Influence of Seasonal Conditions on the Composition and Quality Parameters of Monovarietal Virgin Olive Oils

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ABSTRACT: The aim of this work was to determine the effect of the climatological conditions of the olive crop season on the composition of monovarietal virgin olive oils obtained from the *Arbequina* cultivar with special emphasis on the phenolic fraction, its percent distribution, and related oil quality parameters such as oxidative stability and bitter index. The main differences were due to freeze injuries caused by low temperatures in December 2001. The levels of chlorophylls and carotenoids in olive oil or pulp from frost-damaged olive trees were lower as a consequence of faster ripening. The olive oil extracted from frost-damaged olive pulp had lower contents of secoiridoid and especially lower levels of 3,4-DHPEA-EDA (the dialdehydic form of elenolic acid linked to hydroxytyrosol). In the following crop seasons, a significant increase in phenolic compounds, especially in secoiridoid derivatives such as 3,4-DHPEA-EDA, was observed. This increase may be due to the fact that olive trees that suffered frost damage in December 2001 were more sensitive to stress caused by the water deficit during summer in the subsequent crop seasons, which is usual in this olive-growing region. Moreover, important correlation coefficients were observed between the main secoiridoid derivative compound (3,4-DHPEA-EDA) and oxidative stability and the bitter index.

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KEY WORDS: Crop season, oxidative stability, phenolic compounds, virgin olive oil.

World olive oil production is about 2.8 million tonnes, and represents 2.3% of the total for vegetable oil (1). The main producers are European countries, such as Spain (56%), Italy (26%), and Greece (16%), although the olive tree is cultivated as far away as South Africa, Australia, China, and Japan. During recent years, the perceived health benefits of olive oil, a continuing interest in Mediterranean cuisine, and promotion by the controlling body of the industry and the International Olive Oil Council have stimulated market demand for olive oil, particularly in countries not traditionally associated with this oil, such as the United States and Japan.

Virgin olive oils are the oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal, that do not lead to alterations in the oil; furthermore, these oils have not undergone any treatment other than washing, decantation, centrifugation, and filtration. Varietal virgin olive oils are labeled not only with the producer's name and usually the place of origin but also with the olive fruit varieties used to give them their distinctive character. Olive oil production in Spain has taken an unexpected new direction. In this way, botanists, chemists, oil producers, and chefs have turned their focus on the origins of the oil. Although varieties such as *Arbequina*, *Picual*, *Cornicabra,* and *Hojiblanca* are very popular, new varieties such as *Lechín*, *Manzanilla Cacereña* and *Verdial* are also getting attention. Some of the new generation of oils, identified by the olive varieties, are derived from a single variety. In classic or experimental blends, varietal oils are carefully mixed to give a finely tuned taste profile.

The climatology of the year may be more important than genetic factors in determining the chemical components that characterize oil quality. Different studies with monovarietal virgin olive oils have shown that rainfall, particularly during the growing and ripening of the olive fruit, is one of the most important environmental factors affecting oil composition, mainly the minor components, of virgin olive oils (2–6). A study over four consecutive seasons with monovarietal virgin olive oils obtained from the *Arbequina* cultivar growing in the area of the Protected Designation of Origin (PDO) "Les Garrigues" (Catalonia, Spain) (7) indicated a great influence of climatic conditions. The cumulative rainfall during the summer period affected the composition of FA and of phenolic compounds, whereas low temperatures during the olive harvest period influenced the contents of chlorophyll, carotenoid pigments, and α-tocopherol.

The aim of this work was to determine the effect of the climatological conditions of the olive crop season on the composition of monovarietal virgin olive oils with special emphasis on the phenolic fraction and related oil quality parameters, such as oxidative stability (OS) and the bitter index.

MATERIALS AND METHODS

Olive oil. Seventy virgin olive oil samples from various olive oil mills from all over the region of "Les Garrigues" were obtained in four successive crop seasons, corresponding to 2000/01 [10 samples first harvest period + 10 samples second harvest period (see below)], 2001/02 (10 + 4 samples), 2002/03 (9 + 9 samples), and 2003/04 (9 + 9 samples). In this region, autumn frosts are very common, and the harvest period covers

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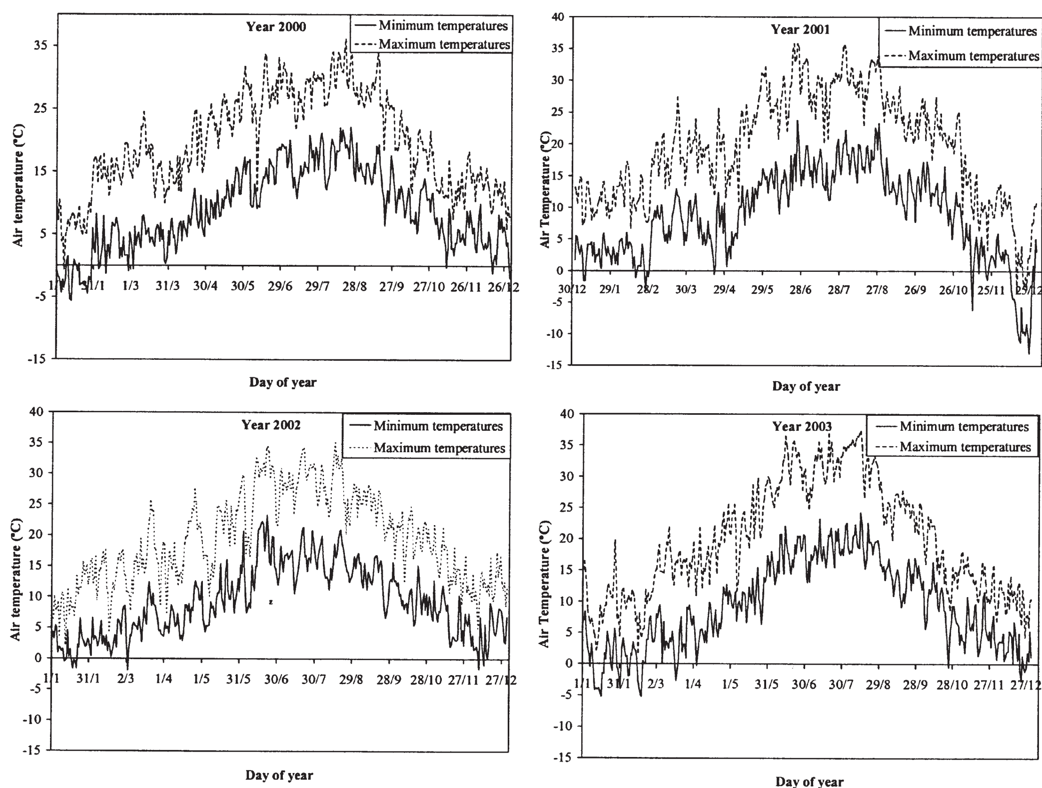

FIG. 1. Annual patterns of air temperature for 2000, 2001, 2002, and 2003.

the 3 mon from November to January. Oils samples collected from November 1 to December 15 were classified as being from the first harvest period, and oils collected between December 15 and January 31 were classified as the second harvest period. The oils were taken directly from the production line under a protocol established by the Regulator Organism of the PDO "Les Garrigues."

Daily climatic data (temperature and rainfall) were obtained during the four experimental years (2000–2003) from the meteorological station situated at La Granadella in the geographical centre of the "Les Garrigues" region. The maximum and minimum temperatures and the amount of precipitation for each day (Figs. 1 and 2) were correlated to oil quality.

HPLC analysis of the phenolic compounds. Phenols were extracted from virgin olive oil (45 g) with methanol/water (60:40, vol/vol) (2 × 20 mL), following the procedure of Tovar *et al.* (8). The phenolic fraction extracted was dissolved in 1 mL of methanol and analyzed by HPLC (loop 20 µL). The HPLC system included a Waters 600 E pump, a Waters column heater, a Waters 717 plus autosampler, and a Waters 996 photodiode array detector. 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, Barcelona, Spain) precolumn. HPLC analysis was performed following the same procedure as in Reference 8. A gradient elution using 0.2% acetic acid (pH 3.1) and methanol with a flow rate of 1.5 mL/min was used. The injection volume was 20 µL. The gradient elution with total run time of 60 min consisted of 95% aqueous phase (0.2% acetic acid) and 5% methanol for 2 min followed by an increase in methanol concentration to 40, 50, and 100% over the following 33-, 10-, and 10-min periods, respectively. Methanol concentration was maintained at 100% for the last 5 min. Empower software (Waters) was used, and chromatograms were obtained at 278 and 339 nm.

Reference compounds. Tyrosol (*p*-HPEA) 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 (3,4-DHPEA) was obtained by acid hydrolysis of oleuropein glucoside (9). The rest of the phenolic compounds were obtained from olive oil phenolic extract using a semipreparative Spherisorb ODS-2 HPLC column (5 µm, 25 cm × 10 mm i.d.; Technokroma) and a flow rate of 4 mL/min. The mobile phases and gradient are described elsewhere (8). 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 peaks in the chromatograms was carried out by determining their absorbance at 280 nm. The results are expressed as mg per kg of oil.

Bitter index. The bitter index (K_{225}) was evaluated by extracting the bitter components of 1.0 ± 0.01 g oil dissolved in 4 mL hexane using a C18 column (125 Å pore size, 80 µm particle size) (Waters Sep-Pack Cartridges), previously activated with methanol (6 mL) and washed with hexane (6 mL) prior to the separation by elution with hexane. First, 10 mL of eluent

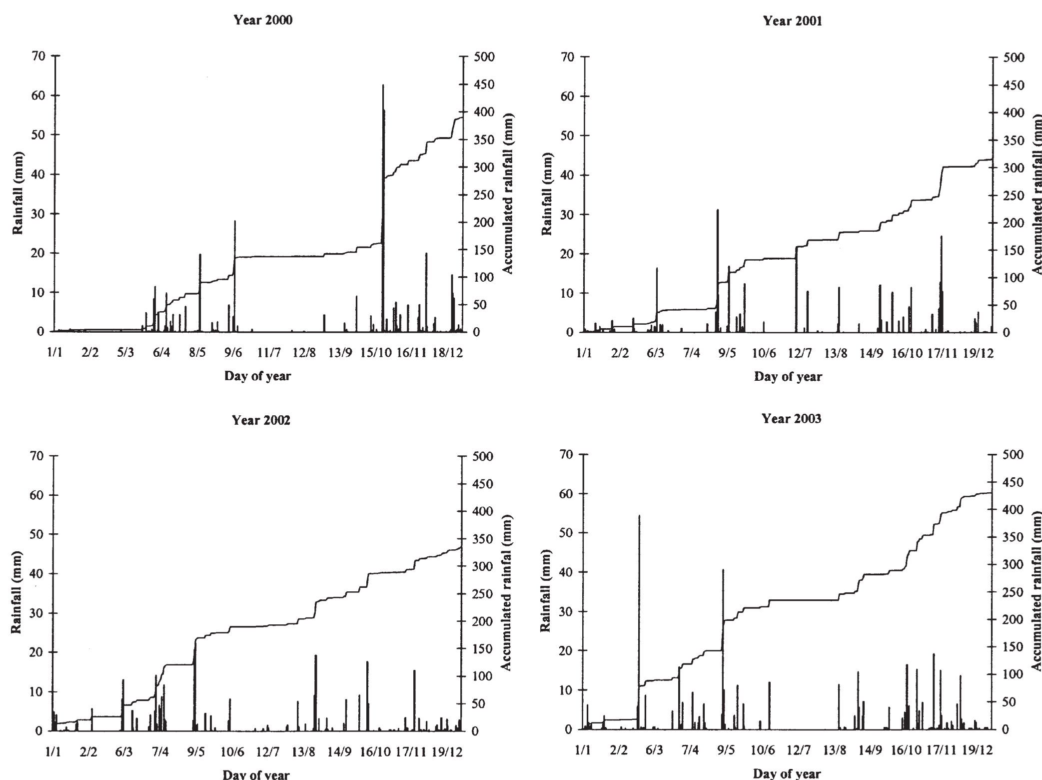

FIG. 2. Annual patterns of daily and accumulated rainfall (mm) for 2000, 2001, 2002, and 2003.

was passed to remove the fat, and then the retained compounds were eluted with 25 mL of methanol/water (1:1). The absorbance of the extract was measured at 225 nm against methanol/water (1:1) in a 1-cm cuvette (10,11).

OS. OS, expressed as the oxidation induction time (h), was measured with a Rancimat 679 apparatus (Metrohm Co., Herisau, Switzerland) using 3 g of oil sample heated to 120°C with 20 L/h air flow. The breakpoint is designated as the intersection point of the two extrapolated straight parts of the curve (12).

Quality parameters. Determination of the FFA content, PV, and UV absorption at 270 nm was carried out following the analytical methods described in the Regulation EEC/2568/91 (13) of the European Union Commission. Results were expressed as a percentage of oleic acid, milliequivalents of active oxygen per kilogram of oil (mequiv O_2/kg), and absorbance at 270 nm (K_{270}), respectively.

Oil composition. α-Tocopherol was quantified by HPLC with direct injection of an oil-in-hexane solution (7). Chromatograms were recorded at 295 nm, and α-tocopherol was quantified by the external standard method. The results are expressed as mg per kg of oil.

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) (14). The chlorophyll and carotenoid contents are expressed as mg of the major pigments, pheophytin *a* and lutein, per kg of oil, 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 the European Union Commission modified Regulation EEC 2568/91 (13). A chromatographic analysis was performed in a Hewlett-Packard 5890 Series II gas chromatograph using a capillary column (SP 2330; Supelco, Bellefonte, PA). The column temperature was maintained at 190°C, and the injector and detector temperatures were 220°C. The FA were identified by comparing retention times with standard compounds. The contents of palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids were determined and expressed as percentages of FAME.

Statistical analysis. The data were subjected to 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 at $P \leq 0.05$. Stepwise linear regression analysis (SLRA) was applied to select the chemical variables that better explained OS and bitter index.

RESULTS AND DISCUSSION

The chromatographic profile of the phenolic fraction and the corresponding structures of the phenolic compounds are shown in Figure 3. The composition of the phenolic fraction (Table 1) of a sample of the monovarietal virgin olive oil from the *Arbequina* cultivar growing in the area of the PDO "Les Garrigues"

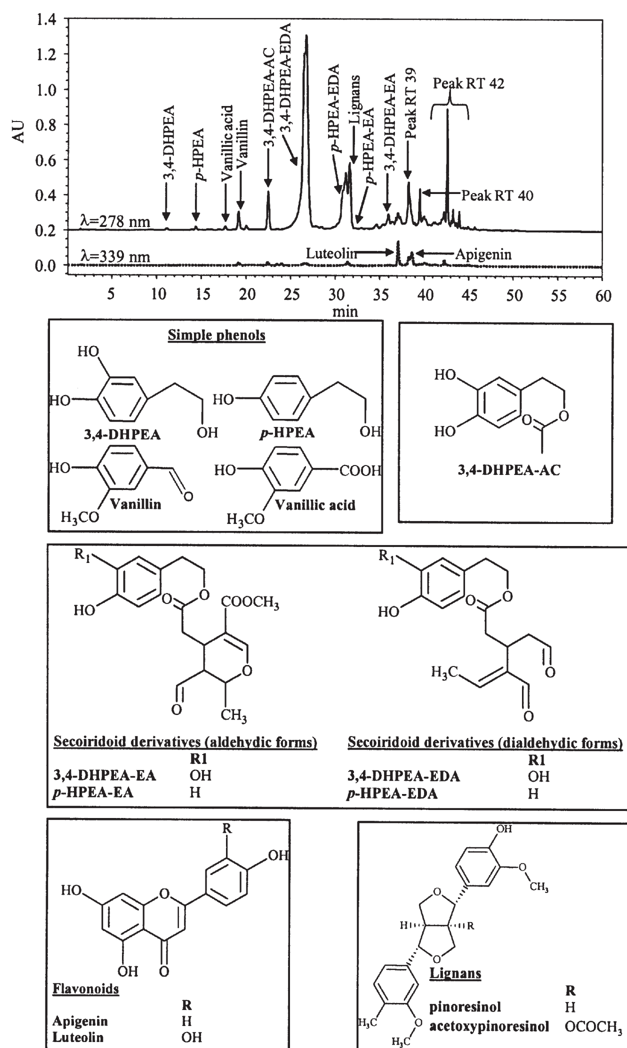

FIG. 3. HPLC chromatogram showing phenolic profile of monovarietal virgin olive oil (*Arbequina* cv) at 278 and 339 nm and the structures of the main phenolic compounds. 3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EA, oleuropein aglycone; *p*-HPEA-EA, the aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EDA, the dialdehydic form of elenoic acid linked to hydroxytyrosol; *p*-HPEA-EDA, the dialdehydic form of elenolic acid linked to tyrosol; RT, retention time.

is characterized by low levels of simple phenols [hydroxytyrosol (3,4-DHPEA), tyrosol (*p*-DHPEA), vanillic acid, and vanillin], with concentrations between 0 and 2 ppm. These values are different from those obtained by García *et al.* (15) for *Arbequina* grown in southern Spain, which averaged about 5 ppm, and other varieties, such as *Picual*, *Hojiblanca,* and *Cornicabra* having values of 17, 12, and 9 ppm, respectively (5,16). The main phenols present in olive oil are the 4-(acetoxyethyl)-1,2-dihydroxybenzene (3,4-DHPEA-AC) and secoiridoid derivatives, such as 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); the aldehydic form of elenolic acid linked to tyrosol (*p*-HPEA-EA); the aldehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EA; oleuropein aglycone); and Peaks RT 39 and RT 40 that showed two absorption maxima at 210 and 278 nm, which are characteristic of secoiridoid derivatives. Peak RT 42 had a UV spectrum similar to that of *trans*-cinnamic acid with a maximum at 276 nm. Flavonoid aglycones such as apigenin and luteolin were also present in small amounts. Lignans showed two UV absorption maxima at 226 and 278 nm. The concentration of lignans in olive oil ranged from 15 to 96 ppm, depending on the crop season and harvesting period.

Although no qualitative differences were observed in the phenolic fraction between crop seasons (Table 1), quantitative differences were observed between phenolic compounds. The main differences were observed in secoiridoid derivatives (3,4-DHPEA-EDA, *p*-HPEA-EDA, *p*-HPEA-EA, and 3,4-DHPEA-EA). The main phenolic compound quantified in the olive oil phenolic fraction, 3,4-DHPEA-EDA, showed a significantly different concentration between crop seasons 2000/01 and 2001/02 and crop seasons 2002/03 and 2003/04. The oils from the 2000/01 and 2001/02 crop seasons showed average concentrations of between 30 and 223 ppm, whereas the values for oils from the 2002/03 and 2003/04 crop seasons rose to between 427 and 610 ppm. The large decrease in the 3,4-DHPEA-EDA concentration that was observed in olive oils from the second harvest period of the 2001/02 crop season may be due to low temperatures (below –5°C) in December 2001 that affected the olive trees (Fig. 1), and caused the olive drupes to freeze on the trees. The drop in the concentration of the main phenolic compounds of the olive components could be due to freeze-fracturing of cell walls in the olives, leading to oxidation of phenolic compounds as described by Morelló *et al.* (17). The concentrations of the remaining secoiridoid derivatives, such as *p*-HPEA-EDA, *p*-HPEA-EA, and 3,4-DHPEA-EA, increased in the 2002/03 and 2003/04 crop seasons after the frost damage that occurred in the 2001/02 crop season. However, no significant differences in lignans (pinoresinol and acetoxypinoresinol) were observed between the first and second harvesting period during the four crop seasons studied.

The percentages of the main phenols in the total fraction (Fig. 4) showed that the secoiridoid derivatives varied between crop seasons as reflected in the level of 3,4-DHPEA-EDA, the main secoiridoid derivative in all the crop seasons. The percentage of the main phenols in the total fraction (Fig. 4) showed that the secoiridoid derivatives varied between crop seasons, as reflected in the level of 3,4-DHPEA-EDA, and this percentaged depended on the year's weather changes including the frost damage from December 2001 (Fig. 1). In the 2001/02 crop season, a significantly different percent composition of the phenolic profile was observed. This was characterized by a lower amount of secoiridoid derivatives and especially of 3,4-DHPEA-EDA, which decreased significantly, while the percentage of lignans increased owing to their high stability, although their concentration remained practically constant over the four crop seasons. That the percentage of 3,4-DHPEA-EDA in olive oils extracted from olives after frost damage decreased probably was due to its antioxidant activity, as reported

TABLE 1
Influence of Crop Season[a] and Harvest Period on the Composition[b] of the Phenolic Fraction (mg/kg) of Monovarietal Virgin Olive Oil from the *Arbequina* Cultivar

		Crop season				
Parameter	Harvest period	2000/01	2001/02	2002/03	2003/04	Sig. level[c]
3,4-DHPEA	First	0.26	0.28	0.27	0.30	NS
	Second	0.18	0.38	0.38	0.20	NS
p-HPEA	First	0.55 a	0.70 a	0.93 b	0.96 b	**
	Second	0.38	0.89	0.62	1.04	NS
Vanillic acid	First	0.03 a,b	0.04 a,b	0.02 b	0.08 a	
	Second	0.02 a	0.11 b	0.03 a	0.11 b	*
						***
Vanillin	First	0.39 a	0.27 a	0.73 b	0.33 a	***
	Second	0.29a	0.50	0.58	0.34	NS
Simple phenols	**First**	**1.23**	**1.29**	**1.95**	**1.67**	
	Second	**1.05**	**1.88**	**1.61**	**1.69**	
3,4-DHPEA-AC	First	30 a	45 b	32 a	48 b	**
	Second	24 a,b	10 b	39 a	35 a	***
3,4-DHPEA-EDA	First	223 a	187 a	610 b	538 b	***
	Second	136 a	30 a	467 b	427 b	***
p-HPEA-EDA	First	54 a	45 b	72 c	83 d	***
	Second	53 a	28 b	74 c	76 c	***
p-HPEA-EA	First	2.7 a	0.2 b	1.1 b	4.5 c	*
	Second	2.5 a,b	0.6 a	1.0 a	4.3 b	*
3,4-DHPEA-EA	First	85 a	84 a	180 b	202 b	***
	Second	70 a	45 a	160 b	188 b	***
Peak 39	First	50	30	38	64	NS
	Second	59	74	49	62	NS
Peak 40	First	88 a	36 b	23 b	79 a	**
	Second	105	85	27	100	NS
Secoiridoid derivatives	**First**	**532.7**	**427.2**	**956.1**	**1018.5**	
	Second	**449.5**	**272.6**	**817**	**892.3**	
Luteolin	First	1.22 a	1.02 a	1.88 b	3.15 c	***
	Second	1.40 a	1.48 a	0.59 b	3.49 c	***
Apigenin	First	0.89 a	0.64 a	0.63 a	1.54 b	***
	Second	1.10 a	1.01 a	0.98 a	1.68 b	***
Flavonoid	First	0.20 a	0.11 a	0.69 b	0.17 a	***
	Second	0.15 a	0.09 a	0.50 b	0.16 a	***
Flavonoids	**First**	**2.31**	**1.77**	**3.20**	**4.86**	
	Second	**2.65**	**2.58**	**2.07**	**5.33**	
Lignans	First	62 a	86 b	28 c	48 a	***
	Second	67 a,b	96 b	15 a	57 a	***
Peak 42	First	55	62	52	35	NS
	Second	64 a	37 b,c	72 a	35 c	*

[a]Samples obtained as follows: 2000/01, 10 samples in first harvest period and 10 samples in second harvest period; 2001/02, 10 + 4 samples; 2002/03, 9 + 9 samples; 2003/04, 9 + 9 samples.
[b]3,4-DHPEA, hydroxytyrosol; *p*-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, the dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, the dialdehydic form of elenolic acid linked to tyrosol; *p*-HPEA-EA, the aldehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycone.
[b]First harvest period, November 1–December 15; second harvest period, December 15–January 31.
[c]Significance level: NS = not significant. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$. Different letters within the same row indicate a significant difference.

in Morelló *et al.* (17). This antioxidant activity is due to the presence of a 3,4-dihydroxy moiety linked to an aromatic ring, and the effect depends on the polarity of the compound (open or closed ring in elenolic acid) (18). Another change observed in the phenolic fraction of the oil was the increase in the 3,4-DHPEA-EDA percentage in the 2002/03 and 2003/04 crop seasons. This increase may be due to the fact that olive trees that suffered frost damage in December 2001 were more sensitive to stress caused by water deficit during summer in the last two crop seasons (especially in the 2002/03 crop season). Although temperatures and rainfall during the four crop seasons (Figs. 1 and 2) were similar (except for December 2001), higher levels of phenolic compounds were detected in olive oils from the two last crop seasons.

The values for the quality indices—FFA content, PV, and the absorbance at 270 nm (K_{270})—were considerably lower than the limit established by EEC legislation for high-quality oils (Table 2). The behavior of secoiridoid derivatives is related to OS and K_{225} (Table 2). The lowest values of OS and bitterness appeared in the 2001/02 crop season, probably due to frost

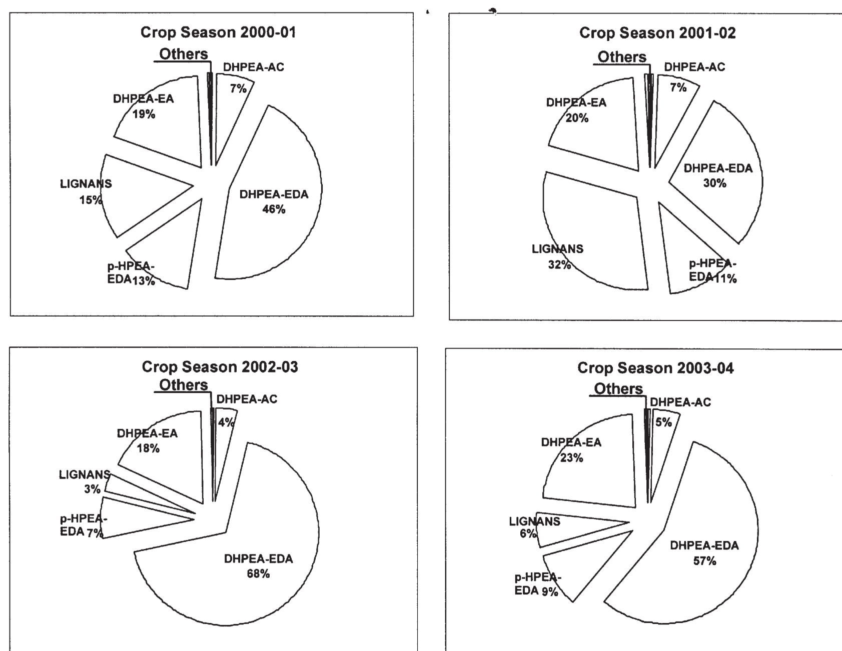

FIG. 4. Composition of the main phenolic compounds on monovarietal virgin olive oil phenolic fraction for crop seasons 2000/01, 2001/02, 2002/03, and 2003/04. For abbreviations see Figure 3.

damage of the olives and the consequent oxidation of cell content, including phenolic compounds that are related to OS and bitterness. Therefore, the high values for OS and bitterness of virgin olive oils from the 2002/03 crop season were probably due to the influence of the December 2001 frost damage on the physiological status of olive trees. The climatological conditions were similar in all crop seasons studied except for the freeze that occurred during December 2001 (Figs. 1, 2).

Significant differences between crop seasons were observed in the α-tocopherol and pigment contents (Table 2). Although α-tocopherol was higher in oils from the 2000/01 crop season, it was lower in the 2002/03 crop season. The changes in chlorophyll and carotenoid content were similar during the four crop seasons studied, with higher levels in the 2000/01 and 2001/02 crop seasons and lower levels during the last two crop seasons. These observations may be related to the ripening index of the olive fruit, which was more advanced as a consequence of the stress suffered by the olive trees during the summer period (19). Although no clear trend was observed for major fatty composition, there were significant differences between crop seasons in the percentage of some FA.

An SLRA procedure was applied to analyze the relationship between OS, as a dependent variable, and all other parameters (independent variables) for oils from the four crop seasons studied. The OS (in hours) was fitted to Equation 1 (adjusted $R^2 = 0.8681$):

$$\text{OS} = 17.3182 + 0.01001\,(3,4\text{-DHPEA-EDA}) + 0.02702\,(\alpha\text{-tocopherol}) - 0.14529\,(\text{PV}) - 1.31641\,(\text{linoleic acid percentage}) \quad [1]$$

where 3,4-DHPEA-EDA and α-tocopherol are expressed as mg per kg of oil and PV as mequiv O_2/kg oil

The regression model selected four parameters as independent variables: PV and linoleic acid, which contributed negatively to virgin olive oil stability, and 3,4-DHPEA-EDA and α-tocopherol, which contributed positively. In comparison with the equation obtained in a previous study on the oils from the same olive-growing area between 1996 and 2000 (7), the main differences in the model proposed in the present study were the noninclusion of carotenoids and the substitution of total phenols variable (measured colorimetrically by the Folin-Ciocalteau reagent) with the 3,4-DHPEA-EDA, which is the most abundant secoiridoid derivative in the phenolic fraction.

The regression model was applied to predict the value of the bitter index (K_{225}), and the equation of the model was as follows (adjusted $R^2 = 0.6027$):

$$K_{225} = 0.0842 + 0.091 \cdot 10^{-3}\,(3,4\text{-DHPEA-EDA}) + 0.866 \cdot 10^{-3}\,(p\text{-HPEA-EDA}) \quad [2]$$

where 3,4-DHPEA-EDA and *p*-HPEA-EDA are expressed as mg per kg of oil.

Results of the previous study with oils between 1996 and 2000 (7) showed that a second-order polynomial model described the relationship between the total phenols (measured by the Folin-Ciocalteau reagent) and the bitter index with adjusted $R^2 = 0.7505$. It seems that the total phenol content better describes the bitter index than 3,4-DHPEA-EDA and *p*-

TABLE 2
Influence of Crop Season on Oil Quality Parameters and Composition of Monovarietal Virgin Olive Oil from the *Arbequina* Cultivar

Quality parameter	Crop season[a] 2000/01	2001/02	2002/03	2003/04	Sig. level[b]
FFA content (% oleic acid)	0.14 a	0.13 a	0.15 a	0.07 b	*
PV (mequiv/kg)	6.48 a	8.73 b	3.68 c	3.95 c	*
UV absorption (K_{270})	0.11 a	0.10 b	0.09 c	0.09 c	*
Oxidative stability (h)	11.69 a	7.98 b	16.60 c	12.33 a	*
Bitter index (K_{225})	0.18 a	0.11 b	0.21 c	0.18 a	*
Oil composition (mg/kg)					
α-Tocopherol	206 a	187 b	138 c	172 b	*
Chlorophylls	8.77 a	7.17 a	5.26 b	5.60 b	*
Carotenoids	9.72 a	7.75 a	5.66 b	6.29 b	*
FA composition (%)					
Palmitic acid ($C_{16:0}$)	12.92 a	13.69 b	11.64 c	11.45 c	*
Palmitoleic acid ($C_{16:1}$)	1.43 a	1.15 b	0.98 c	1.32 d	*
Stearic acid ($C_{18:0}$)	2.24 a	2.09 b	2.24 a	1.82 c	*
Oleic acid ($C_{18:1}$)	72.86 a	71.95 b	74.42 c	72.80 a	*
Linoleic acid ($C_{18:2}$)	9.44 a	10.53 b	9.28 a	9.78 c	*
Linolenic acid ($C_{18:3}$)	0.56 a	0.23 b	0.42 c	0.64 d	*
Arachidic acid ($C_{20:0}$)	0.55 a	0.37 b	0.37 b	0.93 c	*

[a]Samples obtained as follows: 2000/01, 20 samples; 2001/02, 14 samples; 2002.03, 18 samples; 2003/04, 18 samples.
[b]Different letters within the same row indicate a significant difference. Significance level: NS = not significant; (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

HPEA-EDA. This is probably due to the high diversity of phenolic compounds implied in the bitterness of virgin olive oil.

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