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Influence of Olive Storage Period on Oil Quality of Three Portuguese Cultivars of *Olea europea*, Cobrançosa, Madural, and Verdeal Transmontana

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Olives (*Olea europaea* cv. Cobrançosa, Madural, and Verdeal Transmontana) used for oil production were stored, in plastic containers, at 5 ± 2 °C (70% relative humidity) for three different periods before oil extraction: 0, 7, and 14 days (T_0, T_7, and T_{14}, respectively). In the crop year 1997/1998 this procedure was done only for cv. Cobrançosa and in 1998/1999 for the three cultivars. After storage, the oils were extracted from the fruits, and the acidity, peroxide value, coefficients of specific extinction at 232 and 270 nm, stability, color, *p*-anisidine value, fatty acids, and tocopherols compositions were determined. The results confirm that storage of fruits produces losses in the olive oil quality. Acidity and stability to oxidation indicate a progressive deterioration of oil quality as fruit is stored. The storage time affects the total tocopherols contents, namely, α-tocopherol, which clearly decreased during fruit storage. The oil quality of the Verdeal Transmontana cultivar deteriorated more rapidly than that of Cobrançosa and Madural cultivars. This study also shows that cv. Cobrançosa, the main cultivar in the region, is a good choice in terms of final olive oil quality.

KEYWORDS: ***Olea europaea*; olive storage; olive oil; fatty acids; tocopherols; quality**

INTRODUCTION

Virgin olive oil is obtained from crushed olives by press or centrifugation processes, preserving its sensory characteristics and nutritional value. In addition to its high proportion of monounsaturated fatty acids, that is, oleic acid, and the modest presence of polyunsaturated fatty acids, olive oil contains natural antioxidants. Because of these characteristics it is particularly resistant to storage and more suitable for cooking than other vegetable oils (*1*).

It has been known that olive oil quality and behavior can be influenced by the cultivars, the degree of ripeness, and the industrial processes employed for oil extraction, as well as environmental conditions (mineral nutrition, ambient temperature, light, availability of water) and cultural practices (*2*).

Olive processing in principal producing countries is often not well synchronized with crop harvests due to the reduced number and size of the oil extraction facilities (*3*−*5*). Olives are often piled into large heaps and stored at ambient temperature for periods that may range from weeks to months prior to oil extraction (*6*, *7*). During this storage period mechanical, physicochemical, and physiological alterations of the fruit occur, which may cause the breakdown of their cell structures and subsequent alterations (*3*, *4*). The fruit deteriorates rapidly as a result of the joint action of pathogenic microorganisms and the internal processes of senescence. Both processes are accelerated by the temperature increase caused by fruit fermentation and by mechanical damage as a compression consequence. The fruit degradation causes the loss of flesh texture and browning of the skin and finally a complete decomposition (*7*).

Olive oils obtained from damaged olives present a characteristic high acidity, low oxidative stability, and high levels of oxidation, due to the increased peroxide value and specific extinction coefficients at 232 and 270 nm (*5*). They can also develop a high content of volatile acids (acetic or butyric) that causes a typical musty smell (*8*). These olive oils must be refined before consumption (*7*), resulting in higher costs and loss of market value (*4*).

The aims of the present work were to establish the behavior and effects of the olive storage on olive oil quality of three commercially important cultivars (Cobrançosa, Madural, and Verdeal Transmontana) produced in the Trás-os-Montes region of northeastern of Portugal, frequently used blended. Although there have been some data published on the effect of olive storage before oil extraction (*3*−*8*), there is no information concerning these Portuguese cultivars. Also, these studies deal with a reduced number of parameters and, frequently, controlled atmosphere (*3*, *4*), a situation not yet available in the majority of Portuguese oil facilities. This study included the results obtained in two crop years (1997/1998 and 1998/1999) for cv.

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Cobrançosa and in one year for the others. The parameters evaluated were titratable acidity, peroxide value (PV), specific extinction coefficients (K_{232} and K_{270}), oxidative stability, color, *p*-anisidine value (AV), fatty acid composition, and tocopherols content.

MATERIALS AND METHODS

Samples. The *Olea europea* cultivars studied were Cobrançosa, Madural, and Verdeal Transmontana. The trees were identified and carefully marked. For each cultivar five trees have been sampled.

The olive fruits were handpicked in Mascarenhas-Paradela, an orchard 10 km north from Mirandela, in northeastern Portugal (U.T.M. 29 PG5602). The orchard has 6 ha with a planting density of 9 × 9 m; trees are >40 years old and are pruned every 3 years; organic fertilization is made but not fitossanitary treatments; the soil is not irrigated but is mobilized two to three times each year; the more important cultivar is Cobrançosa (80% of the trees).

Sampling simulated the real conditions during harvesting of the olives in the region. It was performed simultaneously with the farmer's harvest and collected from around the whole perimeter of each tree at the operator height. Healthy and damaged fruits were all collected together. Damaged fruits considered were the fruits in the tree that were attacked by *Bactrocera oleae* fly. In the assays the percentage of attacked fruits was adjusted to 30%.

In year crop 1997/1998 the maturation index of the Cobrançosa olive fruits corresponded approximately to an average value of 3.99 and the fruits were handpicked on December 20. In year crop 1998/1999 the maturation indices of the olive fruits corresponded approximately to average values of 4.04, 5.73, and 3.06 for Cobrançosa, Madural, and Verdeal Transmontana, respectively, and the fruits were handpicked on December 15. The maturation index was determined according to the proposals of the Estación de Olivicultura y Elaiotecnia, Jaén (Spain), and was a function of fruit color in both skin and pulp. The following classes were considered: class 0, olives with intense green or dark green epidermis; class 1, olives with yellow or yellowish green epidermis; class 2, olives with yellowish epidermis, with reddish spots or areas; class 3, olives with reddish or light violet epidermis; class 4, olives with black epidermis and pulp totally white; class 5, olives with black epidermis and violet pulp to the midpoint; class 6, olives with black epidermis and violet pulp almost to the pit; class 7, olives with black epidermis and dark pulp. MI were calculated by $MI = (a \times 0 + b \times 1 + c \times 2 + d \times 3 + e \times 4 + f \times 5 + g \times 6 + h \times 7)/100$, where letters are the number of fruits in each class (9).

After harvest, olive fruits were immediately transported to the laboratory. There, ~75 kg of each variety and crop year was randomly taken. In crop year 1997/1998 the olives of Cobrançosa were distributed in four groups, in open plastic containers having a capacity of 14 kg. In crop year 1998/1999 the olives of the three cultivars were distributed in five groups each and stored in plastic containers as described. All groups were stored at 5 ± 2 °C to simulate the mean ambient temperature in the region, in December, with a relative humidity of 70%. Olive fruit storage times prior to oil extraction were 0 (T_0), 7 (T_7), and 14 days (T_{14}) for all of the cultivars and groups formed. From each group a 1 kg sample was randomly taken after the determined storage period and submitted separately to oil extraction.

Oil Extraction. An Abencor analyzer (Comercial Abengoa S.A., Sevilla, Spain) was used to process the olives in a pilot extraction plant. The unit consists of three essential elements: the mill, the thermobeater, and the pulp centrifuge. After being processed in the mill, the oil was separated by decanting, transferred into dark glass bottles, and stored in the dark at 4 °C. Before the analytical procedures, the samples were dehydrated with anhydrous sodium sulfate and subsequently filtered through filter paper.

In crop year 1997/1998, 12 Cobrançosa samples were analyzed (4 from each storage time T_0, T_7, and T_{14}) and titratable acidity, peroxide value, coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}), oxidative stability, and fatty acid composition were evaluated. In crop year 1998/1999, 45 samples were analyzed (5 from each storage time T_0, T_7, and T_{14} and from each cultivar, Cobrançosa, Madural, and Verdeal Transmontana). The same parameters were evaluated together with *p*-anisidine value, color and chromatic characteristics, and total tocopherol contents.

The titratable acidity, the peroxide value, and the coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) were determined according to European Union standard methods (Annexes II and IX in European Community Regulation EEC/2568/91) (*10*).

Acidity value, expressed as percent of oleic acid, was determined by titration of a solution of oil in ethanol/ether 1:1 (v/v) with ethanolic potash.

Peroxide value, given in milliequivalents of active oxygen per kilogram of oil (mequiv/kg), was determined as follows: a mixture of oil and chloroform/acetic acid 3:2 (v/v) was left to react in darkness with saturated potassium iodine solution; the free iodine was then titrated with a sodium thiossulfate solution.

K_{232} and K_{270} extinction coefficients [absorption of 1% solution (m/v) in iso-octane at 232 and 270 nm, respectively, with 1 cm of pass length] were measured using a UV spectrophotometer (Hitachi 150-20).

Stability was evaluated by measuring the oxidation induction time, on a Rancimat apparatus (Metrohm CH series 679). Air (20 L/h) was bubbled through the oil (2.5 g) heated at 110 ± 0.2 °C, the volatile compounds were collected in water, and the increasing water conductivity was continually measured. The time taken to reach the conductivity inflection time was recorded.

Color was evaluated following the NP-937 (1987) method (*11*). It consists of the determination of the transmittance values at 445, 495, 560, 595, and 625 nm of a sample solution (oil and carbon tetrachloride with transmittance values between 0.2 and 0.8), in a spectrophotometer (Hitachi 150-20).

Anisidine value was determined following the NP-1819 (1984) method (*12*). It consists of the determination of absorvance increase, measured at a 350 nm, of a sample solution of 0.4−4.0 g of olive oil (m) in iso-octane (25 mL), before (A_1) and after reaction with *p*-anisidine (A_2) (0.25 g of 4-methoxyaniline/1000 mL of acetic acid) in the dark. The anisidine value is equal to $25\ (1.2\ A_2 - A_1)/m$.

Fatty acids were measured as their methyl esters after hydrolysis with 11 g L^{-1} methanolic potassium hydroxide solution, esterification with BF_3/MeOH, and extraction with *n*-heptane. The fatty acid profile was analyzed with a Chrompack CP 9001 chromatograph equipped with a split−splitless injector, an FID, an autosampler Chompack CP-9050, and a 50 m × 0.25 mm i.d. fused silica capillary column coated with a 0.19 μm film of CP-Sil 88 (Chrompack). Helium was used as carrier gas at an internal pressure of 12 kPa. The temperatures of the detector, injector, and oven were 250, 230, and 185 °C, respectively. The split ratio was 1:50 and the injected volume 1 μL. The results are expressed in relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area (*13*). The fatty acid methyl esters (FAME) (>99%) used for identification were purchased from Sigma Chemical Co. Standards included the following FAME: dodecanoate 12:0; tetradecanoate 14:0; pentadecanoate 15:0; hexadecanoate 16:0; 9-palmitoelaidate 16:1*t*; *cis*-9-hexadecenoate 16:1*c*; heptadecanoate 17:0; octadecanoate 18:0; *trans*-9-octadecenoate 18:1*t* (elaidate); *cis*-9-octadecenoate 18:1*c* (oleate); *cis*-9,*cis*-12-octadecadienoate 18:2*cc*; *trans*-9,*trans*-12-octadecadienoate 18:2*tt*; *cis*-9,*trans*-12-octadecadienoate 18:2*ct*; *trans*-9,*cis*-12-octadecadienoate 18:2*tc*; eicosanoate 20:0; 9,12,15-octadecatrienoate 18:3*ccc*; 11-eicosenoate 20:1; docosanoate 22:0; 13-docosenoate 22:1; and tetracosanoate 24:0.

Tocopherol composition was evaluated following the method of Gama et al. (*14*). A 0.1 g sample of olive oil was blended with 10 mL of *n*-hexane and homogenized by stirring. Sample preparation was conducted in the dark, and tubes containing the samples were always wrapped in aluminum foil. The mixture was filtered by membrane (Schleicher & Shuell, 0.2μm; ∅ 13 mm, pure polyamide) and analyzed by HPLC. The chromatographic separation of the compounds was achieved with a normal-phase LiChrosorb SI 60 (5 μm; 25 × 0.4 cm) column from Merck (Darmstadt, Germany). The effluent used was a mixture of *n*-hexane and 2-propanol (99.7:0.3). Elution was performed at a solvent flow rate of 1.7 mL/min. The effluent was monitored with a diode array detector and with a fluorometric detector, at 290 and 330 nm as excitation and emission wavelengths, respectively, connected in series. Data were analyzed in the Borwin PDA Controller software

Table 1. Determination of Acidity, Peroxide Value (PV), K_{232} and K_{270}, Anisidine Value (AV), and Stability in the Three Cultivars (Cobrançosa, Madural, and Verdeal Transmontana) after Harvest (T_0) and after 7 and 14 Days of Storage (T_7 and T_{14}) (Mean ± SD)

	Cobrançosa						Madural			Verdeal Transmontana		
	1997/1998 ($n = 8$)			1998/1999 ($n = 10$)			1998/1999 ($n = 10$)			1998/1999 ($n = 10$)		
	T_0	T_7	T_{14}	T_0	T_7	T_{14}	T_0	T_7	T_{14}	T_0	T_7	T_{14}
acidity	0.7 ± 0.1^a	0.7 ± 0.0^a	1.5 ± 0.6^b	0.3 ± 0.0^a	0.6 ± 0.2^b	1.5 ± 0.2^c	0.3 ± 0.0^a	0.4 ± 0.2^b	1.1 ± 0.1^c	0.4 ± 0.1^a	0.9 ± 0.2^b	3.8 ± 0.5^c
PV	16.5 ± 3.4^a	20.0 ± 1.4^a	9.8 ± 0.5^b	13.7 ± 1.9^a	23.0 ± 3.0^b	14.5 ± 2.0^a	14.7 ± 0.3^a	25.6 ± 3.9^b	13.9 ± 2.7^{ac}	23.1 ± 6.5a	20.0 ± 1.7^a	13.5 ± 1.6^b
K_{232}	1.83 ± 0.1^a	1.64 ± 0.1^b	1.51 ± 0.1^c	2.38 ± 0.1^a	2.42 ± 0.2^a	2.43 ± 0.1^a	2.66 ± 0.2^a	2.67 ± 0.3^a	2.64 ± 0.1^a	1.57 ± 0.1^a	1.95 ± 0.1^b	1.89 ± 0.1^b
K_{270}	0.24 ± 0.0^a	0.22 ± 0.0^a	0.21 ± 0.0^a	0.24 ± 0.0^a	0.22 ± 0.0^a	0.21 ± 0.0^a	0.22 ± 0.0^a	0.22 ± 0.0^a	0.21 ± 0.0^a	0.17 ± 0.0^a	0.17 ± 0.0^a	0.24 ± 0.0^a
AV				17.0 ± 1.2^a	5.1 ± 0.5^b	4.2 ± 1.8^b	8.0 ± 3.2^a	2.5 ± 0.5^b	1.6 ± 0.2^b	5.2 ± 1.9^a	1.2 ± 0.1^b	1.4 ± 0.4^b
stability (h)	38.4 ± 5.4^a	23.5 ± 0.9^b	22.1 ± 6.8^b	32.7 ± 3.0^a	13.7 ± 3.6^b	12.4 ± 3.6^b	11.6 ± 3.0^a	3.7 ± 1.5^b	3.9 ± 0.7^b	28.6 ± 8.1^a	8.1 ± 1.1^b	10.3 ± 1.6^b

$^{a-c}$ Statistically different ($p \leq 0.05$).

(JMBS). Tocopherols (α, β, γ, and δ) and tocotrienols (α, β, and γ) were identified by chromatographic comparisons with authentic standards, by coelution, and by their UV spectra.

Statistical Analysis. The determined parameters were carried out in duplicate, in each group. The results are shown as tables of mean values and standard deviations for all of the cultivars and groups formed ($n = 8$ and 10, respectively, in 1997/1998 and 1998/1999 crops). The differences between the plots in each parameter on the different cultivars were analyzed using the analysis of variance, after the homogeneity of variance had been tested, followed by a Tukey test.

RESULTS AND DISCUSSION

According to the information presented under Material and Methods, the sampling occurred simultaneously in all cultivars in the study. Thus, the differences presented, by cultivars, in the maturation index may be considered an intrinsic characteristic of each cultivars because this parameter is not much influenced by climatic conditions. Cv. Madural presented the earlier maturity stage (the highest value) compared to the others and cv. Verdeal Transmontana the later. In the two year crops evaluated the cv. Cobrançosa presented the same value.

Table 1 gives the values, determined in the three cultivars, of acidity, peroxide value (PV), K_{232} and K_{270}, anisidine value (AV), and oxidative stability, presented after harvest (T_0) and after 7 and 14 storage days (T_7 and T_{14}, respectively).

Change in Acidity. The olive oils obtained in T_0 and T_7 were all of extra virgin quality (<1%). In crop 1997/1998 the T_0 acidity value was 2-fold that determined in the 1998/1999 crop but also <1%. The behaviors presented by the cultivars were somewhat different. In the 1998/1999 crop, when T_0 values were compared, Madural presented a slight rise but the others presented a 2-fold increase. In Verdeal Transmontana the value determined (0.9%) was near the limit of extra virgin olive oil (EEC 2568/91 regulation). In the 1997/1998 crop, the olive storage during 7 days did not affect the acidity value, a behavior that was not in accordance with that verified in the 1998/1999 crop with the same cultivar but was in accordance with the behavior presented by Madural. One possible justification for the differences in Cobrançosa values between the two crops is different climatic conditions, which imply a compositional change and a different susceptibility to triacylglycerols hydrolysis after harvest (T_0), as the percentage of damaged fruits was the same.

When olives were stored during 14 days (T_{14}), the olive oils lost the extra virgin classification. In the two crop years of Cobrançosa the final values were similar (1.5%). Verdeal Transmontana presented the highest value (3.8%) and Madural the lowest (1.1%). These different behaviors presented by cultivars can be important information in the prevision of the acidity of olive oil blends, characteristic in this region. Although Verdeal Transmontana olive oil T_7 must be refined (acidity > 3.3%) to be consumed as is, the oils from Madural and Cobrançosa were classified as "current" (<2%) and can be commercialized without processing. From a chemical point of view, considering the major importance of Cobrançosa, in quantitative terms, the high values presented by Verdeal Transmontana can be diluted in blended lots of olive oil and normally consumed.

In crop year 1997/1998 no significant differences between the values presented in T_0 and T_7 were determined; there were significant differences between T_0/T_7 and T_{14} ($p \leq 0.05$). In crop year 1998/1999 the acidity values showed significant differences ($p \leq 0.05$) concerning the cultivars in the study and the different storage times evaluated. Considering that acidity values are the result of lipolytic action of enzymes present in the fruit and in the microorganisms around, it is clear that Verdeal Transmontana was the cultivar more susceptible to this action. Whenever possible, these olives must be processed after harvest. The acidity values are also related with the storage temperature and the percentage of damaged olives (*4*, *8*, *7*). Thus, it is important to consider these factors in order to improve olive oil quality.

Change in Peroxide Value (PV). The verified behavior was unexpected. With the exception of Verdeal Transmontana, PV increased from T_0 to T_7 (T_0 values were significantly lower, $p \leq 0.01$, compared with T_7) and decreased in T_{14}. In T_7 PV values are ≥20, meaning the loss of the extra virgin category. In Verdeal Transmontana, T_0 presented a significantly higher PV ($p \leq 0.05$) than T_7 and T_{14} and the T_0 oils were already out of the extra virgin category.

Considering this parameter it seems that the storage time is beneficial to oil quality, which is in disagreement with the acidity values. The increase in PV (from T_0 to T_7) can be explained as a consequence of the action of fruit lipoxygenases, but it does not explain the behavior presented in the second week of storage. On the other hand, the decrease of the enzyme lipoxygenase activity during ripening of the olives is described in the literature (*15*). According to the values presented in **Table 1**, the oxidation status of the oils obtained after the different storage periods of the olives was not affected significantly. This can be seen in the case of K_{270}, anisidine value, and PV values. The pejorative effect of the storage was clear in the stability determination. Probably, the decrease verified in this parameter is due to the consumption of minor compounds such as phenols and tocopherols that make the formation of peroxides difficult.

Change in Specific Extinction Coefficients K_{232} and K_{270}. The values of K_{232} tended to fall slightly or remain constant during the storage period in Cobrançosa and Madural olives. The behavior of Verdeal Transmontana was quite the opposite (the values increased), but it did not result in the highest values. Cv. Madural olive oils cannot be considered to be in the extra

Table 2. Color Characteristics of Olive Oil Samples in the Crop Year 1998/1999 (Mean ± SD)[a]

	Cobrançosa			Madural			Verdeal Transmontana		
color	T_0	T_7	T_{14}	T_0	T_7	T_{14}	T_0	T_7	T_{14}
x	0.39 ± 0.0	0.36 ± 0.0	0.36 ± 0.0	0.37 ± 0.0	0.35 ± 0.0	0.35 ± 0.0	0.40 ± 0.0	0.35 ± 0.0	0.36 ± 0.0
y	0.41 ± 0.0	0.38 ± 0.0	0.37 ± 0.0	0.38 ± 0.0	0.36 ± 0.0	0.36 ± 0.0	0.42 ± 0.0	0.36 ± 0.0	0.37 ± 0.0
transparency (*Y*%)	74.1 ± 4.2	76.3 ± 2.5	73.7 ± 3.2	75.2 ± 1.9	76.3 ± 2.0	73.9 ± 3.0	70.0 ± 3.6	75.8 ± 2.4	75.4 ± 2.5
purity (σ%)	46.5 ± 12.0	30.1 ± 1.7	27.6 ± 2.7	32.7 ± 5.1	23.4 ± 5.1	21.1 ± 8.4	53.8 ± 7.5	22.4 ± 2.2	27.1 ± 1.7

[a] $n = 10$.

Table 3. Fatty Acid Composition of Olive Oil Samples in the Crop Years 1997/1998 and 1998/1999 (Mean ± SD)

	Cobrançosa						Madural			Verdeal Transmontana		
	1997/1998 ($n = 8$)			1998/1999 ($n = 10$)			1998/1999 ($n = 10$)			1998/1999 ($n = 10$)		
fatty acid	T_0	T_7	T_{14}	T_0	T_7	T_{14}	T_0	T_7	T_{14}	T_0	T_7	T_{14}
$C_{14:0}$	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.03	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$C_{16:0}$	12.11 ± 0.24	12.05 ± 0.23	12.03 ± 0.10	9.57 ± 0.05	9.64 ± 0.09	9.68 ± 0.08	10.28 ± 0.12	10.35 ± 0.19	10.22 ± 0.09	9.65 ± 0.23	9.70 ± 0.20	9.65 ± 0.15
$C_{16:1}c$	0.95 ± 0.05	0.96 ± 0.03	0.97 ± 0.02	0.40 ± 0.01	0.43 ± 0.02	0.41 ± 0.03	0.26 ± 0.01	0.27 ± 0.01	0.23 ± 0.01	0.33 ± 0.02	0.30 ± 0.02	0.27 ± 0.01
$C_{17:0}$	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.11 ± 0.00	0.10 ± 0.01	0.08 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.12 ± 0.02	0.08 ± 0.02	0.07 ± 0.01
$C_{17:1}$	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.22 ± 0.00	0.23 ± 0.01	0.21 ± 0.01	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.25 ± 0.03	0.21 ± 0.01	0.18 ± 0.01
$C_{18:0}$	3.99 ± 0.16	3.92 ± 0.11	3.86 ± 0.15	5.05 ± 0.10	4.94 ± 0.08	4.98 ± 0.08	2.33 ± 0.04	2.38 ± 0.07	2.43 ± 0.05	3.20 ± 0.20	3.21 ± 0.12	3.23 ± 0.15
$C_{18:1}c$	73.72 ± 0.28	74.24 ± 1.42	73.60 ± 0.15	76.68 ± 0.01	76.27 ± 0.42	76.18 ± 0.18	71.65 ± 0.36	71.20 ± 0.15	71.93 ± 0.17	81.05 ± 0.54	80.84 ± 0.44	81.05 ± 0.19
$C_{18:2}cc$	6.93 ± 0.13	7.19 ± 0.17	7.25 ± 0.27	5.89 ± 0.11	6.11 ± 0.83	6.57 ± 0.08	12.96 ± 0.17	13.30 ± 0.02	13.06 ± 0.16	3.23 ± 0.38	3.52 ± 0.11	3.56 ± 0.10
C_{20}	0.47 ± 0.02	0.47 ± 0.02	0.48 ± 0.02	0.47 ± 0.01	0.46 ± 0.05	0.45 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.45 ± 0.02	0.44 ± 0.03	0.46 ± 0.01
$C_{18:3}c$	0.97 ± 0.02	1.01 ± 0.03	1.03 ± 0.03	0.64 ± 0.02	0.71 ± 0.04	0.69 ± 0.03	0.78 ± 0.02	0.85 ± 0.04	0.78 ± 0.02	0.59 ± 0.05	0.60 ± 0.04	0.55 ± 0.02
$C_{20:1}$	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.13 ± 0.01	0.25 ± 0.01	0.23 ± 0.01	0.18 ± 0.01	0.21 ± 0.01	0.17 ± 0.02	0.15 ± 0.01
$C_{22:0}$	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.00	0.10 ± 0.02	0.12 ± 0.02	0.13 ± 0.01
$C_{24:0}$	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.65 ± 0.10	0.5 7 ± 0.08	0.47 ± 0.09	1.05 ± 0.38	0.79 ± 0.06	0.71 ± 0.05	0.72 ± 0.11	0.75 ± 0.14	0.64 ± 0.02
SFA	16.9 ± 0.3	16.8 ± 0.1	16.7 ± 0.1	15.9 ± 0.1	15.8 ± 0.2	15.8 ± 0.1	14.1 ± 0.3	13.9 ± 0.3	13.7 ± 0.1	14.3 ± 0.2	14.3 ± 0.2	14.2 ± 0.1
UFA	83.1 ± 0.3	83.9 ± 1.5	83.4 ± 0.4	84.1 ± 0.1	84.0 ± 0,8	84.2 ± 0.1	86.0 ± 0.3	86.0 ± 0.2	86.3 ± 0.1	85.7 ± 0.2	85.7 ± 0.3	85.8 ± 0.1
PUFA	7.9 ± 0.2	8.2 ± 0.2	8.3 ± 0.3	6.5 ± 0.1	6.8 ± 0.8	7.3 ± 0.1	13.7 ± 0.2	14.2 ± 0.3	13.8 ± 0.2	3.8 ± 0.4	4.1 ± 0.2	4.2 ± 0.1
MUFA	75.2 ± 0.3	75.7 ± 1.4	75.1 ± 0.1	77.5 ± 0.1	77.1 ± 0.4	76.9 ± 0.2	72.3 ± 0.4	71.8 ± 0.2	72.4 ± 0.2	81.9 ± 0.6	81.5 ± 0.4	81.7 ± 0.2
trans isomers	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.02 ± 0.01	0.05 ± 0.03	0.03 ± 0.01	0.02 ± 0.01

virgin category because they presented values exceeding the limit (2.5). K_{270} presented similar values in all cultivars, without significant variations but near the limit for extra virgin olive oils (10).

Considering that these coefficients are related to the primary (K_{232}) and secondary (K_{270}) oxidation products (*4*, *7*, *16*), it is possible to conclude that, with the exception of Verdeal Transmontana, the olive oil oxidation status was maintained during the storage times. Comparing the two crop years of Cobrançosa, in 1997/1998 the oxidation status determined was lower than that obtained in 1998/1999 (the ratios K_{232}/K_{270} were about 7 and 11, respectively).

Change in Anisidine Value (AV). This parameter was determined only in the crop year 1998/1999. The values provide information about the oxidation status of the oil, namely, the presence of alkenals, and simultaneously can help in the appreciation of the PV, K_{232}, and K_{270} evolution.

All cultivars in the study presented a similar behavior, with a marked decrease during storage (especially from T_0 to T_7, $p \leq 0.05$). Considering this parameter as an empirical measure of secondary oxidation products, it seems to be more sensitive than K_{270}.

Cobrançosa olive oils revealed higher values (17.02 at T_0) followed by Madural (8.04 at T_0). Verdeal Transmontana olive oils presented the lowest values (at T_0, T_7, and T_{14}) as with PV and K_{232}.

Change in Stability. In the three cultivars under study, oil stability decreased during olive storage. The more important change occurred in the first period of storage (from T_0 to T_7) with losses of ~60−70% with the exception of Cobrançosa for the 1997/1998 crop year, which had losses of only ~40%. The differences presented in the two crop years can have the same explanation as the acidity and peroxide values.

Changes verified from T_7 to T_{14} were insignificant. In the two crop years of Cobrançosa the behaviors presented were similar, with a decrease along the storage period. In Madural and Verdeal Transmontana a slight increase was verified in this period.

Madural was the cultivar more affected by the olive storage (4 h) compared with Cobrançosa (12 h) and Verdeal Transmontana (10 h). These values are in accordance with some references in the literature (*4*, *5*, *7*).

Change in Color. Table 2 gives the values, determined in the three cultivars, of the color parameters, chromatic coordinates (*x* and *y*), transparency (*Y*%), and purity (σ%). *x* and *y* are the chromatic coordinates of the superficial point of the chromaticity diagram that corresponds to the light transmitted by the oil. Transparency corresponds to the values of the light transmitted after passing through the oil layer. Purity is the percentage of light transmitted by the oil with the prevailing wavelength.

Only purity presented significant changes during storage period. There was a marked decrease from T_0 to T_7 in all cultivars under study. From T_7 to T_{14} in Verdeal Transmontana the purity increased while in the other cultivars it decreased. The purity (σ%) showed higher values in Cobrançosa and decreased significantly ($p \leq 0.05$) with the olive storage time.

Change in Fatty Acid Composition. Table 3 gives the fatty acids composition (percent) for the three cultivars studied. It can be seen that Cobrançosa presented the highest values in saturated fatty acids (SFA) and the lowest in unsaturated fatty acids (UFA). Madural presented the lowest values in monounsaturated fatty acids (MUFA) and the highest values in polyunsaturated fatty acids (PUFA), which can explain the stability values determined (*8*). In the case of Cobrançosa and

Table 4. Tocopherol Content of Olive Oil Samples in the Crop Year 1998/1999 (Mean ± SD, $n = 10$)

	Cobrançosa			Madural			Verdeal Transmontana		
tocopherols	T_0	T_7	T_{14}	T_0	T_7	T_{14}	T_0	T_7	T_{14}
total (mg/kg)	205.2 ± 17.6^a	129.8 ± 26.0^b	101.5 ± 35.7^c	162.7 ± 26.4	134.1 ± 16.2	147.3 ± 9.4	131.6 ± 26.4^a	110.8 ± 10.5^b	97.9 ± 18.0^b
α- (mg/kg)	199.6 ± 17.2^a	124.7 ± 24.9^b	97.5 ± 34.6^c	160.1 ± 26.2	131.8 ± 16.0	146.3 ± 9.2	128.2 ± 26.6^a	106.9 ± 10.3^b	94.3 ± 17.5^b
β- (mg/kg)	1.5 ± 0.1^a	1.5 ± 0.7^a	1.0 ± 0.3^b	1.0 ± 0.14	0.9 ± 0.12	0.8 ± 0.1	0.5 ± 0.1^a	0.5 ± 0.1^a	0.5 ± 0.1^a
γ- (mg/kg)	4.0 ± 0.4^a	3.7 ± 0.6^a	3.0 ± 0.8^b	1.7 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	2.8 ± 0.4^a	3.4 ± 0.4^b	3.1 ± 0.6^{ab}

[a–c] Statistically different at $p < 0.05$.

Table 5. Chemical Parameters Correlation betweem Cultivars Cobrançosa, Madural, and Verdeal Transmontana and Storage Times

chemical parameters and storage time	crop year	Cobrançosa		Madural		Verdeal Transmontana	
		r	*p*[a]	*r*	*p*	*r*	*p*
acidity	1997/1998	0.798	x				
	1998/1999	0.939	xxx	0.955	xxx	0.982	xxx
PV	1997/1998	0.916	xxx				
	1998/1999	0.883	xxx	0.891	xxx	0.723	xxx
K_{232}	1997/1998	0.940	xxx				
	1998/1999	0.215	ns	0.056	ns	0.809	xx
K_{270}	1997/1998	0.284	ns				
	1998/1999	0.393	ns	0.181	ns	0.869	xxx
stability	1997/1998	0.847	xx				
	1998/1999	0.950	xxx	0.902	xxx	0.905	xxx
palmitic acid	1997/1998	0.662	ns				
	1998/1999	0.543	xxx	0.152	ns	0.196	ns
oleic acid	1997/1998	0.173	ns				
	1998/1999	0.604	xxx	0.788	xxx	0.244	ns
linoleic acid	1997/1998	0.305	ns				
	1998/1999	0.890	xxx	0.616	xxx	0.573	xxx
linolenic acid	1997/1998	0.104	ns				
	1998/1999	0.694	xxx	0.737	xxx	0.482	xx
AV	1998/1999	0.981	xxx	0.981	xxx	0.880	xxx
tocopherols							
α-	1998/1999	0.844	xxx	0.542	xxx	0.599	xxx
β-	1998/1999	0.489	xx	0.542	xxx	0.175	ns
γ-	1998/1999	0.588	xxx	0.415	x	0.493	x
total	1998/1999	0.841	xxx	0.543	xxx	0.595	xxx

[a] x, $p \leq 0.05$ (significant differences); xx, $p \leq 0.01$ (very significant differences); xxx, $p \leq 0.001$ (extremely significant differences); ns, not significant differences.

Verdeal Transmontana the stability values presented were intermiediate between MUFA and PUFA levels.

Oleic acid ($C_{18:1}$) was the main monounsaturated fatty acid found. The average oleic acid contents were 81% in cv. Verdeal Transmontana, 75% in cv. Cobrançosa, and 72% in cv. Madural.

Palmitic acid ($C_{16:0}$) was the main saturated fatty acid determined and was similar in all of the cultivars (average values of 10%).

The maximum limit of linolenic acid ($C_{18:3}c$) considered in legislation is 0.9% (*10*). In the crop year 1998/1999 all cultivars were within the limit, but in the crop year 1997/1998 Cobrançosa presented higher values.

By comparison of the fatty acid compositions determined in the two crop years of Cobrançosa, it can be seen that the differences are in accordance with the ones verified in the other parameters and can have the same justification. It is known that the composition varies slightly with many factors, namely, climatic conditions.

Concerning the presence of *trans* isomers of unsaturated fatty acids, only traces were detected in all cultivars.

Change in Tocopherols Contents. Table 4 gives the results obtained in the tocopherol determination for the three cultivars studied. The cultivars had characteristic values; and Cobrançosa presented the highest (205 mg/kg) and Verdeal Transmontana the lowest (132 mg/kg). The tocopherols detected were α-, β-, and γ-tocopherol, and the more representative was α-tocopherol.

During storage a marked decrease in total tocopherols contents was verified ($p \leq 0.05$). The cultivar with higher contents was the one that presented the highest losses (~50%). Cv. Madural behaved differently with an increase from T_7 to T_{14}. Thus, the α-tocopherol content is less affected by storage in this cultivar despite the initial content.

These compounds have both antioxidant and vitamin action (*16*), and in this cultivar an association between a low stability value and higher tocopherols content was verified.

Study of the Correlation of the Different Chemical Parameters and Storage Time. Table 5 summarizes the results previously discussed and presents the correlation coefficients (*r*) and significance values (*p*) between the chemical parameters and the storage time for the three cultivars studied.

LITERATURE CITED

(1) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49*, 2532−2538.

(2) Tovar, M. J.; Motilva, M. J.; Romero, M. P. 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.* **2001**, *49*, 5502−5508.

(3) Castellano, J. M.; García, J. M.; Morilla, A.; Perdiguero, S.; Gutierrez, F. Quality of Picual olive fruits stored under controlled atmospheres. *J. Agric. Food Chem.* **1993**, *41*, 537−539.

(4) Gutierrez, F.; Perdiguero, S.; García, J. M.; Castellano, J. M. Quality of oils from olives stored under controlled atmosphere. *J. Am. Oil Chem. Soc.*, **1992**, *69*, 1215−1218.

(5) García, J. M.; Gutiérrez, F.; Barrera, M. J.; Albi, M. A. Storage of mill olives on an industrial scale. *J. Agric. Food Chem.* **1996**, *44*, 590−593.

(6) Kiritsakis, A. K. *Olive Oil from the Tree to the Table*; Food and Nutrition Press: Trumbull, CT, 1998; 348 pp.

(7) García, J. M.; Gutiérrez, F.; Castellano, J. M.; Perdiguero, S.; Morilla, A.; Albi, M. A. Influence of storage temperature on fruit ripening and olive oil quality. *J. Agric. Food Chem.* **1996**, *44*, 264−267.

(8) Agar, I. T.; Hess-Pierce, B.; Sourour, M. M.; Kader, A. A. Quality of fruit and oil of black-ripe olives is influenced by cultivar and storage period. *J. Agric. Food Chem.* **1998**, *46*, 3415−3421.

(9) Hermoso, M.; Uceda, M.; García, A.; Morales, B.; Frias, M. L.; Fernández, A. *Elaboración de Aceite de Calidad*; Consejeria de Agricultura y Pesca, Serie Apuntes 5/92; Sevilla, Spain, 1991.

(10) Commission Regulation 2568/91, July 11. *Off. J. Eur. Communities* **1991**, *L 248*, 1−82.

(11) CT39 (IQA) NP-937. Edible fats and oils. Determination of oils colour and its chromatic characteristics, 1987.

(12) CT39 (IQA) NP-1819. Edible fats and oils. Determination of *p*-anisidine valve, 1984.

(13) Oliveira, M. B.; Alves, M. R.; Ferreira, M. A. Multivariate analysis of fatty acid *cis* and *trans* isomers in margarines determined by HRGC/FID/capillary column. *J. Chemom.* **2001**, *15*, 71−84.
(14) Gama, P.; Casal, S.; Oliveira, M. B. P. P.; Ferreira, M. A. Development of an HPLC/diode-array/fluorimetric detector method for monitoring tocopherols and tocotrienols in edible oils. *J. Liq. Chromatogr. Relat. Technol*. **2000**, *23*, 3011−3022.
(15) Gutiérrez, F.; Jímenez, B.; Ruíz, A.; Albi, M. A. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. *J. Agric. Food Chem.* **1999**, *47*, 121−127.
(16) Ranalli, A.; Angerosa, F. Integral centrifuges for olive oil extraction. The qualitative characteristics of products. *J. Am. Oil Chem. Soc*. **1996**, *73*, 417−422.

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