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ARTICLE

Effect of Preprocessing Olive Storage Conditions on Virgin Olive Oil Quality and Composition

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The quality of virgin olive oil (VOO) is intimately related to the characteristics and composition of the olive fruit at the moment of its milling. In this study, the determination of suitable olive storage conditions and feasibility of using this preprocessing operation to modulate the sensory taste of VOO are reported. Several olive batches were stored in different conditions (from monolayer up to 60 cm thickness, at 20 and 10 °C) for a period of up to three weeks, and the quality and composition of minor constituents, mainly phenols and volatiles, in the corresponding VOO were monitored. Cornicabra cultivar VOO obtained from drupes stored for 5 or 8 days at 20 or 10 °C, respectively, retained the "extra virgin" category, according to chemical quality indices, since only small increases in free acidity and peroxide values were observed, and the bitter index of this monovarietal oil was reduced by 30−40%. Storage under monolayer conditions at 10 °C for up to two weeks is also feasible because "off-odor" development was delayed, a 50% reduction in bitterness was obtained, and the overall good quality of the final product was preserved.

KEYWORDS: Olive; virgin olive oil; storage; quality; phenolic compounds

INTRODUCTION

The quality of virgin olive oil (VOO) is intimately related to the characteristics and composition of the olive fruit (*Olea europaea* L.) at the moment of its processing. After harvesting, the preprocessing storage time is, therefore, critical to ensure the final quality of the product (*1*). However, when the fruit reaches the oil mill, processing can sometimes be delayed when the mill's capacity is exceeded by the amount of harvested olives (*2*, *3*). Consequently, storing olive fruits can sometimes be required, and this is generally carried out in piles outside the factory for some days without any special care. During this period, the weight of the stored olives damages the tissues of the drupe, resulting in the secretion of fluids from the fruits which favors the growth of undesirable microorganisms (*4*). The increased temperature can also augment the respiratory activity of the drupe (*5*), leading to undesirable metabolic processes that accelerate fruit deterioration and result in poor quality final VOOs (characterized by the presence of the "fusty" sensory defect).

Recent studies have proposed using a lower temperature (4 °C) (*6*) or modified atmosphere (CO_2) during storage to preserve the quality of the freshly harvested olive fruits (*7*, *8*). However, oil mill factories are often unable to store olives in refrigerated chambers, particularly when processing large amounts of raw materials (hundreds of tons per day). Garcia et al. (*9*) have also reported that using perforated plastic containers (60 × 40 × 40 cm, 64 kg capacity) and low temperatures (between 5 and 8 °C) allowed the production of extra virgin olive oil (EVOO) after more than 1 month of storage. Indeed, these containers avoid excessive stress on the fruits because of their limited weight capacity and allow normal fruit respiration, reducing the increase of temperature inside the olive pile.

During storage, the olives also lose phenolic compounds, which affects the final olive oil's stability (*10*) and bitterness (*9*, *11*). Therefore, this operation may be useful for modifying the taste of the final product in phenol-rich varieties such as Cornicabra cultivar. This variety is widely grown in the Spanish region of Castilla-La Mancha (*12*) and is characterized by an intense bitter taste that could affect the consumer's preference.

The objective of this study was, therefore, to not only establish suitable storage conditions for olive fruits before processing but also investigate the feasibility of using preprocessing storage to modulate the sensory taste of phenol-rich VOOs, in particular, in Cornicabra cv. monovarietal VOO. To this end, several olive batches were stored in different conditions (from monolayer up to 60 cm thickness, at 20 and 10 °C) for a period of up to 3 weeks and the quality and composition of minor constituents, mainly phenols and volatiles, in the corresponding VOO were monitored and discussed.

MATERIALS AND METHODS

Olive Fruit Storage. The study was carried out using Cornicabra cv. olives from the 2007/2008 crop season. Unwashed olives, with an IOOC ripeness index (*13*) of approximately 4.0, were stored in different thicknesses using perforated plastic containers of 60 × 30 × 40 cm (50 kg capacity): olives spread in one layer (monolayer; ML), 10 cm thickness (10T), 20 cm thickness (20T), and 60 cm (60T; using a taller container and

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a different olive batch). Two sets were stored in darkness at two different temperatures and relative humidity (RH) conditions (20 ± 1 °C and 50% RH and 10 ± 1 °C and 75% RH). The total storage time depended on the temperature used (15 days at 20 °C and 20 days at 10 °C).

Olive Oil Samples. An Abencor laboratory scale system was used to extract the olive oil. For each assay, 700 g of olive paste were used. The oil obtained was separated by decanting and the amount measured according to Martinez Suarez et al. (*14*). All samples were processed under the same malaxation temperature and time conditions (28 °C and 45 min), without adding microcrystalline talc (Mg-silicate) or water. The samples obtained were dehydrated with anhydrous sodium sulfate, filtered through filter paper, and stored at 4 °C until analysis.

Analysis of Olive Fruits. *Water and Oil Content*. The water content of the olive fruit was determined by desiccation, and the fat content was determined by Soxhlet extraction according to the UNE Spanish Standard method 55032 (*15*). The industrial oil yield was expressed as a percentage of dry olive paste weight according to the method described by Martínez Suarez et al. (*14*).

Drupe Biophenols. A sample of olive pulp (4.0 g) was homogenized with a mixture of methanol/water (80:20 v/v) (40 mL) for 2 min with an Ultraturrax homogenizer (14000 rpm). The suspension obtained was shaken (20 min, 150 rpm, <4 °C in darkness) and then centrifuged (10 min, 5000 rpm, 4 °C). The hydromethanolic phase was recovered and filtered with a 0.45 μm nylon syringe filter. The phenolic fraction extracted was analyzed by high-performance liquid chromatography (HPLC) using an Agilent Technologies 1100 series system equipped with an automatic injector, column oven, and diode array UV detector. A Zorbax SB-C18 column (250 × 4.6 id mm, 5 μm particle size) (Agilent Technologies, USA), maintained at 30 °C, was used with an injection volume of 20 μL and a flow rate of 1.0 mL/min. The mobile phase consisted of a mixture of water/acetic acid (95:5 v/v; solvent A), methanol (B) and acetonitrile (C): from 95% (A)-2.5% (B)-2.5% (C) to 34% (A)-33% (B)-33% (C) for 50 min. Chromatograms were recorded at 280, 340, and 520 nm. Hydroxytyrosol, oleuropein, and demethyloleuropein were quantified at 280 nm, anthocyanins at 520 nm, and verbascoside and flavonoids at 340 nm. Phenolic compounds were quantified using a five-point calibration curve based on the corresponding standard substances, with the exception of hydroxytyrosol which was quantified as tyrosol.

Identification of biophenols was carried out by comparing their retention times, UV−visible characteristics, and MS spectra with their standard substances. The mass detector used was a LCQ Deca XP Plus (Thermo Electron Corporation, Waltham, MA, USA) equipped with an electrospray ionization system. Nitrogen was used as the nebulizing gas at a flow rate of 14 (arbitrary units). The temperature and voltage of the capillary were 250 °C and 4.50 kV, respectively. Data were acquired in the negative ionization mode. Fragmentation experiments were performed using helium as the collision gas with collision energy between 30−40%.

Analysis of Virgin Olive Oil. All reagents used were analytical, HPLC, or spectroscopic grade, and were supplied by Merck (Darmstadt, Germany).

Quality Indices. Free acidity (FA), given as a percentage of oleic acid, peroxide value (PV) expressed as milliequivalents of active oxygen per kilogram of oil (meqO_2/kg), and K_{232} and K_{270} extinction coefficients calculated from absorption at 232 and 270 nm were measured following the analytical methods described in the European Commission (EC) standard methods (*16*).

Tocopherols. Tocopherols were evaluated following the AOCS method (*17*). A solution of oil in hexane was analyzed on an Agilent Technologies HPLC (1100 series) on a silica gel Lichrosorb Si-60 column (particle size 5 μm, 250 mm × 4.6 mm id; Sugerlabor, Madrid, Spain) which was eluted with hexane/2-propanol (98.5:1.5) at a flow rate of 1 mL/min. A fluorescence detector (Thermo-Finnigan FL3000) was used with excitation and emission wavelengths set at 290 and 330 nm.

Phenolic Compounds. A solution of the internal standard (250 μL of 15 mg/kg of syringic acid in methanol) was added to a 2.5 g sample of VOO, and the solvent was evaporated with a rotary evaporator at 35 °C under a vacuum. The oil was then dissolved in 6 mL of hexane, and a diol-bonded phase cartridge (Supelco Co., Bellefonte, USA) was used to extract the phenolic fraction. The cartridge was first conditioned with methanol (6 mL) and then with hexane (6 mL). The oil solution was then applied, and the SPE column was washed with hexane (2 × 3 mL) and with hexane/ethyl acetate (85:15, v/v; 4 mL). Finally, the phenols were eluted with methanol (15 mL) and the solvent was removed with a rotary evaporator at 35 °C under a vacuum until dry. The phenolic residue was dissolved in methanol/water (1:1 v/v; 250 μL).

HPLC analysis was performed using an Agilent Technologies 1100 series system equipped with an automatic injector, column oven and diode array UV detector. A Zorbax SB-C18 column (250 × 4.6 id mm, 5 μm particle size) (Agilent Technologies, USA) was used, maintained at 30 °C, with an injection volume of 20 μL and a flow rate of 1.0 mL/min. The mobile phase was a mixture of water/acetic acid (95:5 v/v; solvent A), methanol (B), and acetonitrile (C): from 95% (A)-2.5% (B)-2.5% (C) to 34% (A)-33% (B)-33% (C) in 50 min. Phenolic compounds were quantified at 280 nm using syringic acid as the internal standard and the response factors determined according to Mateos et al. (*18*).

Bitterness Index (K_{225}). This was determined by the method described by Gutiérrez-Rosales et al. (*19*), which consists of extracting the bitter components from a sample of 1.0 g of oil dissolved in 4 mL of hexane passed through a C_{18} column (Bakerbond SPE, J.T. Baker, Phillipsburg, NJ, USA) previously activated with methanol and washed with hexane. After elution, 10 mL of hexane was passed through to eliminate the oil residues 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.

Volatile Compounds (*Adapted from Vichi et al. (20)*). Solid phase microextraction (SPME) followed by GC was used to analyze the volatile compounds in the VOO samples. 1.5 g of olive oil spiked with 4-methyl-2-pentanol (as the internal standard) to a concentration of 1.5 μg/g was placed in a 10 mL vial fitted with a silicone septum. SPME sampling was performed by exposing the DVB/Carboxen/PDMS fiber (50/30 μm, 2 cm long from Supelco) for 30 min in the headspace of the sample, maintained at 40 °C, and then retracted into the needle and immediately transferred and desorbed for 5 min into the injection port of a gas chromatograph equipped with an FID. Compounds were resolved on a Supelcowax-10 column (30 m × 0.25 mm × 0.25 μm, Supelco Inc., Bellefonte, PA, USA) under the following conditions: injection port temperature 260 °C; helium flow 0.8 mL/min; oven temperature ramp: 35 °C for 10 min, 3 °C/min up to 160 °C and then 15 °C/min up to 200 °C (maintained for 5 min). Volatile compounds were identified by comparing the retention times and mass chromatograms of the standard substances (Sigma-Aldrich) added to the refined olive oil. The equipment used was an Agilent 5975C Series mass spectrometer (Agilent Technologies, USA) equipped with an electron ionization (EI+) detector and coupled to an Agilent 6850 Series gas chromatograph. The capillary column used was a DB-Wax (30 m × 0.25 mm × 0.25 μm, J&W Scientific, USA). Helium was employed as the carrier gas at a flow rate of 0.8 mL/min. The transfer line temperature was 280 °C, and the temperature of the ionization source and the quadrupole were 230 and 150 °C, respectively, with an electromultiplier voltage of +941 eV.

The analytical determinations were carried out at least in duplicate.

Statistical Analysis. Analysis of ANOVA was performed using SPSS version 15.0 statistical software (SPSS Inc., Chicago, IL). Duncan′s test ($p \leq 0.05$) was used to discriminate among the mean values.

RESULTS AND DISCUSSION

Effect on VOO Quality Indices. The evolution of the FA of the oils obtained from the olives stored in the different preprocessing storage conditions studied is depicted in **Figure 1**. As expected, VOO quality parameters were clearly affected by the thickness of stored olives and the storage temperature and time employed before processing the raw materials.

During the first week of storage, the increase in FA was moderate, and only small differences were observed between storage thickness (monolayer, ML; 10 and 20 cm thickness, 10T and 20T) and temperature (at 20 and 10 °C), with the exception of 60T (60 cm thickness) oils which quickly increased their FA and reached the upper limit for the "extra virgin" olive oil category (0.8%) in just 5 days. After the first week of storage, the FA of the oils obtained from olives stored at 20 °C increased faster compared with that from olives stored at 10 °C. The thickness of the stored olives also influenced this quality index.

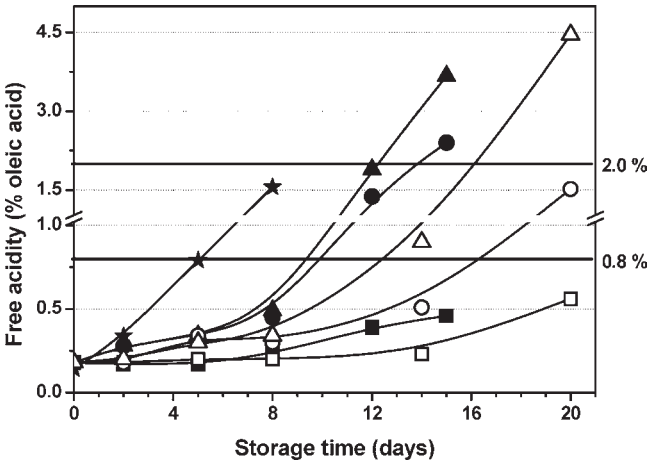

Figure 1. Free acidity in VOO obtained from olives stored in different conditions. open symbols, 10 °C; solid symbols, 20 °C; □, ML; ○, 10T; △, 20T; ■, ML; ●, 10T; ▲, 20 T; ★, 60T.

A higher thickness produced a faster increase in FA value. After 15 days stored at 20 °C, the ML, 10T, and 20T oils increased their FA values by 2.5, 13, and 20 times, respectively. This passed established EC limits for the "extra virgin" and "virgin" categories (0.8% and 2.0%, respectively). In the cases of 10T (2.4%) and 20T (3.7%), this produced the lowest category of VOO (lampante), which is inadequate for human consumption. At the lower storage temperature (10 °C), a similar increase in FA (from 0.18 to 0.56% in ML, 1.52% in 10T and 3.67% in 20T) was reached after 20 days. ML-stored olives produced oils with a FA value below 0.8% and, therefore, maintained the "extra virgin" category for the entire storage period studied at the lower temperature (**Figure 1**).

PV increased in all conditions during the storage period (data not shown); however, its value remained below EC limits for EVOO (20 meq/kg) even in the case of the 60T oils, which showed the highest rise in PV (2-fold in eight days from 3.8 to 7.5 $meqO_2kg^{-1}$). Nonetheless, there were clear differences in the PV increment rate according to storage conditions: 0.09 ± 0.00 $meqO_2kg^{-1}$/day at 10 °C and 0.15 ± 0.00 $meqO_2kg^{-1}$/day at 20 °C in VOO extracted from fruits kept at ML, 10T, and 20T conditions as an average, with the exception of 60T oils (0.42 $meqO_2kg^{-1}$/day). The specific extinction coefficients at 232 and 270 nm (K_{232} and K_{270}) did not vary noticeably with storage time (data not shown) and remained below EVOO EC limits.

Therefore, the increase in FA was the limiting parameter as far as the regulated chemical quality of the oil is concerned. In this way, the hydrolysis deterioration process, produced by endogenous and exogenous lipases, was more significant than the oxidation process in the storage conditions studied. Moreover, using ML-stored conditions the "chemical" EVOO quality indices were kept along with the complete assay (2−3 weeks storage).

The initial humidity of the olive fruit (47%) was not much affected by the different storage conditions studied. In fact, a final water content of between 39% (10T at 20 °C) and 42% (20T, 10 °C) was observed in all cases at the end of the storage period with the exception of ML at 20 °C, where an important decrease was observed (26% final humidity).

Concerning the oil content (expressed as dry weight), no significant differences were observed during the fruit storage. However, the industrial oil yield, measured using the Abencor system, was affected by the storage conditions. A slight increase, from the initial 37% up to 39% (as dry weight), was observed for ML at both 10 and 20 °C; on the contrary, a slight decrease (down to 34%) was found for the other conditions studied with the exception of the highest thickness (60 cm) in which a greater reduction in the industrial oil yield (30%) was obtained, probably because of the higher damage of the fruit and the emulsion formed with water.

Behavior of VOO Minor Constituents. *Natural Antioxidants.* Regarding the behavior of the content in tocopherols during storage of the drupes, its concentration did not vary in oils obtained from olives stored at different temperatures and thicknesses (data not shown). However, a low and similar decrease at 20 and 10 °C (content from 220 to about 200 mg/kg at the end of the storage, as an average in VOO extracted from fruits stored at ML, 10T and 20T) was observed, probably because of the oxidation process. A similar behavior was obtained in the oils from fruits stored at 60T, only slightly reducing its content from 205 to 197 mg/kg on the eighth day.

Besides tocopherols, phenolic compounds are the chief antioxidant components naturally contained in VOOs. Concentrations, expressed as mg/kg of dry weight, of the major biophenols found in the Cornicabra olive fruits under the different storage conditions are reported in **Table 1**.

Oleuropein was the main oleoside found in the drupes, and the main factor responsible for the characteristic olive fruit bitterness (*21*, *22*). In fact, oleuropein concentration in Cornicabra olives (*23*) at the beginning of the assay was very high (22 g/kg), and oleuropein was the biophenol most affected by the storage conditions studied. It showed a decrease during the first week of storage in fruits kept both at 20 and 10 °C probably because of its oxidative degradation (**Table 1**). Oleuropein diminished in ML-stored fruits at 20 °C from 22 706 mg/kg to 17 314 mg/kg (23%) on the fifth day of storage and at 10 °C from 22 706 mg/kg to 10 244 mg/kg (55%) on the eighth day of storage. This trend was constant in ML-stored olives (at 20 °C down to 8411 mg/kg on the 15th day of the storage and at 10 °C down to 7197 mg/kg on the 20th day). On the contrary, an increase in oleuropein concentration was observed at the end of the assay in 10T and 20T fruits (**Table 1**). In olive fruits stored at 20 °C in 10T conditions, the oleuropein content significantly increased from 21 081 mg/kg at day 5 to 24 576 mg/kg at day 15 and at 10 °C from 13 085 at day 8 to 21 072 mg/kg on the 20th day of the storage. This behavior is attributable to the release of the oleuropein initially bound to different subtracts in the fruit, such as polysaccharides (*24*), which could be degraded by exogenous enzymes produced by the microbial growth in olives stored in 10T and 20T at the end of the assay. In fact, olive fruits kept in ML conditions showed a continuous decrease in oleuropein concentration along the assay, probably because of lower microbial growth, meaning the biophenol content depended mainly on the oxidation process rate. At 60T, a behavior similar to that seen at 10T and 20T was observed, although it was faster because of the higher hydrolysis rate in these conditions. Thus, after a severe decrease on the second storage day of about 64% (from 25 169 mg/kg to 8943 mg/kg), a sudden increase on the eighth day to 23 969 mg/kg was observed. Kalua et al. (*6*) reported similar changes in oleuropein levels in Frantoio cv. olive fruits stored for three weeks at 4 °C.

The initial content of hydroxytyrosol (3,4-DHPEA) diminished on average by 64% and 50% along the assay from fruits stored at ML, 10T, and 20T at 20 and 10 °C, respectively (**Table 1**), probably because of its oxidative degradation. At 60T, a similar reduction was reported on the eighth storage day (60%, from 823 mg/kg to 337 mg/kg).

The content of flavonoids and anthocyanins in the olive fruits showed no clear trend at the beginning of the storage because of their stable structure and high oxidation resistance (*25*, *26*). These

Table 1. Behavior of Olive Biophenols (mg/kg Dry Weight) under Different Storage Conditions[a]

			20 °C, 50% RH			10 °C, 75% RH		
time (day)	0		2	5	15/8**	2	8	20
oleuropein	22706 ± 878	ML	24987 ± 526[b]	17314 ± 864[a]	8411 ± 74[a]	23334 ± 27[b]	10244 ± 2655[a]	7197 ± 606[a]
		10T	20357 ± 972[a]	21081 ± 1549[a]	24576 ± 1691[c]	13848 ± 1503[a]	13085 ± 678[a]	21072 ± 2252[b]
		20T	24401 ± 104[b]	15743 ± 2567[a]	16626 ± 889[b]	22437 ± 246[b]	13846 ± 694[a]	24885 ± 2202[b]
	25169 ± 986	60T	8943 ± 1102	12348 ± 1845	23969 ± 77**			
3,4-DHPEA	774 ± 35	ML	452 ± 6[a]	384 ± 62[a]	205 ± 81[a]	570 ± 66[a]	729 ± 267[a]	584 ± 125[b]
		10T	612 ± 118[a]	294 ± 51[a]	346 ± 32[a]	869 ± 172[a]	510 ± 39[a]	253 ± 83[a]
		20T	594 ± 181[a]	356 ± 0[a]	283 ± 50[a]	680 ± 45[a]	397 ± 75[a]	316 ± 46[a]
	823 ± 55	60T	810 ± 68	483 ± 21	337 ± 2**			
rutin	701 ± 5	ML	734 ± 26[b]	441 ± 7[a]	395 ± 38[a]	420 ± 77[a]	477 ± 49[a]	602 ± 103[a]
		10T	313 ± 62.3	729 ± 163[a]	398 ± 24[a]	365 ± 29[a]	452 ± 80[a]	697 ± 25[a]
		20T	768 ± 24[b]	495 ± 58[a]	495 ± 16[b]	512 ± 44[a]	550 ± 88[a]	637 ± 33[a]
	741 ± 41	60T	615 ± 68	561 ± 1	1040 ± 139**			
luteolin-7-*O*-glucoside	502 ± 38	ML	662 ± 43[b]	476 ± 1[a]	502 ± 24[a]	442 ± 65[a]	412 ± 71[a]	410 ± 88[a]
		10T	408 ± 66[a]	680 ± 147[a]	535 ± 6[a,b]	378 ± 13[a]	415 ± 75[a]	787 ± 39[b]
		20T	589 ± 12[b]	517 ± 79[a]	566 ± 0[b]	497 ± 16[a]	533 ± 41[a]	615 ± 55[b]
	584 ± 20	60T	312 ± 24	461 ± 0	666 ± 63**			
apigenin-7-*O*-glucoside	79 ± 1	ML	167 ± 6[b]	108 ± 13[a]	103 ± 23[a]	65 ± 19[a]	71 ± 19[a]	85 ± 35[a]
		10T	63 ± 15[a]	142 ± 55[a]	87 ± 5[a]	46 ± 1[a]	72 ± 19[a]	225 ± 32[b]
		20T	128 ± 17[b]	113 ± 41[a]	115 ± 1[a]	89 ± 1[b]	97 ± 0[a]	145 ± 3[b]
	92 ± 3	60T	54 ± 10	76 ± 7	150 ± 26**			
cyanidin-3-*O*-glucoside	796 ± 54	ML	707 ± 103[a]	326 ± 61[a]	147 ± 40[a]	431 ± 86[b]	167 ± 68[a]	49 ± 26[a]
		10T	444 ± 173[a]	1017 ± 273[b]	725 ± 59[b]	196 ± 91[a]	265 ± 65[a]	533 ± 222[b]
		20T	822 ± 512[a]	324 ± 57[a]	182 ± 88[a]	505 ± 1[b]	551 ± 307[a]	1578 ± 60[c]
	837 ± 213	60T	401 ± 94	724 ± 110	1848 ± 6**			
cyanidin-3-*O*-rutinoside	6648 ± 223	ML	6372 ± 23[a]	3285 ± 1070[a]	2483 ± 342[a]	4278 ± 904[b]	1803 ± 739[a]	719 ± 106[a]
		10T	3827 ± 919[a]	9361 ± 2496[b]	6991 ± 455[b]	1488 ± 680[a]	2962 ± 460[a]	4313 ± 978[b]
		20T	6057 ± 622[b]	3650 ± 3287[a]	2376 ± 461[a]	4964 ± 98[b]	5026 ± 1034[a]	12349 ± 473[c]
	8706 ± 1203	60T	4295 ± 657	8046 ± 1623	7900 ± 197**			

[a] Different letters a−c within a compound in a column indicate significant differences ($p < 0.05$) with respect to storage conditions (ML, 10T, and 20T) for the same day. **Stored for eight days at 20 °C in 60T conditions. ML, monolayer; 10T, 10 cm; 20T, 20 cm (20T); 60T, 60 cm thickness. 3,4-DHPEA, hydroxytyrosol.

Table 2. Content in Phenolic Compounds (mg/kg) in Virgin Olive Oil Obtained from Olives Stored in Different Conditions[a]

			20 °C, 50% RH			10 °C, 75% RH		
time (day)	0		2	5	15/8**	2	8	20
3,4-DHPEA-EDA	353 ± 12	ML	273 ± 52[a]	145 ± 6[b]	5 ± 0[b]	266 ± 24[a]	187 ± 12[b]	5 ± 0[c]
		10T	207 ± 9[a]	96 ± 14[a]	4 ± 0[a]	231 ± 24[a]	157 ± 10[a,b]	4 ± 0[b]
		20T	191 ± 27[a]	84 ± 14[a]	4 ± 0[a]	228 ± 41[a]	132 ± 11[a]	3 ± 0[a]
	314 ± 4	60T	124 ± 5	3 ± 0	2 ± 0**			
3,4-DHPEA-EA	140 ± 6	ML	132 ± 4[b]	87 ± 2[b]	5 ± 0[b]	127 ± 21[a]	109 ± 5[b]	12 ± 0[a]
		10T	107 ± 3[a]	66 ± 10[a]	2 ± 0[a]	109 ± 12[a]	104 ± 3[b]	11 ± 1[a]
		20T	103 ± 11[a]	61 ± 1[a]	2 ± 1[a]	115 ± 12[a]	86 ± 3[a]	12 ± 1[a]
	124 ± 0	60T	58 ± 2	23 ± 1	12 ± 0**			
3,4-DHPEA	5.7 ± 3.3	ML	2.9 ± 0.6[a]	1.9 ± 0.7[a]	0.2 ± 0.0[a]	4.6 ± 2.7[a]	2.5 ± 0.0[a]	1.0 ± 0.2[a]
		10T	7.9 ± 0.2[b]	4.0 ± 0.6[b]	7.9 ± 1.0[b]	7.0 ± 0.8[a]	3.2 ± 0.3[b]	6.3 ± 0.3[b]
		20T	8.5 ± 1.9[b]	4.6 ± 0.3[b]	8.4 ± 0.4[b]	3.9 ± 1.9[a]	3.7 ± 0.2[b]	14.1 ± 0.7[c]
	2.3 ± 0.3	60T	4.3 ± 0.1	1.2 ± 0.1	0.6 ± 0.1**			
p-HPEA-EDA	151 ± 39	ML	140 ± 19[a]	109 ± 2[b]	10 ± 1[a]	113 ± 12[a]	85 ± 1[a]	22 ± 1[a]
		10T	104 ± 5[a]	63 ± 2[a]	14 ± 1[b]	104 ± 1[a]	88 ± 7[a]	43 ± 2[c]
		20T	104 ± 13[a]	53 ± 6[a]	13 ± 1[b]	101 ± 6[a]	85 ± 3[a]	33 ± 2[b]
	136 ± 0	60T	65 ± 3	9 ± 0	9 ± 0**			
p-HPEA-EA	19 ± 2	ML	22 ± 2[a]	16 ± 3[a]	19 ± 1[a]	19 ± 1[a]	15 ± 1[a]	21 ± 0[b]
		10T	16 ± 3[a]	14 ± 3[a]	17 ± 1[a]	17 ± 1[a]	18 ± 3[a]	20 ± 0[b]
		20T	18 ± 1[a]	13 ± 0[a]	19 ± 1[a]	19 ± 5[a]	16 ± 0[a]	15 ± 1[a]
	14 ± 0	60T	9 ± 0	7 ± 3	8 ± 2**			
p-HPEA	4.9 ± 1.4	ML	3.5 ± 0.3[a]	2.6 ± 0.3[a]	3.5 ± 0.4[a]	4.4 ± 0.7[a]	3.4 ± 0.1[a]	7.9 ± 0.4[a]
		10T	5.4 ± 0.0[b]	4.6 ± 0.6[b]	12.6 ± 0.0[b]	5.9 ± 0.2[a]	4.5 ± 0.4[a,b]	11.9 ± 0.8[b]
		20T	5.9 ± 0.4[b]	5.4 ± 0.2[b]	13.1 ± 0.1[b]	4.1 ± 1.0[a]	5.4 ± 0.4[b]	15.7 ± 0.9[c]
	4.3 ± 0.1	60T	5.0 ± 0.5	5.3 ± 0.2	7.2 ± 0.1**			

[a] Different letters a−c within a compound in a column indicate significant differences ($p < 0.05$) with respect to storage conditions (ML, 10T, and 20T) for the same day. **Stored for eight days at 20 °C in 60T conditions. ML, monolayer; 10T, 10 cm; 20T, 20 cm (20T); 60T, 60 cm thickness. 3,4-DHPEA, hydroxytyrosol; 3,4-DHPEA-EA and 3,4-DHPEA-EDA, aldehydic and dialdehydic forms of the oleuropein aglycon; *p*-HPEA, tyrosol; *p*-HPEA-EA and *p*-HPEA-EDA, aldehydic and dialdehydic forms of the ligstroside aglycon.

groups of phenols often decreased in ML conditions; nonetheless, an increase in non-ML-stored fruits was observed, in particular for rutin, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside, in the final steps of the storage (**Table 1**), probably because of cell

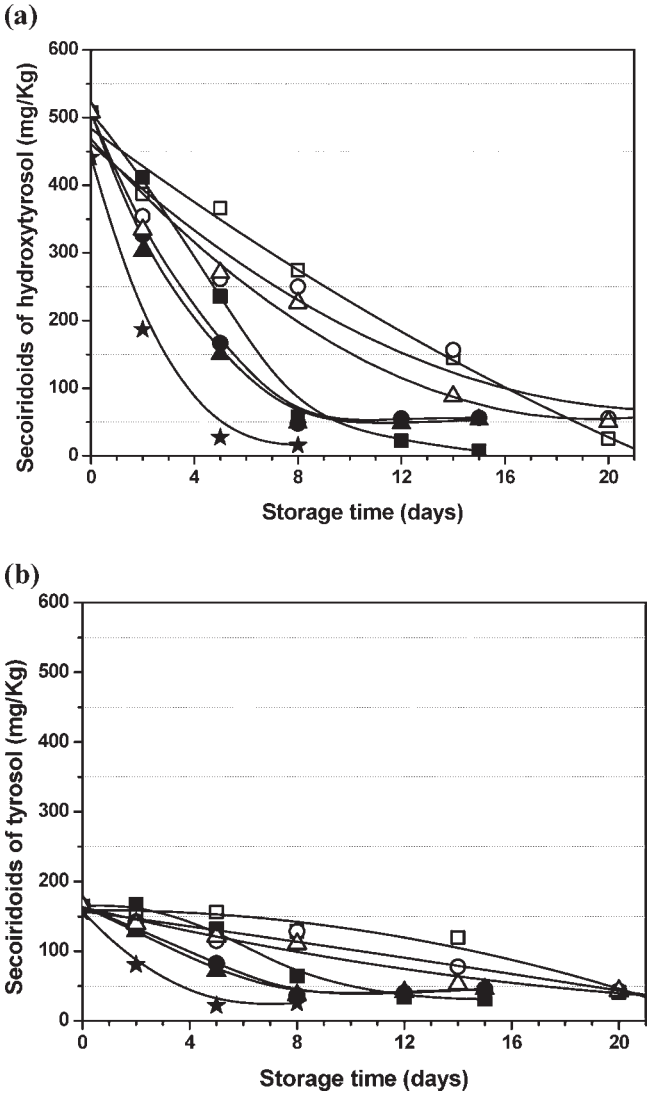

Figure 2. Content in secoiridoid derivatives of hydroxytyrosol (a) and tyrosol (b) in VOO obtained from olives stored in different conditions. open symbols, 10 °C; solid symbols, 20 °C; □, ML; ○, 10T; △, 20T ; ■, ML; ●, 10T; ▲, 20T; ★, 60T.

structure destruction and the release of the bound phenolic compounds, as previously mentioned.

VOO biophenols are mainly derivatives of the oleosides and lignans contained in olive fruits. The concentrations of the main phenolic compounds, expressed as mg per kg, found in oils obtained from Cornicabra olives during storage at different conditions are reported in **Table 2**.

The secoiridoid derivatives of hydroxytyrosol (3,4-DHPEA-EDA and -EA) and tyrosol (*p*-HPEA-EDA and -EA) were the major phenolic fractions in all the oil samples (*27*) and clearly decreased along the storage assay. However, hydroxytyrosol derivatives, apart from the higher initial oil content, were more affected by storing the fruit (**Figure 2a**). The decrease rate in the concentration of both phenol families at 20 °C was higher than at 10 °C (40−45% reduction at 10 °C and 55−60% at 20 °C after five days for both 10T and 20T conditions). Stored olive thickness also influenced their degradation rate, which was lower in ML oils than in 10T and 20T once, although these two conditions showed similar behavior. As expected, 60T oils showed the fastest decrease in both secoiridoid families, reaching in 8 days values similar to those obtained on the 15th and 20th storage days at 20 and 10 °C, respectively.

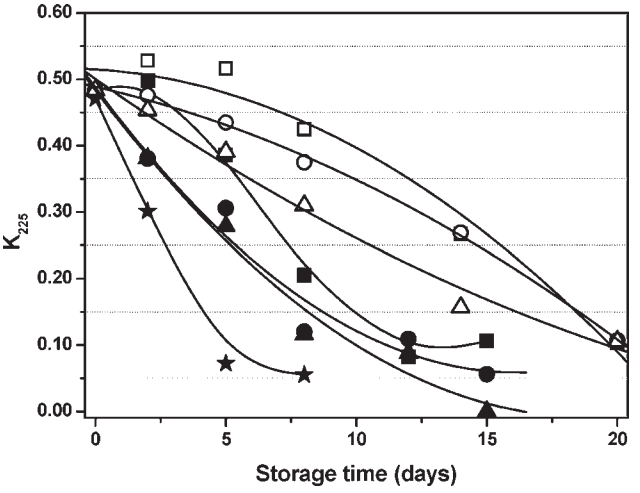

Figure 3. Reduction in K_{225} in VOO obtained from olives stored in different conditions. open symbols, 10 °C; solid symbols, 20 °C; □, ML; ○, 10T; △, 20T ; ■, ML; ●, 10T; ▲, 20T; ★, 60T.

The dialdehydic form of oleuropein aglycone (3,4-DHPEA-EDA) was the oil phenolic compound most affected by storing the fruit. This reduced its initial values at 20 °C by almost half on the second storage day (from 353 to 207 mg/kg at 10T, to 191 mg/kg at 20T and from 314 to 124 mg/kg at 60T; **Table 2**). On the 15th storage day, this phenol almost disappeared completely, remaining at only about 1% in all thicknesses. This effect was achieved quickest at 60T on the fifth storage day (from 353 to 3 mg/kg; **Table 2**). At 10 °C, the 3,4-DHPEA-EDA decreased at a lower rate than at 20 °C (down to 157 mg/kg in 10T on the eighth day) and did not disappear until the 20th day (**Table 2**). Similar behavior was observed in the case of 3,4-DHPEA-EA.

Secoiridoids of tyrosol (mainly *p*-HPEA-EDA since *p*-HPEA-EA was present in very low concentrations) showed lower degradation rates compared with those of hydroxytyrosol. Significant differences were observed between different thicknesses for the *p*-HPEA-EDA in oils obtained from fruits stored at 20 °C on the fifth day (from 151 mg/kg to 109 mg/kg in ML, to 63 mg/kg in 10T and to 53 mg/kg in 20T). At 10 °C, similar significant reductions were obtained on the 14th storage day (**Figure 2b**), while 60T oils showed a total disappearance of this compound on the eighth day (from 136 mg/kg to 9 mg/kg; **Table 2**). The very low levels of *p*-HPEA-EA almost showed no differences between thicknesses both at 20 and 10 °C during storage, since its concentration was very low (19 mg/kg) and practically constant during the assay (**Table 2**).

VOO bitterness has often been estimated by the analytical bitter index (K_{225}), which correlates with sensory bitterness as well as with the total polar phenol content (*19*, *28*), the main bitter compounds in VOO (*29*−*31*).

Reduction in bitterness in phenol-rich olive oil varieties such as Cornicabra cv. (or Picual and Coratina) is one of the goals of this study. As expected from the behavior of the phenolic compounds, the reduction in K_{225} at 20 °C was faster than at 10 °C (**Figure 3**), particularly under 60T conditions (K_{225} diminished by about 85% from 0.47 to 0.07 in 5 days). On the eighth storage day at 20 °C, ML oils reduced their initial analytical bitterness by approximately half (58% from 0.48 to 0.21), and 4-fold in the cases of 10T and 20T oils (from 0.48 to 0.12). As expected, at 10 °C a longer period is required to observe similar results (down to 0.27 after 14 days for ML conditions). The K_{225} index was reduced by about 80% in all olive storage conditions after 20 days (**Figure 3**).

Table 3. Content in LOX Volatiles (ppm internal standard) in Virgin Olive Oil Obtained from Olives Stored in Different Conditions[a]

			20 °C, 50% RH			10 °C, 75% RH		
time (day)	0		2	5	15/8**	2	8	20
hexanal	0.45 ± 0.01	ML	0.45 ± 0.07[a]	0.54 ± 0.07[a]	1.35 ± 0.01[c]	0.49 ± 0.11[a]	0.69 ± 0.00[a]	0.89 ± 0.01[b]
		10T	0.50 ± 0.01[a]	0.64 ± 0.06[a]	0.53 ± 0.01[b]	0.61 ± 0.01[a]	0.69 ± 0.00[a]	0.88 ± 0.01[b]
		20T	0.34 ± 0.17[a]	0.65 ± 0.03[a]	0.43 ± 0.01[a]	0.61 ± 0.01[a]	0.71 ± 0.01[b]	0.51 ± 0.01[a]
	0.44 ± 0.01	60T	0.47 ± 0.01	0.63 ± 0.02	0.42 ± 0.02**			
hexan-1-ol	0.25 ± 0.01	ML	0.21 ± 0.01[a]	0.12 ± 0.01[a]	0.14 ± 0.01[a]	0.31 ± 0.14[a]	0.18 ± 0.00[b]	0.13 ± 0.01[a]
		10T	0.21 ± 0.01[a]	0.13 ± 0.01[a]	0.36 ± 0.01[b]	0.31 ± 0.00[a]	0.17 ± 0.01[b]	0.27 ± 0.01[b]
		20T	0.20 ± 0.01[a]	0.13 ± 0.01[a]	0.41 ± 0.01[c]	0.28 ± 0.01[a]	0.15 ± 0.01[a]	0.30 ± 0.01[c]
	0.17 ± 0.01	60T	0.26 ± 0.01	0.23 ± 0.01	0.52 ± 0.01**			
hexyl acetate	<0.01	ML	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		10T	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		20T	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	60T	<0.01	<0.01	<0.01**			
E-2-hexenal	2.80 ± 0.10	ML	4.05 ± 0.34[a]	5.08 ± 0.61[a]	3.79 ± 0.03[c]	4.06 ± 0.25[a]	5.23 ± 0.06[a]	3.58 ± 0.04[b]
		10T	4.54 ± 0.12[a]	6.03 ± 0.43[a]	2.83 ± 0.03[b]	4.06 ± 0.08[a]	6.45 ± 0.06[b]	4.33 ± 0.01[c]
		20T	3.78 ± 1.04[a]	6.26 ± 0.29[a]	2.25 ± 0.01[a]	4.84 ± 0.06[b]	7.08 ± 0.04[c]	1.30 ± 0.01[a]
	1.91 ± 0.10	60T	3.63 ± 0.15	3.77 ± 0.13	1.39 ± 0.19**			
	0.75 ± 0.01	ML	0.31 ± 0.00[a]	0.13 ± 0.01[a]	0.05 ± 0.01[a]	0.49 ± 0.25[a]	0.10 ± 0.00[a]	0.09 ± 0.01[a]
Z-3-hexen-1-ol		10T	0.31 ± 0.01[a]	0.11 ± 0.01[a]	0.11 ± 0.01[b]	0.54 ± 0.02[a]	0.12 ± 0.01[a]	0.11 ± 0.01[a]
		20T	0.27 ± 0.02[a]	0.11 ± 0.01[a]	0.11 ± 0.01[b]	0.41 ± 0.01[a]	0.11 ± 0.01[a]	0.20 ± 0.01[b]
	0.75 ± 0.01	60T	0.33 ± 0.01	0.14 ± 0.01	0.12 ± 0.01**			
E-2-hexen-1-ol	0.05 ± 0.04	ML	0.04 ± 0.01[a,b]	0.06 ± 0.01[a]	0.19 ± 0.01[a]	0.03 ± 0.01[a]	0.08 ± 0.00[a]	0.18 ± 0.01[a]
		10T	0.05 ± 0.01[b]	0.13 ± 0.01[b]	0.91 ± 0.01[b]	0.03 ± 0.01[a]	0.12 ± 0.01[b]	0.61 ± 0.01[b]
		20T	0.03 ± 0.00[a]	0.13 ± 0.01[b]	0.95 ± 0.01[c]	0.04 ± 0.01[a]	0.14 ± 0.01[b]	0.64 ± 0.01[c]
	0.04 ± 0.02	60T	0.04 ± 0.01	0.51 ± 0.06	1.52 ± 0.08**			
Z-3-hexenyl acetate	<0.01	ML	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		10T	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		20T	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01	60T	<0.01	<0.01	<0.01**			

[a] Different letters a−c within a compound in a column indicate significant differences ($p < 0.05$) with respect to storage conditions (ML, 10T, and 20T) for the same day. **Stored for eight days at 20 °C in 60T conditions. ML, monolayer; 10T, 10 cm; 20T, 20 cm (20T); 60T, 60 cm thickness.

Volatile Compounds. The major aromatic components present in high quality VOO are C6 volatile compounds, produced by means of the enzymatic pathway of the lipoxygenase (LOX pathway), which are mainly responsible for the green and fruity sensory notes of VOOs (*32*). The content of these volatiles in oils obtained from olives stored in the different conditions studied (ML to 60T; 10 and 20 °C) expressed as mg of the internal standard (4-methyl-2-pentanol) per kg of oil are reported in **Table 3**.

The hexanal content of C6 aldehydes, which is related to the apple and green fruity attributes (*31*), showed a 3-fold increase in ML oils at 20 °C (from 0.45 to 1.35 mg/kg as internal standard, IS) after 15 days of storage, but a 2-fold increase in ML and 10T oils at 10 °C (from 0.45 to 0.88−0.89 mg/kg IS) after 20 days. No clear change in initial hexanal content was observed in other storage conditions, including 60T, meaning the greater hexanal content in ML oils is probably because of the higher oxidation rate produced under these conditions (**Table 3**).

The *E*-2-hexanal, which is responsible for the green and bitter almonds notes (*32*), showed a Gaussian-type curve trend during storage. The highest content was reached in the middle of the storage period at both temperature conditions (on the fifth and eighth days at 20 and 10 °C, respectively) with a 2-fold increase in the initial concentration in all the thicknesses studied (2.80−5.87 mg/kg IS; **Table 3**). Koprivnjak et al. (*33*) observed a similar trend for this volatile in Bjelica olive fruits during 10 days of storage in cool dry air and apportioned this diminution of its concentration to the decrease of hydroperoxide lyase (HPL) activity in the olive fruits during storage (*34*).

The C6 alcohols, which are also related to fruity, green, grassy, and sweet sensory notes in olive oils, showed different trends during storage. The initial content of *Z*-3-hexen-1-ol (0.75 mg/kg IS) strongly diminished (by about 85% on average, down to 0.13−0.11 mg/kg IS) on the fifth and eighth storage days at 20 and 10 °C in all storage conditions (**Table 3**). On the contrary, an increase in *E*-2-hexen-1-ol in 10T, 20T, and 60T was observed at the end of the assay (from 0.05 to 0.91 mg/kg IS at 20 °C and to 0.61 mg/kg IS at 10 °C in 10T oils; **Table 3**). Similar results have been reported by other researchers (*6*, *33*), who associated this behavior to the enhancement of alcohol dehydrogenase (ADH) activity during storage.

The initial content in C6 esters was very low (< 0.01 mg/kg) and showed no change during storage.

Besides the evolution and changes observed in the desirable LOX pathway C6 fraction, there are other negative volatile compounds (off-flavors) formed during storage responsible for several defects in the olive oil. These compounds originate from the metabolic action of yeasts, such as ethyl acetate and acetic acid, and molds, such as propan-1-ol, 2-methyl propan-1-ol, 3-methyl butan-1-ol and their corresponding aldehydes, acids, and esters (*35*). The evolution of these off-flavors in the oils obtained from olive batches stored in different conditions is depicted in **Figure 4**. Olive oils obtained from fruits stored at 20 °C showed a higher off-flavor formation rate compared with those stored at 10 °C. Moreover, ML oils showed a significantly higher "off-flavor" content at this higher temperature, especially after 4−5 days. Its initial content increased about 12-fold on the 15th day (from 0.15 to 1.75 mg/kg IS), whereas both 10T and 20T oils increased about 9-fold (up to 1.33 mg/kg IS) during the same period. In contrast, the formation of these undesirable volatiles in oils from fruits stored at 10 °C was irrelevant, in particular during the first week of storage. In ML oils, "off flavors" rose 4-fold (from 0.15 to 0.61 mg/kg IS), in 10T about 3-fold (up to 0.48 mg/kg IS) and in 20T by 5-fold (up to 0.73 mg/kg) on the 14th storage day. Therefore, the development of "off-flavors" mainly related

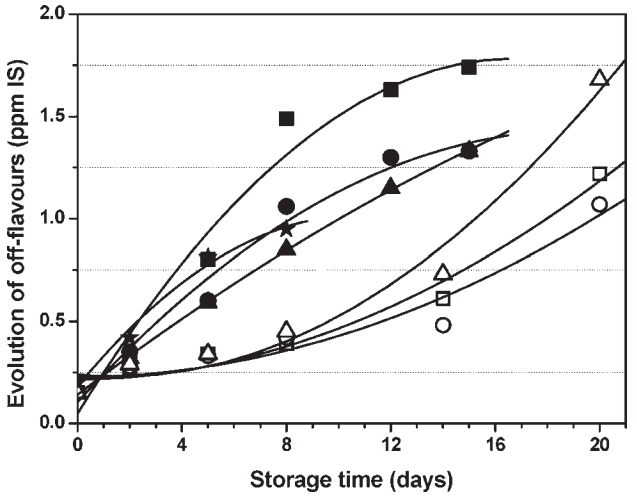

Figure 4. Formation of off-flavors in VOO obtained from olives stored in different conditions. open symbols, 10 °C; solid symbols, 20 °C; □, ML; ○, 10T; △, 20T; ■, ML; ●, 10T; ▲, 20T; ★, 60T. *Sum of 2-methyl-1-propanol, ethyl 3-methylbutanoate, ethyl 2-methylbutanoate, 3-methylbutanal, and 2-methylbutanal.

with the sensory defects such as the "fusty" and "musty" perceptions generally associated with pile storage and fungal and mold growths, was satisfactorily delayed during storage at 10 °C.

Finally, according to the results obtained in this study, Cornicabra cv. VOOs obtained from drupes stored for 5 or 8 days at 20 or 10 °C, respectively, maintained their "extra virgin" status − according to chemical quality indices − because of the small increases in FA, and PV observed, independent of the storage conditions used (ML, 10T, or 20T) with the exception of 60T olives which produced lower quality VOO. Moreover, the bitter index of this monovarietal oil was positively reduced by about 30−40% under 10T and 20T storage conditions. Storing fruits for up to two weeks under ML conditions at 10 °C is also feasible because the delay in "off-odor" development meant the overall good quality of the final product was preserved and led to a 50% reduction in bitterness. Therefore, storing olive fruits under controlled conditions before milling could be useful not only for extending the expiry date of raw materials but also modulating the bitterness of phenol-rich varieties such as Cornicabra (or Picual and Coratina) with the aim of improving the consumer's acceptance of this monovarietal VOO.

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