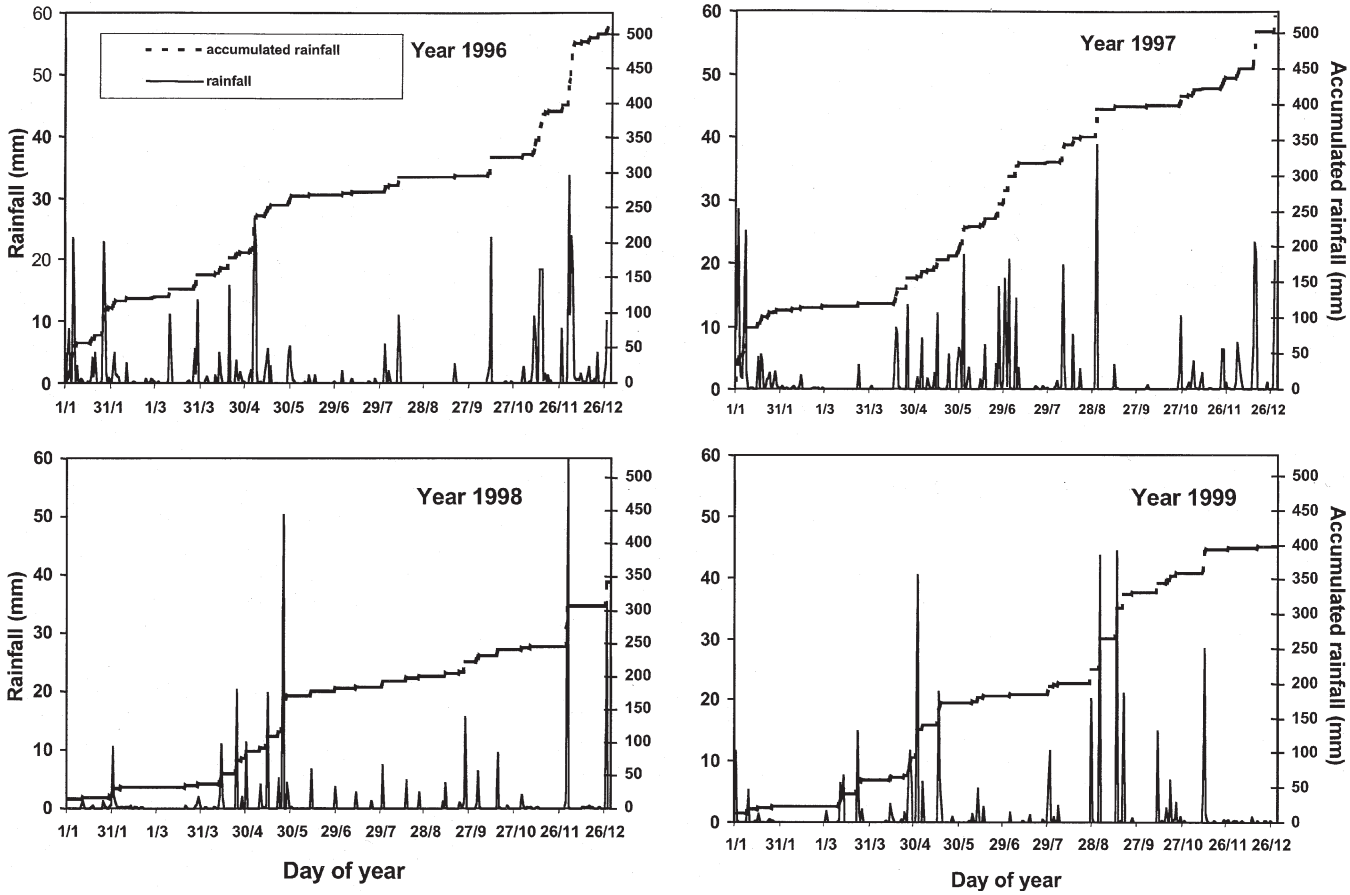

FIG. 2. Annual patterns of daily rainfall (mm) and accumulated rainfall (mm) for 1996, 1997, 1998, and 1999.

Chemical Co. (St. Louis, MO). Six FA were considered in this study: palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3), expressed as percentages of FAME.

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) (9). The chlorophyll and carotenoid content are expressed as mg of pheophytin *a* and lutein per kg of oil, respectively.

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. Oil samples were examined without dilution to avoid color variation, and the tristimulus values X, Y, and Z were calculated for illuminant C from the absorption spectrum (10). The oil color is expressed as chromatic ordinates a*, b*, and L*.

α-Tocopherol. α-Tocopherol was evaluated by HPLC with direct injection of an oil-in-hexane solution: 1.5 ± 0.01 g of oil dissolved in hexane to 10 mL. The standard was obtained from Sigma Chemical Co. Detection and quantification was carried out in a Waters 600 apparatus with a photodiode detector array (Waters 996) set at 295 nm. The 25-cm × 4-mm i.d. column used was filled with Supelcosil LC-NH_2, 5 µm (Supelco, Inc.). The volume of injection was 20 µL. The mobile phase consisted of hexane/ethyl acetate (70:30) at a flow rate of 1 mL/min. α-Tocopherol was quantified by the external standard method. Results are given as milligrams of α-tocopherol per kilogram of oil.

Phenolic content. The polar fraction of the oils was obtained using the modified method described by Vázquez-Roncero *et al.* (11). Oil samples (10 g) dissolved in hexane (50 mL) were extracted with methanol/water (60:40, vol/vol, 3 × 10 mL). The aqueous fractions were collected in a volumetric flask (50 mL) to obtain the total polyphenol extract. Total phenols were measured colorimetrically at 725 nm after adding the Folin–Ciocalteau reagent to the extract. *Ortho*-diphenols were measured colorimetrically at 370 nm after adding 5% (wt/vol) sodium molybdate in 50% ethanol to the extract (12). Results are given as milligrams of caffeic acid per kilogram of oil.

Oil stability. A stability test was carried out using a 679 Rancimat apparatus (Metrohm Co., Basel, Switzerland) at 120°C and 20 L h^{-1} air flow (13). The oil stability is expressed as the induction time (h) of hydroperoxide decomposition.

Bitter index (K_{225}). K_{225} was evaluated by extraction of the bitter components of samples of 1.0 ± 0.01 g oil dissolved in 4 mL hexane and passed through an octadecyl (C_{18}) column (Sep-Pack Cartridges; Waters, Milford, MA), previously

activated with methanol (6 mL) and washed with hexane (6 mL). After elution, 10 mL of hexane was passed through to eliminate fat, and then the retained compounds were eluted with methanol/water (1:1) to 25 mL (14). The absorbance of the extract was measured at 225 nm against methanol/water (1:1) in a 1-cm cuvette.

The results for the α-tocopherol content correspond to the 1997, 1998, and 1999 crop seasons, and those for the bitter index to the 1998 and 1999 crop seasons, when the methods were validated.

Statistical analysis. The data were subjected to ANOVA using Version 8.1 of the SAS system package (SAS Institute Inc., Cary, NC). Separation of the means was obtained using the LSD test at the 95% level. Unless otherwise stated, significant differences are at the 5% level. A regression procedure was used to establish the relationship between the chromatic ordinates and the oil pigment content. Stepwise linear regression analysis (SLRA) was applied to select the chemical variables that better explained the oil stability.

RESULTS AND DISCUSSION

Figures 1 and 2 show annual patterns of air temperature and daily and accumulated rainfall, respectively, during the four years studied (1996, 1997, 1998, and 1999). The weather in 1996 was characterized by occasional frosts in November and December and a very dry summer. Total cumulative rainfall in 1996 was 513 mm, markedly higher than average for this area (350–400 mm). In 1997, rainfall fell mainly in the spring and summer (total accumulated rainfall was 523 mm). The main climatic characteristics in 1998 were the persistent frosts during November and December and the scarce rainfall, especially during the summer. Accumulated rainfall in this year was 342 mm. The 1999 weather was characterized by very severe frosts (below –5°C) and a total absence of rainfall during the harvest period (November and December). Maximum accumulated rainfall was during September (total accumulated rainfall in this year was 397 mm).

The oil quality parameters are summarized in Table 1. In all seasons, oils from the first harvest period showed the lowest FFA content and UV absorption (K_{270}). PV remained almost constant throughout the harvest period in each crop season. The effect of crop season was significant ($P < 0.001$) for all parameters analyzed, particularly for PV, being significantly higher in oils from the 1997/98 season. However, the average quality parameter values of all the oils in this study were considerably lower than the limits established by the European Union Commission (7) for the highest-category extra virgin olive oils.

The FA composition of the oils was not affected by harvest period (Table 2). However, season had a very important effect; oils from the 1997/98 crop season showed a significant difference ($P < 0.001$) in FA composition in relation to oils from the other crop seasons. The percentages of stearic, oleic, and linolenic acids were lowest and palmitic, palmitoleic, and linoleic acids were highest in the oils from the 1997/98 crop season. This could be attributed to a modification of lipid biosynthesis coinciding with the wet summer of 1997. Environmental factors, such as light, temperature, and water stress, affect lipid levels and metabolism in the olive fruit (15), and some authors have observed that oleic acid, some TAG, and the oleic/linoleic acid ratio are connected to the rainfall in the summer period (16). The rainfall regime in 1997 could have affected the *de novo* FA biosynthesis that occurs in plant plastids and that needs the concerted activity of two enzymes, acetyl-CoA carboxylase and FA synthase, to regulate a further chain elongation cycle in *de novo* FA biosynthesis. This step is particularly relevant because it determines the C_{16}/C_{18} ratio, and this is directly related to the degree of unsaturation of the final oil product (17). The highest C_{16}/C_{18} ratio was 0.21 in oils from the 1997/98 season (Table 2), and 0.17, 0.15, and 0.16 in oils from 1996/97, 1998/99, and 1999/00 crop seasons, respectively. This ratio seems to be related to the accumulated rainfall regime in the summer of each year. In considering the accumulated rainfall (Fig. 2) during June–August, the maximum corresponds to

TABLE 1
Oil Quality Parameters and Significant Differences in the Means of the Same Harvest Period in Relation to Crop Season and Between Harvest Period[a]

Parameter	Harvest period	Crop season 1996/97	1997/98	1998/99	1999/00	Sig. level
FFA content	First	0.12[a]	0.15[b]	0.13[a,b]	0.12[a]	**
(% oleic acid)	Second	0.14	0.17	0.15	0.17	NS
	Sig. level	*	*	*	**	
PV	First	6.0[a]	7.9[b]	5.0[a]	6.2[a]	***
(meq $O_2 \cdot kg^{-1}$)	Second	6.2[a]	7.5[b]	5.6[a]	6.4[a]	***
	Sig. level	NS	NS	NS	NS	
UV absorption	First	0.09[a]	0.10[b]	0.10[b]	0.09[a]	**
(K_{270})	Second	0.12[a]	0.10[b]	0.12[a]	0.10[b]	**
	Sig. level	**	NS	**	**	

[a]Significance level by row and column for each parameter: NS = not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Different letters within the same row indicate a significant difference.

TABLE 2
Oil FA Composition (expressed as %) and Significant Differences in the Means of the Same Harvest Period in Relation to Crop Season and Between Harvest Period[a]

FA	Harvest period	Crop season 1996/97	1997/98	1998/99	1999/00	Sig. level
Palmitic acid	First	12.8[a]	15.5[b]	12.5[a]	12.8[a]	***
	Second	13.1[a]	15.0[b]	11.7[c]	12.6[d]	***
	Sig. level	NS	NS	NS	NS	
Palmitoleic acid	First	1.44[a]	1.65[b]	1.32[a]	1.01[a]	***
	Second	1.58[a]	1.62[a]	1.14[b]	0.96[c]	***
	Sig. level	NS	NS	NS	NS	
Stearic acid	First	1.92[a]	1.58[b]	2.01[a]	2.12[c]	***
	Second	1.74[a]	1.54[b]	2.11[c]	2.10[c]	***
	Sig. level	NS	NS	NS	NS	
Oleic acid	First	73.6[a]	69.0[b]	73.4[a,c]	74.6[c]	***
	Second	73.1[a]	69.7[b]	74.3[a,c]	74.8[c]	***
	Sig. level	NS	NS	NS	NS	
Linoleic acid	First	9.6[a]	11.4[b]	9.9[a]	9.0[c]	***
	Second	9.8[a]	11.5[b]	10.0[a]	9.1[c]	***
	Sig. level	NS	NS	NS	NS	
Linolenic acid	First	0.33[a]	0.19[a]	0.61[a]	0.45[a]	***
	Second	0.30[a]	0.17[b]	0.53[c]	0.46[d]	***
	Sig. level	NS	NS	NS	NS	
C_{16}/C_{18} ratio		0.17	0.21	0.15	0.16	

[a]Significance level by row and column for each parameter: NS = not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Different letters within the same row indicate a significant difference.

1997, with 350 mm at the end of the period, 300 mm in 1996, and 200 mm in 1998 and 1999. These climatological conditions could cause modification in the degree of unsaturation of the oils.

The color of an oil affects the consumer's perception of quality. This property can also be used as an estimate of pigment content. Table 3 shows the pigment concentration and the chromatic ordinates of oils from the four seasons studied. The effect of harvest period on the chlorophyll and carotenoid contents is different in relation to the crop season. Thus, the main differences were observed in oils from the 1997/98 and 1999/00 seasons. This is probably a consequence of frosts in November 1997 and 1999 (Fig. 1) that led to deterioration of the olive fruit and pigment degradation, mainly in the chlorophyll fraction. Oils from the 1998/99 crop season showed no significant differences between the first and second harvest periods. The minimum air temperature during the harvest period (November 1998) remained around 0°C without heavy frosts that could have produced pigment degradation in the olive fruit. The color measurement by tristimulus coordinates CIELAB (b*, a*, and L*) obtained from the absorption spectra of the oils showed significant differences related to harvest period. The color changes in oils during the harvest could be attributed to a reduction of the pigment concentration. The values of b* correspond to the yellow zone and decreased with the harvest period, mainly in oils from the 1997/98 and 1999/00 crop seasons, similarly to the values observed in the pigment content. The values of a* are found in the green zone, and the variation was minimal throughout the harvest. In general, the luminosity values (L*) increased significantly in the second harvest period, probably as a consequence of the reduction of the pigment content in the oils, as pigments would capture part of the light instead of transmitting it.

There were significant differences in the pigment content and the color of the oils in relation to the year (Table 3). Oils from the 1999/00 crop season showed the lowest chlorophyll content. As mentioned above, the 1999 harvest period was characterized by frequent, heavy frosts that may have initiated the degradation of the olive pigments, mainly the chlorophyll fraction. Very significant differences between seasons were also observed in color parameters. Oils from the 1996/97 crop season showed the lowest b* values. There were no differences among oils from the other seasons. Luminosity (L*) of oils showed differences. The lowest value corresponded to oils from the 1996/97 crop, and the highest to oils from the 1997/98 and 1999/00 seasons. Thus, with respect to pigment content and oil color, the main effect was the minimum air temperature during the harvest period (November–December); rainfall regime was a secondary effect.

Because the tristimulus color measurement is easy to obtain, this was used to estimate the pigment composition. Thus, a correlation analysis between color and pigment was given.

TABLE 3
Oil Pigment Content (chlorophyll and carotenoid) and Color (expressed as chromatic ordinates b*, a*, and L*) and Significant Differences in the Means of the Same Harvest Period in Relation to Crop Season and Between Harvest Period[a]

Parameter	Harvest period	Crop season 1996/97	1997/98	1998/99	1999/00	Sig. level
Chlorophyll (mg kg^{-1})	First	9.83[a]	12.24[b]	10.42[a,b]	7.11[c]	***
	Second	7.49[a]	6.78[a]	7.75[a]	5.13[b]	*
	Sig. level	*	***	NS	**	
Carotenoid (mg kg^{-1})	First	8.60[a]	8.60[a]	11.08[b]	8.20[a]	***
	Second	6.88[a]	6.10[a]	9.26[b]	6.29[a]	***
	Sig. level	*	***	NS	**	
b*	First	95.8[a]	108.8[b]	108.3[b]	105.9[b]	***
	Second	90.0	93.3	96.5	92.6	NS
	Sig. level	NS	***	*	***	
a*	First	–1.59[a]	–2.99[b]	–0.61[a]	–1.03[a]	***
	Second	–2.30[a]	–3.53[b]	–0.85[a]	–2.12[a]	***
	Sig. level	NS	NS	NS	*	
L*	First	76.4[a]	85.9[b]	81.8[c]	84.7[b]	***
	Second	82.3[a]	89.3[b]	87.2[b,c]	86.2[c]	***
	Sig. level	***	***	*	*	

[a]Significance level by row and column for each parameter: NS = not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Different letters within the same row indicate a significant difference.

The best correlation coefficient was obtained between carotenoid pigments and the b*/L* ratio. A polynomial regression analysis was applied to analyze the relationship between the b*/L* ratio and the carotenoid pigment concentration in the oils, with samples from the 1996/97 to 1999/00 crop seasons. The best-fitted model, corresponding to a second-order polynomial model (adjusted $R^2 = 0.80$), was

$$\text{carotenoid} = 22.8992 - 38.4461\ (\text{b*/L*}) + 21.3727\ (\text{b*/L*})^2 \quad [1]$$

Table 4 shows the main components that are related to oil stability. The tocopherol content of virgin olive oil is important to protect lipids against autoxidation and, thereby, to increase

TABLE 4
Oil α-Tocopherol and Phenolic (total and *o*-diphenol) Content, Bitter Index and Stability and Significant Differences in the Means of the Same Harvest Period in Relation to Crop Season and Between Harvest Period[a]

Parameter	Harvest period	Crop season 1996/97	1997/98	1998/99	1999/00	Sig. level
α-Tocopherol (mg kg^{-1})	First	—	215.8[a]	289.4[b]	136.7[c]	***
	Second	—	199.1[a]	265.5[b]	123.3[c]	***
	Sig. level		***	NS	***	
Total phenols (mg kg^{-1})	First	272.5[a]	106.3[b]	242.0[a]	175.8[c]	***
	Second	215.2[a]	84.0[b]	171.9[a]	88.0[c]	***
	Sig. level	***	*	NS	***	
o-Diphenols (mg kg^{-1})	First	—	8.57[a]	25.6[b]	8.29[a]	***
	Second	—	7.28[a]	16.0[b]	1.04[c]	***
	Sig. level		NS	NS	***	
Stability (h)	First	16.7[a]	9.61[b]	15.7[a]	10.0[b]	***
	Second	13.4[a]	8.24[b]	12.6[a]	5.4[c]	***
	Sig. level	***	NS	NS	***	
Bitter index (K_{225})	First	—	—	0.19	0.17	NS
	Second	—	—	0.15	0.12	NS
	Sig. level			NS	***	

[a]Significance level by row and column for each parameter: NS = not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Different letters within the same row indicate a significant difference.

its storage life and value as a wholesome food. The range of α-tocopherol contents in olive oil from PDO "Les Garrigues" for each crop season and harvest period is wide. There were significant ($P < 0.001$) differences in the α-tocopherol content between the crop seasons studied, and the effect of the harvest period was significant ($P < 0.001$) in oils from the 1997/98 and 1999/00 seasons. Oils from the 1998/99 crop showed no significant differences between harvest periods, but a decrease was observed in the second harvest period. As with the pigment contents (Table 3), oils from the 1999/00 crop season showed the lowest tocopherol content. As mentioned above, the minimum air temperature in the area during the 1999 harvest period (November–December) was characterized by frequent, heavy frosts that produced a considerable degradation of the olives. As a consequence, the fleshy tissue of the olives softened during this crop season and underwent browning, probably derived from lipoxygenase activity.

The amount of phenolic compounds in virgin olive oil is an important factor when evaluating its quality, given that the natural phenols improve its resistance to oxidation and, to a certain extent, are responsible for its sharp bitter taste. The total phenols in the oils analyzed in this study varied considerably and a significant ($P < 0.001$) effect of the year could be observed (Table 4). Thus, oils from the 1997/98 crop season had the lowest total phenol concentration (106.3 to 84.0 mg kg^{-1}), and the highest values were in oils from the 1996/97 and 1998/99 seasons (from 272.5 to 215.2 and from 242.0 to 171.9 mg kg^{-1} for each year and harvest period, respectively). A relation was observed between the total phenols content of the oil and accumulated rainfall by year (Fig. 2). Thus, the weather in 1997, which corresponded to the year with the lowest polyphenol content in the olive oils, was characterized by nearly 400 mm accumulated rainfall during the summer period (June–August), above the average for the area. In contrast, during this period, there was a very low rainfall accumulation in 1996 and 1998, and the oils from these years showed the highest polyphenol contents. It has long been known that the level of phenolics in plant tissues can be influenced by environmental factors such as ambient temperature and water availability. With regard to the latter factor, a water deficit tends to generate a stress situation that induces the production of phenolics (18), and this factor could be related to the increase in the polyphenol content of the oils from the 1996/97 and 1998/99 seasons.

The effect of harvest period differed by crop season, but the level of polyphenols in the oils studied followed a similar pattern in all the years, showing higher values in the first period (Table 4). Their level usually decreases with maturation of the olives, which could explain the lower phenolic concentration observed in our study in oils from the second harvest period. *o*-Diphenol concentrations were also studied, except in oils from the 1996/97 season. Significant differences were found among *o*-diphenol contents of all the samples from the different harvesting periods and seasons analyzed, similar to those observed in the total phenol contents.

The oxidative stability of oils, measured as the induction time determined using the Rancimat method, showed the same trend in relation to crop season and harvest period as the total phenol and *o*-diphenol contents of the oils (Table 4). Significant ($P < 0.001$) differences were found between the stability of oils from the 1996/97 and 1998/99 seasons (16.7 to 13.4 and 15.7 to 12.6 h, respectively) and oils from the 1997/98 and 1999/00 seasons (9.6 to 8.2 and 10.0 to 5.4 h, respectively). The effect of harvest period was very significant ($P < 0.001$) in oils from the first and last seasons, as it was for phenolic content. It is important to emphasize that the stability of the oils from the 1997/98 season, which showed the lowest total phenols concentration, was similar to that of oils from the 1999/00 season. This fact could be explained by a degradation of *o*-diphenol compounds, mainly implicated in oil stability, as a consequence of the frequent, heavy frosts in the area during the 1999 harvest period (November–December).

Bitter taste is one of the characteristic attributes of virgin olive oil. In our study, the bitter index (K_{225}) was analyzed in oils from the 1998/99 and 1999/00 crop seasons with values from 0.19 to 0.15 and from 0.17 to 0.12 in the first and second harvest period and each crop season, respectively (Table 4). There were no differences in K_{225} between seasons, and the effect of harvest period was only significant ($P < 0.001$) during the 1999/00 season, similar to that observed in oil phenol content and stability. Gutierrez *et al*. (14) suggested that K_{225} values of the order of 0.14 or lower correspond to oils with a slight bitterness intensity, corresponding to oils from the area of the PDO "Les Garrigues," and values close to 0.36 correspond to quite bitter oils.

A stepwise linear regression analysis (SLRA) procedure was applied to analyze the relationship between oxidative stability, as a dependent variable, and all parameters analyzed (independent variables) in oils corresponding to the seasons studied. The values of *F*-to-enter and *F*-to-remove statistical variables were selected from the *F*-table at 0.99. Under these conditions, SLRA selected the following chemical variables: total phenols, carotenoids, α-tocopherol, linolenic acid, and PV. Thus, oil stability (OS in hours) was fitted to the following equation (adjusted $R^2 = 0.9275$):

$$\text{OS} = 1.2773 + 0.0463\ (\text{total phenols}) + 0.2033\ (\text{carotenoid}) + 0.0171\ (\alpha\text{-tocopherol}) - 5.4215\ (\text{linolenic acid}) - 0.1111\ (\text{PV}) \quad [2]$$

SRLA selected five parameters as independent variables—total phenol, carotenoid, and α-tocopherol content, which contribute positively to Arbequina virgin olive oil stability, and the percentage of linolenic acid and PV, which contribute negatively. These results agree with the studies by Aparicio *et al*. (19) in Picual and Hojiblanca cultivars, which showed a clear influence of total phenols on olive oil stability and a much lower contribution of α-tocopherol and linoleic acid.

The SLRA procedure was applied to predict the value of the bitter index. The model includes only the phenol content, so a polynomial regression was applied. The results showed that a second-order polynomial model describes the relationship between polyphenol content and the bitter index. The

equation of the model was (adjusted $R^2 = 0.7505$):

$$K_{225} = 0.0377 + 1.0167 \cdot 10^{-3} \text{ (total phenols)} - 1.3812 \cdot 10^{-6} \text{ (total phenols)}^2 \quad [3]$$

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REFERENCES

1. European Union Commission, Regulation EEC 1107/96 on the Registration of Geographical Indications and Designations of Origin Under the Procedure Laid Down in Article 17 of Council Regulation (EEC) 2081/92, 1996.
2. Motilva, M.J., I. Jaria, I. Bellart, and M.P. Romero, Estudio de la Calidad del Aceite de Oliva Virgen de la Denominación de Origen "Les Garrigues" (Lleida) Durante la Campaña 1995/96, *Grasas Aceites 49*:425–433 (1998).
3. Kiritsakis, A., *Olive Oil,* Food & Nutrition Press, Trumbull, CT, 1998.
4. Osman, M., I. Metzidakis, D. Gerasopoulos, and A. Kiritsakis, Qualitative Changes in Olive Oil Collected from Trees Grown at Two Altitudes, *Riv. Ital. Sost. Grasse 71*:187–190 (1994).
5. Panelli, G., M. Servili, R. Selvaggini, M. Baldioli, and G.F. Montedoro, Effect of Agronomic and Seasonal Factors on Olive (*Olea europaea* L.) Production and on the Qualitative Characteristics of the Oil, *Acta Hortic. 356*:239–244 (1994).
6. García, J.M., S. Seller, and M.C. Pérez-Camino, Influence of Fruit Ripening on Olive Oil Quality, *J. Agric. Food Chem. 44*: 3516–3520 (1996).
7. European Union Commission, Regulation EEC 2568/91 on the Characteristics of Olive and Olive Pomace Oils and Their Analytical Methods, *Off. J. Eur. Communities L248,* 1991.
8. León Camacho, M., and A. Cert, Recomendaciones para la Aplicación de Algunos Métodos Analíticos Incluidos en el Reglamento CEE 2568/91 Relativo a las Características de los Aceites de Oliva y de Orujo de Oliva, *Grasas Aceites 47*: 395–401 (1994).
9. Mínguez Mosquera, M.I., L. Rejano, B. Gandul, A.H. Sánchez, and J. Garrido, Color–Pigment Correlation in Virgin Olive Oil, *J. Am. Oil Chem. Soc. 68*:332–336 (1991).
10. CIE Publication No. 15.2, Colorimetry (Technical Report). CIE Central Bureau, Vienna, 1986.
11. 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).
12. Gutfinger, T., Polyphenols in Olive Oils, *J. Am. Oil Chem. Soc. 58*:966–968 (1981).
13. 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).
14. Gutiérrez, F., S. Perdiguero, R. Gutiérrez, and J.M. Olías, Evaluation of the Bitter Taste in Virgin Olive Oil, *Ibid. 69*:394–395 (1992).
15. Harwood, J.L., Effects of Environment on the Acyl Lipids of Algae and Higher Plants, in *Structure, Function and Metabolism of Plant Lipids*, edited by P.A. Siegenthaler and W. Eichenberger, Elsevier, Amsterdam, 1984, pp. 543–550.
16. Angerosa, F., L. di Giacinto, C. Basti, and A. Serraiocco, Influenza della Variabile "Ambiente" sulla Composizione degli Oli Vergini di Oliva, *Riv. Ital. Sost. Grasse 73*:461–467 (1996).
17. Salas, J.J., J. Sánchez, U.S. Ramli, A.M. Manaf, M. Williams, and J.L. Harwood, Biochemistry of Lipid Metabolism in Olive and Other Oil Fruits, *Prog. Lipid Res. 39*:151–180 (2000).
18. Parr, J.A., and G.P. Bolwell, Review. Phenols in the Plant and in Man. The Potential for Possible Nutritional Enhancement of the Diet by Modifying the Phenols Content or Profile, *J. Sci. Food Agric. 80*:985–1012 (2000).
19. Aparicio, R., L. Roda, M.A. Albi, and F. Gutiérrez, Effect of Various Compounds on Virgin Olive Oil Stability Measured by Rancimat, *J. Agric. Food Chem. 47*:4150–4155 (1999).

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