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Reduction of Virgin Olive Oil Bitterness by Fruit Cold Storage

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Green mature olives (*Olea europaea* L. cv. 'Manzanilla', 'Picual', and 'Verdial') were stored at 5 °C, and the oil extracted from them showed a middle intensity level of sensory-evaluated bitterness. The storage times necessary for this reduction were different for the three varieties tested, requiring 4, 6, and 8 weeks, respectively, for 'Manzanilla', 'Picual', and 'Verdial' olives. The level of commercial quality of the extracted oil did not deteriorate as a consequence of previous fruit storage. Olives matured during refrigeration at 5 °C, as the increase of maturation index and the decrease of color index and fruit firmness indicated. Similarly, as the fruit storage period progressed, the total phenolic compound content of the extracted oils decreased. Although the use of green mature olives may require a more prolonged storage time, it allows for a better postharvest handling of the fruits, which are more resistant to physical damage or fungal infections than the riper ones.

KEYWORDS: Oil quality; *Olea europaea*; olive maturity; phenolic compounds; refrigeration

INTRODUCTION

The excessive intensity of bitter taste in virgin olive oil (VOO) determines its rejection by the consumers of important markets (Japan, Canada, U.S.A., Australia, China, or Northern European countries) accustomed to the milder taste of refined oils, obtained by solvent extraction. VOO is extracted from crushed olives exclusively through physical processes, such as press or centrifugation, preserving its sensory characteristics and nutritional value, as a natural fruit juice. Control of VOO bitterness by the addition of chemical agents is not allowed. Postharvest heat treatments applied to olive drupes before oil extraction can reduce the level of bitterness of the subsequently extracted oils without significantly affecting the physicochemical parameters established to evaluate the level of oil quality (*1, 2*). However, an inadequate use of these treatments can provoke the emulsion of the oil during the extraction process, causing a dramatic loss in oil yield, or can induce off-flavor development in the VOO extracted.

Cold storage of the mill olive can be an alternative solution. This technique is based on a reduction of temperature, which is translated into a reduction and slowing down of the speed of metabolic activities. The low temperatures have the additional advantage of decreasing the growth rate of the pathogenic microorganisms and generally complicating any type of parasitizing. During cold storage, the olive continues maturing, although more slowly than on the tree, and it is supposed that the intensity of oil bitterness will decrease with storage. Work performed previously on a laboratory scale with the varieties 'Gordal' and 'Picual' (*3*−*5*) or 'Koroneiki and 'Coratina' (*6, 7*) and on industrial scale with 'Blanqueta' and 'Villalonga' (*8, 9*) demonstrated that it was possible to maintain the quality of virgin olive oil within the extra category after maintaining the fruit at 5 °C. More recently, Kalua et al. (*10*) have found that the cold storage of 'Frantoio' mill olives at 4 °C for 3 weeks may be beneficial, increasing oil yield and moderating the sensory quality of VOO subsequently extracted.

A reduction of the excessive presence of bitterness should be very interesting to improve the commercialization of the oils extracted from different Spanish varieties. Thus, 'Manzanilla' olives are only harvested at their green mature stage for table use, but every season about 10% of this production is rejected, because of inappropriate size or appearance. This significant amount of drupes is then destined to the mill industry, where they produce a low yield (<10%) of very bitter and pungent oil, which cannot be directly marketed without a previous blending with other softer VOO. The 'Picual' variety, the mill olive most cultivated in Spain, is well-known for producing oils with a high level of bitterness, even if they are extracted from fruits at advanced stages of maturation (*11*). To obtain 'Picual' oils with an adequate level of bitterness, it is often necessary to delay fruit harvesting until drupes exhibit black skin. This extension to the duration of the drupes remaining on the tree can cause a loss in production because of spontaneous fruit fall, meteorology agents, and different macro- or microbiological parasitism. Furthermore, the oil contained in the olive cell deteriorate even by the action of its own metabolism, which can produce hydrolytic (lipase) or oxidative (lypoxygenase) alterations in VOO triglycerides (*12*). Finally, 'Verdial' olives are habitually used in both the table and mill industries. In the orchards, a part of the 'Verdial' trees is harvested when the drupes exhibit the green mature stage of maturation, similar to

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'Manzanilla' olives, and the rest of the production is harvested 2 months later, when fruits show black skin, similar to 'Picual' olive harvesting. Consequently, this variety presents all of the problems of the other varieties cited before. The aim of this work is to determine if the cold storage of these varieties necessarily results in VOOs with a tolerable level of bitterness, maintaining the original level of quality of the oil extracted from green mature fruits.

MATERIALS AND METHODS

Olive Cold Storage. Olive fruits (*Olea europaea* L. cvs 'Manzanilla', 'Picual', and 'Verdial') were hand-harvested at the green mature stage of ripening in Villarrasa (Andalusia, Spain) during the 2002−2003 season and immediately transported to the laboratory into perforated plastic 30 kg boxes (30 min travel). Healthy olives of each variety were independently distributed into eight perforated plastic 12 kg boxes and stored in a refrigerated room at 5 °C in darkness over a period of up to 8 weeks. Weekly, a plastic box of each variety was taken, and its fruits were distributed into three samples of 4 kg each.

Fruit Analysis. From each sample, 100 drupes were randomly taken to evaluate the level of maturity by the maturity index (MI), habitually used in the mill olive industry. This method, developed in Venta Del Llano (Jaén, Spain), is based on the sensory evaluation of the olive skin and flesh colors (*13*). Previously, the color of these fruits was determined at the equatorial zone, using a Minolta CR200 (Minolta Camera Co., Osaka, Japan) chromameter, with a measuring area of 8 mm in diameter, diffuse illumination, and a viewing angle of 0° (*14*). The CIE $L^*a^*b^*$ color notation system was applied to determine the parameters L^*, a^*, and b^*, where L^* indicates lightness, a^* refers to the color axis from green to red, and b^* refers to the color axis from blue to yellow. By means of these parameters, a color index (CI) was calculated according to the formula

$$CI = L^*(b^* - a^*)/100$$

The firmness of these fruits was also evaluated at their equatorial zone by resistance of flesh to penetration, using a Zwick 3300 hand densimeter (Zwick GmbH & Co., Ulm, Germany). The consistence of the fruit was measured without rupture by pressure of a 5 mm diameter disk (*8*). The measurement was expressed in N/cm^2.

Oil Extraction. The olives of each 4 kg box were milled and distributed into 4 batches. A sample of 700 g paste was taken from each batch and weighed in a metallic pitcher. The paste of each pitcher was homogenized using a spatula, and the oil was extracted using an "Abencor" analyzer (Comercial Abengoa S.A., Seville, Spain). This unit, consisting of three basic elements: a mill, a thermobeater (30 °C for 30 min), and a pulp centrifuge (1500*g*, during 1 min), simulates the three-phase industrial process of VOO production (*15*). During malaxation, 10% water was added to the olive paste, and after centrifuging, the oil was decanted into a graduated cylinder to measure the volume (mL) obtained and to calculate the oil yield, which was expressed as the percentage of fresh weight, considering the value 0.915 g mL^{-1} as the value of olive oil density. After the yield was evaluated, each group of four extracted oils was filtered, joined, and stored at −20 °C under a N_2 atmosphere until analysis.

Oil Quality Analysis. The titratable acidity, peroxide value, and the extinction coefficients at 232 and 270 nm (K_{232} and K_{270}) were determined on the extracted oils according to the European Union standard methods (*16*). The overall sensory quality of each oil sample was evaluated by a panel of eight trained (≥5 years experience) tasters according to a nine-point scale, with "1" being the poorest quality possible and "9" being the best. The bitterness intensity was determined by the same panel using a structured scale of five points, where "0" means the absence of attribute, "1" means simple perception, "2" means slight presence, "3" means middle presence, "4" means strong intensity, and "5" means the highest intensity. When the oil extracted from the stored fruit of a variety showed an acceptable level of bitterness (middle presence), the period of cold storage was interrupted and the rest of the boxes of the same variety were removed from the refrigerated room. Oxidative stability was measured by using the Rancimat method, which evaluates the time (h) of resistance to oxidation of 3 g oil samples exposed to streams of dry air heated to 100 °C (*17*). The pigment contents of the oils were evaluated by determining their absorbances at 470 and 670 nm for carotenoids and chlorophyll, respectively, and the results were expressed as mg kg^{-1} (*18*).

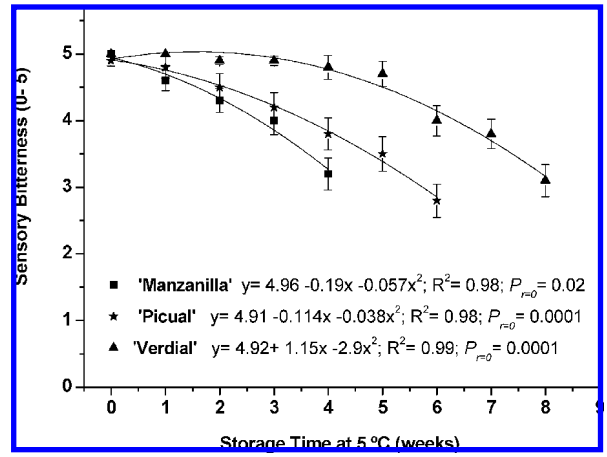

Figure 1. Changes of bitterness intensity presented by oils extracted from olive fruits of different varieties, previously stored for increased periods at 5 °C. Each point corresponds to the mean value, in triplicate, of eight trained tasters to evaluate the sensorial quality of virgin olive oils. Bitterness intensity was evaluated using a structured scale of five points, where "0" means the absence of attribute, "1" means simple perception, "2" means slight presence, "3" means middle presence, "4" means strong intensity, and "5" means the highest intensity. Vertical bars mean ± standard deviation (SD).

Phenolic Compound Analysis. The phenolic fraction was isolated by solid-phase extraction and analyzed by reversed-phase high-performance liquid chromatography (HPLC) using a diode-array UV detector (*19*). Quantification of phenolic compounds (except ferulic acid) was carried out at 280 nm using *p*-hydroxyphenylacetic acid as an internal standard, while flavones and ferulic acid were quantified at 335 nm using *o*-coumaric acid as an internal standard. The results were expressed in mmol kg^{-1} (*20*).

Statistical Data Analysis. Regression between changes in cold storage time and sensory bitterness was studied. Analysis of variance (ANOVA) was carried out for the rest of the data for each variety independently. A 5% level of least significant difference (LSD), calculated by Duncan's multiple range test was used to establish differences between the mean values of each variable, when ANOVA detected a significant ($p \leq 0.05$) effect because of the cold storage time.

RESULTS AND DISCUSSION

Effect of Olive Cold Storage on Oil Bitterness. The level of bitterness gradually diminished in the oils extracted according to the progress of the time of fruit storage at 5 °C (**Figure 1**). Although the regressions between both parameters followed a similar profile of second-degree polynomial curves in the three varieties tested, the results from each one of them were very different. Whereas 'Manzanilla' olives only needed 4 weeks of cold storage to produce middle intensity of bitterness in oils (value "3" in the scale of "0−5"), 'Picual' and 'Verdial' drupes needed 6 and 8 weeks, respectively, to obtain oils with a similar intensity of this attribute. Consequently, 'Verdial' green mature fruits needed double the time of cold storage than the "Manzanilla" olives to achieve a similar reduction in bitterness. Therefore, the particular characteristics of each variety clearly determined the conditions of the process, each requiring a specific study. Kalua et al. (*10*) observed a similar reduction in the bitterness of the oil extracted from 'Frantoio' drupes after only 2 weeks of cold storage at 4 °C. However, these olives were initially stored with a higher MI (2.6), close to that

Table 1. Changes in Maturity Index, Firmness, Color, and Yield of 'Manzanilla' Olives Stored for Different Periods at 5 °C and in Different Parameters of the Oils Later Extracted

	storage time at 5 °C (weeks)				
parameter[a]	0	1	2	3	4
maturity index	0.6 c	0.7 bc	0.9 b	0.9 b	1.2 a
firmness (N cm^{-2})	47.3 a	48.5 a	48.7 a	48.3 a	41.2 b
color index [$L(b - a)$/100]	39.4 a	38.9 a	37.4 ab	35.8 b	34.8 b
yield (%)	4.6	4.6	4.6	4.8	4.8
acidity (% oleic)	0.23 b	0.21 b	0.29 a	0.31 a	0.30 a
peroxide value (mEq of O_2 kg^{-1})	8.78 a	9.79 a	6.89 b	6.12 b	8.40 a
K_{232}	1.69	1.77	1.62	1.75	1.67
K_{270}	0.16	0.17	0.15	0.17	0.17
panel test[b]	7.8	7.6	7.9	7.7	7.6
carotenoids (mg kg^{-1})	22.61	22.66	22.57	23.61	22.09
chlorophylls (mg kg^{-1})	35.92 b	39.22 a	39.02 a	39.49 a	41.59 a
stability (h)	118.5	119.4	114.8	113.2	110.5

[a] Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. [b] A value of 1 indicates the worst sensory quality possible, and a value of 9 indicates the best sensory quality possible.

normally recommended for mill olive harvesting (3.0), when on average the drupes had completely changed their skin color (*21*). To fix a determined storage time for obtaining a significant reduction in bitterness is risky. The particular culture conditions for each season could induce changes in the olive physiology and in the level of bitterness of the oils extracted. It is well-known that the level of irrigation of the olive trees in a determined season induces changes in the maturation speed of the fruits and on the level of bitterness of the oils extracted from them (*22*).

Effect of Olive Cold Storage on Fruit Maturation. The progressive increase in MI and the gradual decreases in CI along with firmness values demonstrate that the olives matured during their storage at 5 °C (**Tables 1−3**). Nevertheless, the evolution of these parameters occurred differently for each variety. The 'Picual' olive presented a more rapid increase in MI and decrease in CI than 'Manzanilla' olives. The ripening of the 'Verdial' olives during cold storage was slower than the other two varieties tested. Similar differences among varieties concerning the development of fruit ripening during cold storage were previously observed by Garcia et al. (*8*), who found that 'Villalonga' olives matured sooner than 'Blanqueta' olives during their storage at 5 °C.

Effect of Olive Cold Storage on Oil Yield. The oil yield values obtained from 'Manzanilla' olives were lower than those extracted from 'Picual' and 'Verdial' drupes. During cold storage, the oil yield of 'Manzanilla' olives did not exhibit any significant change. On the contrary, 'Picual' and 'Verdial' fruits showed a very similar response for this parameter to cold storage. During the first 2 weeks of storage, both varieties exhibited a significant reduction in oil yield but, subsequently, it gradually increased, until even reaching values significantly higher than the initial ones. Because 'Manzanilla' olive has been specifically selected as a table variety, it is not rare that it should present lower values for oil yield than the drupes of the other two varieties, which are used for oil production. Gutierrez et al. (*23*) and Castellano et al. (*14*) also observed an increase in the oil yield of 'Picual' olives stored for 60 days at 5 °C. More recently, Kalua et al. (*10*) also observed an increase in oil yield in the first 2 weeks of storage, followed by a decrease.

Effect of Olive Cold Storage on Oil Quality. The physicochemical quality parameters of the olive oils extracted from the cold-stored olives fell within the limits established for the best commercial category of VOOs ("extra"). The free acidity of the three varieties experienced a slight increase as storage time progressed. In other works on olive cold storage, the values of acidity presented by oils extracted from fruits stored for a period ≥30 days surpassed the ones obtained in this present work. Thus, Gutierrez et al. (*23*) found values of acidity of 0.33, 0.69, and 1.88 in oils extracted from ripe 'Picual' olives (MI = 3.0) stored at 5 °C after 30, 45, and 60 days, respectively; 4 years later, Garcia et al. (*5*), using olives (MI = 2.2) of the same variety and after the same storage periods at 5 °C, obtained values for acidity of 0.35, 0.84, and 2.91, respectively, and more recently, Pereira et al. (*24*) using 'Cobrançosa', 'Madural', and 'Verdeal Transmontana' drupes (MI = 4.04, 5.73, and 3.06, respectively) stored at 5 °C for 14 days found that the storage of fruits produced a significant increase in oil acidity, which determined the loss of the "extra" category. On the contrary, Kiritsakis et al. (*6*) and Clodoveo et al. (*7*) using 'Koroneiki' and 'Coratina' mature olives, respectively, found that olive oil from fruit stored at 5 °C for 30 days had acceptable acidity. On an industrial scale, Garcia et al. (*8*), using the varieties 'Blanqueta' and 'Villalonga' (MI = 2.2, each), obtained values for this parameter of 0.6, 1.7, and 3.5 for the first variety and of 0.32, 1.22, and 1.37 for second one, after 32, 49, and 60 days of storage at 5 °C, respectively. Possibly, the lower values of acidity obtained in the present work are due to the use of

Table 2. Changes in Maturity Index, Firmness, Color, and Yield of 'Picual' Olives Stored for Different Periods at 5 °C and in Different Parameters of the Oils Later Extracted

	storage time at 5 °C (weeks)						
parameter[a]	0	1	2	3	4	5	6
maturity index	0.7 d	0.8 d	1.0 c	1.1 c	1.5 b	1.7 b	2.3 a
firmness (N cm^{-2})	47.4 a	44.2 b	44.4 b	44.2 b	44.2 b	43.9 b	43.9 b
color index [$L(b - a)$/100]	33.8 a	31.5 b	30.8 b	29.4 c	27.1 d	25.5 e	23.6 f
yield (%)	18.2 b	16.5 c	17.1 c	18.3 b	18.6 b	20.4 a	20.9 a
acidity (% oleic)	0.18 b	0.17 b	0.17 b	0.23 a	0.24 a	0.26 a	0.24 a
peroxide value (mEq of O_2 kg^{-1})	6.4 a	5.7 a	3.2 b	3.1 b	2.9 b	5.6 a	5.7 a
K_{232}	1.27 b	1.57 a	1.51 a	1.64 a	1.58 a	1.30 b	1.27 b
K_{270}	0.15	0.14	0.15	0.14	0.16	0.14	0.15
panel test[b]	7.7	7.5	7.6	7.4	7.5	7.4	7.4
carotenoids (mg kg^{-1})	14.44 a	10.82 c	11.28 c	10.97 c	14.13 a	14.76 a	12.92 ab
chlorophylls (mg kg^{-1})	24.26 b	25.30 b	27.10 b	33.74 a	32.63 a	35.29 a	26.39 b
stability (h)	174.0	169.9	170.4	164.6	172.1	166.5	165.7

[a] Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. [b] A value of 1 indicates the worst sensory quality possible, and and value of 9 indicates the best sensory quality possible.

Table 3. Changes in Maturity Index, Firmness, Color, and Yield of 'Verdial' Olives Stored for Different Periods at 5 °C and in Different Parameters of the Oils Later Extracted

	storage time at 5 °C (weeks)								
parameter[a]	0	1	2	3	4	5	6	7	8
maturity index	0.7 d	0.8 cd	0.8 cd	0.9 cd	1.0 c	1.0 c	1.2 bc	1.4 b	1.8 a
firmness (N cm^{-2})	46.4 a	46.6 a	45.8 a	45.7 a	45.2 ab	44.9 ab	43.5 b	40.2 c	38.2 d
color index [$L(b - a)/100$]	29.4 a	28.7 a	28.8 a	27.6 ab	27.2 ab	26.7 b	24.6 c	22.5 d	20.4 e
yield (%)	20.0 c	18.3 d	18.5 d	21.2 b	21.1 b	22.4 a	22.2 a	22.1 a	22.2 a
acidity (% oleic)	0.28	0.27	0.26	0.30	0.32	0.34	0.32	0.36	0.38
peroxide value (mEq of O_2 kg^{-1})	5.4 c	5.6 c	4.2 d	5.1 c	5.4 c	5.4 c	8.8 a	6.8 b	3.0 e
K_{232}	1.51	1.56	1.61	1.53	1.48	1.47	1.52	1.50	1.61
K_{270}	0.15	0.15	0.15	0.16	0.13	0.13	0.15	0.16	0.15
panel test[b]	7.5 a	7.4 a	7.4 a	7.5 a	7.3 a	7.4 a	7.3 a	7.2 ab	7.0 b
carotenoids (mg kg^{-1})	22.07 a	15.70 c	16.21 c	16.19 c	15.93 c	18.70 b	17.14 b	17.47 b	18.92 b
chlorophylls (mg kg^{-1})	40.26 a	23.1 b	22.87 b	22.16 b	22.60 b	22.57 b	21.97 b	21.36 b	21.59 b
stability (h)	76.0	75.6	74.8	75.2	74.1	74.4	73.8	73.2	72.6

[a] Each point is the mean value of three replicates. In each file, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. [b] A value of 1 indicates the worst sensory quality possible, and a value of 9 indicates the best sensory quality possible.

Table 4. Changes in Content of Different Phenolic Compounds (mmol kg^{-1}) of Virgin Oils Obtained from 'Manzanilla' Olives Previously Stored at 5 °C for Different Periods[a]

	storage time at 5 °C (weeks)				
phenolic compound (mmol kg^{-1})[b]	0	1	2	3	4
hydroxytyrosol	0.020	0.049	0.037	0.015	0.035
tyrosol	0.04	0.058	0.04	0.031	0.050
vanillic acid	0.004	0.003	0.003	0.003	0.004
vanillin	0.000	0.000	0.000	0.000	0.000
p-coumaric acid	0.000	0.000	0.000	0.000	0.000
hydroxytyrosol acetate	0.065 a	0.014 b	0.014 b	0.019 b	0.021 b
DFOA[c]	0.885 a	0.468 b	0.475 b	0.542 b	0.466 b
tyrosol acetate	0.000	0.000	0.000	0.000	0.000
DFLA[d]	0.712 a	0.439 b	0.389 b	0.478 b	0.352 b
pinoresinol	0.012	0.000	0.007	0.013	0.013
cinamic acid	0.003	0.004	0.003	0.002	0.002
acetoxy-pinoresinol	0.012	0.021	0.015	0.013	0.012
AFOA[e]	0.852 b	1.160 a	1.011 a	0.743 b	0.735 b
AFLA[f]	0.569 b	0.703 a	0.587 b	0.487 c	0.438 c
ferulic acid	0.005	0.005	0.007	0.003	0.008
luteolin	0.006	0.007	0.006	0.007	0.009
apigenin	0.004	0.004	0.004	0.005	0.006
total phenols	3.188 a	2.935 b	2.610 c	2.368 d	2.154 e
total orthodiphenols	1.828 a	1.698 ab	1.546 bc	1.330 d	1.267 d
total secoiridoids	3.019 a	2.770 b	2.466 c	2.247 d	1.992 e

[a] Quantitation was performed using *p*-hydroxyphenylacetic acid and *o*-coumaric acid as internal standards. [b] For each phenolic compound, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. Each value is the mean value of three replicates. [c] DFOA = dialdehydic form of the oleuropein aglycon. [d] DFLA = dialdehydic form of the ligstroside aglycon. [e] AFOA = aldehydic form of the oleuropein aglycon. [f] AFLA = aldehydic form of the ligstroside aglycon.

olives with a lower level of maturation. An increase in acidity is closely related to the increase in the incidence of decay of the fruits from which the oil is extracted. Pereira et al. (*24*) did not select only healthy fruits for cold storage, evaluating the presence of 30% fly-attacked olives in the initial sample. The rapid deterioration of this commodity was not a surprise. Only healthy fruit can be refrigerated to obtain a high-quality olive oil. Consequently, this system is only compatible with the careful harvesting, packing, and transport of the product. It is a recognized fact that, the more immature a fruit is, the more resistant it is to suffering mechanical damages or infections (*11, 12*). No incidence of fruit decay has been detected in the present work (data not shown), and finding only a slight increase in the free acidity values during fruit storage can be considered normal. Possibly, the slight increase in this parameter was due to the internal lipase activity of the fruit itself. Garcia et al. (*11*), first, and Yousfi et al. (*22*), afterward, observed a similarly slight, although significant, increase in oil acidity during the progression of olive maturation on the tree.

The changes in peroxide value did not show any clear tendencies regarding the progress of fruit cold storage time. Most likely, this parameter depends upon factors that cannot be controlled in the experiment (for instance, the time of oil filtration). However, it is sufficiently clear that the storage of the fruit in a refrigerated room does not significantly affect the oil quality parameter, which always exhibited much lower values than the limit established for "extra" virgin oil (20 mEq of O_2 kg^{-1}).

In general, K_{232} and K_{270} values of the oils did not significantly vary with the progress of fruit cold storage. Only the values of K_{232} obtained from 'Picual' variety oils showed a slight but significant increase during the first 4 weeks of cold storage, but subsequently, this absorbance decreased until presenting the same value as the initial one. Previously, different authors, Garcia et al. (*5*), Kiritsakis et al. (*6*), Clodoveo et al. (*7*), and Kalua et al. (*10*), had found no effect induced by fruit cold storage on the UV absorbance of the olive oils subsequently extracted.

During olive cold storage, the overall grading of the sensory quality significantly diminished in the oils extracted from 'Verdial' olives only. However, the reduction was very slight, from 7.5 to 7.0, and in any case, the values for this parameter always remained within the limit established for the "extra" quality ($\geq$6.5). Consequently, the oils extracted from the stored fruit did not develop any negative sensory attributes, conserving the commercial category of quality "extra virgin" at all times. These results are similar to the ones obtained by García et al. (*5*), who observed how oils extracted from riper 'Picual' olives stored for 45 days at 5 °C were evaluated on the overall grading of sensory quality similar to the ones extracted initially.

Changes in the carotenoid and chlorophyll contents of the oil extracted during the conservation of the fruit were different in each analyzed variety. In oils extracted from 'Manzanilla' drupes, the carotenoids content maintained similar values during the 4 weeks at 5 °C. In those extracted from 'Picual' olives, the carotenoid content registered a clear reduction during the first 3 weeks of refrigeration, but subsequently, it increased until recovery of the initial values at the end of the storage period. Finally, the oils extracted from 'Verdial' olives showed, at the

Table 5. Changes in Content of Different Phenolic Compounds (mmol kg^{-1}) of Virgin Oils Obtained from 'Picual' Olives Previously Stored at 5 °C for Different Periods[a]

	storage time at 5 °C (weeks)						
phenolic compounds (mmol kg^{-1})[b]	0	1	2	3	4	5	6
hydroxytyrosol	0.015	0.026	0.016	0.011	0.010	0.017	0.013
tyrosol	0.017	0.016	0.012	0.009	0.010	0.013	0.018
vanillic acid	0.002	0.006	0.006	0.004	0.003	0.007	0.006
vanillin	0.000	0.000	0.000	0.000	0.000	0.000	0.000
p-coumaric acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000
hydroxytyrosol acetate	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DFOA[c]	0.266 a	0.273 a	0.264 a	0.256 a	0.228 a	0.259 a	0.135 b
tyrosol acetate	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DFLA[d]	0.235 a	0.158 b	0.118 c	0.171 b	0.107 c	0.108 c	0.083 c
pinoresinol	0.000	0.000	0.000	0.000	0.000	0.000	0.000
cinamic acid	0.005	0.004	0.003	0.003	0.003	0.003	0.003
acetoxy-pinoresinol	0.035 a	0.022 b	0.020 b	0.017 b	0.016 b	0.016 b	0.014 b
AFOA[e]	1.601 a	1.534 a	1.696 a	1.267 b	1.342 b	1.273 b	1.139 b
AFLA[f]	1.332 a	0.713 b	0.611 c	0.530 d	0.516 d	0.516 d	0.502 d
ferulic acid	0.003	0.004	0.002	0.002	0.001	0.003	0.003
luteolin	0.021	0.022	0.026	0.027	0.022	0.019	0.023
apigenin	0.012	0.012	0.012	0.011	0.011	0.011	0.011
total phenols	3.544 a	2.789 b	2.785 b	2.309 c	2.268 c	2.244 c	1.950 d
total orthodiphenols	1.903 a	1.854 a	2.001 a	1.562 b	1.602 b	1.568 b	1.310 c
total secoiridoids	3.433 a	2.678 a	2.688 b	2.225 c	2.193 c	2.156 c	1.859 d

[a] Quantitation was performed using *p*-hydroxyphenylacetic acid and *o*-coumaric acid as internal standards. [b] For each phenolic compound, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. Each value is the mean value of three replicates. [c] DFOA = dialdehydic form of the oleuropein aglycon. [d] DFLA = dialdehydic form of the ligstroside aglycon. [e] AFOA = aldehydic form of the oleuropein aglycon. [f] AFLA = aldehydic form of the ligstroside aglycon.

Table 6. Changes in Content of Different Phenolic Compounds (mmol kg^{-1}) of Virgin Oils Obtained from 'Verdial' Olives Previously Stored at 5 °C for Different Periods[a]

	storage time at 5 °C (weeks)								
phenolic compounds (mmol kg^{-1})[b]	0	1	2	3	4	5	6	7	8
hydroxytyrosol	0.032	0.036	0.025	0.025	0.032	0.029	0.019	0.011	0.023
tyrosol	0.111 a	0.129 a	0.128 a	0.120 a	0.113 a	0.062 b	0.068 b	0.054 b	0.051 b
vanillic acid	0.009	0.008	0.006	0.007	0.008	0.008	0.005	0.006	0.004
vanillin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
p-coumaric acid	0.003	0.000	0.000	0.002	0.001	0.002	0.001	0.001	0.004
hydroxytyrosol acetate	0.050	0.027	0.023	0.014	0.017	0.015	0.010	0.011	0.005
DFOA[c]	0.458 bc	0.621 a	0.677 a	0.530 b	0.387 c	0.553 b	0.335 c	0.264 d	0.237 d
tyrosol acetate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DFLA[d]	0.425 c	0.546 b	0.643 a	0.527 b	0.327 d	0.561 b	0.288 d	0.300 d	0.278 d
pinoresinol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
cinamic acid	0.002	0.003	0.003	0.005	0.003	0.003	0.004	0.003	0.003
acetoxy-pinoresinol	0.020	0.026	0.023	0.031	0.021	0.019	0.027	0.020	0.016
AFOA[e]	1.912 a	1.679 b	1.223 c	1.255 c	1.015 d	0.766 e	0.980 d	0.829 e	0.760 e
AFLA[f]	1.957 a	1.653 b	1.195 d	1.316 c	1.087 e	0.826 g	1.085 e	0.938 f	0.848 fg
ferulic acid	0.004	0.004	0.004	0.003	0.004	0.004	0.003	0.004	0.003
luteolin	0.010	0.011	0.007	0.009	0.011	0.007	0.010	0.008	0.007
apigenin	0.007	0.007	0.005	0.006	0.007	0.004	0.002	0.006	0.005
total phenols	5.001 a	4.749 b	3.962 c	3.851 c	3.034 d	2.859 e	2.837 e	2.455 f	2.244 g
total orthodiphenols	2.462 a	2.374 a	1.956 b	1.834 b	1.463 c	1.370 d	1.354 d	1.123 e	1.032 f
total secoiridoids	4.753 a	4.499 b	3.739 c	3.629 c	2.817 d	2.705 d	2.688 d	2.331 e	2.123 f

[a] Quantitation was performed using *p*-hydroxyphenylacetic acid and *o*-coumaric acid as internal standards. [b] For each phenolic compound, the absence of small letters means the absence of a significant ($p \leq 0.05$) effect of the treatments according to ANOVA and values followed by the same small letter are not statistically different ($p \leq 0.05$) according to Duncan's multiple range test. Each value is the mean value of three replicates. [c] DFOA = dialdehydic form of the oleuropein aglycon. [d] DFLA = dialdehydic form of the ligstroside aglycon. [e] AFOA = aldehydic form of the oleuropein aglycon. [f] AFLA = aldehydic form of the ligstroside aglycon.

beginning, a similar behavior to the one exhibited by 'Picual' oils, but they did not achieve the initial carotenoid content at the end of fruit storage.

The chlorophyll content showed a continuous increase in the samples of 'Manzanilla' and 'Picual' oils during fruit cold storage, although this parameter decreased abruptly in the 'Picual' oil samples after the sixth storage week, whereas those extracted from 'Verdial' drupes exhibited $a \geq 40\%$ loss of chlorophyll content after the first week of storage, maintaining a similar level of this pigment during the remaining storage time. Previously, Kiritsakis et al. (*6*) reported that oil extracted from 5 °C, 60 days stored 'Koroneiki' olives had slightly lower chlorophyll content than oil obtained from freshly harvested olives. The interpretation of these results becomes very difficult, given the complexity of the factors that determine the presence of these pigments in the oil.

Stability against oxidation of the oils was slightly reduced as a consequence of fruit refrigeration, decreasing after the period of storage <10% of the initial value in each variety tested. This fact can be considered normal, because the fruit matured during its cold storage and the oil stability decreased with olive maturation (*22*). Previously, García et al. (*8*) and Kiritsakis et

al. (*6*) had found that oil extracted from olives stored at 5 °C for ≥30 days showed a significantly lower value for oil stability against oxidation than the initial sample.

Effect of Olive Cold Storage on the Phenolic Content and Composition of the Extracted Oils. The total phenolic content of the oils extracted decreased as the fruit storage period progressed (**Tables 4−6**). In 'Picual' oils, the reduction of the secoiridoids derivatives content (45.8%) was proportionally higher than that of the *o*-diphenols (31.2%), specially because of the loss registered in the contents of the dialdehydic form of the ligstroside aglycon (DFLA) and in the aldehydic form of the ligstroside aglycon (AFLA). The reduction observed in the content of the different phenolic groups was more homogeneous in the oils extracted from 'Manzanilla' and 'Verdial' olives. The gradual decrease of bitterness observed in the oils extracted during olive storage coincided better with the contents exhibited by the total phenolics or the groups formed by *o*-diphenols or secoiridoids than with the content of each phenolic compound individually considered in the three varieties tested. It would be complicated to establish a relationship between the changes in the contents of the different phenolic compounds and the changes in the bitter intensity in the oil during fruit refrigeration. With only these results, it is not possible to identify exactly which individual compound is mainly responsible for bitterness, because the existence of saturation values for human senses complicates the determination of accurate correlations. According to Mateos et al. (*20*), concentrations of 0.5 mmol kg^{-1} of the aldehydic form of the oleuropein aglycon (AFOA) in the oil determined saturation in the sensory perception of the bitter attribute. In the present work, the extracted oils of the three varieties tested showed values higher than this limit but they only exhibited a middle intensity ("3") for this sensory attribute.

The loss in stability exhibited by the oils did not proportionally coincide with the loss in phenolic compounds. Thus, under the perspective of relative values, the higher loss of stability corresponded to the oils extracted from 'Manzanilla' drupes with 6.8%, followed by 'Picual' with 5.7%, and finally by those of 'Verdial' with 4.5% reduction. Nevertheless, the highest relative loss in phenolic compounds corresponded to the oils extracted from 'Verdial' olives with 50%, followed by 'Picual' with 45%, and finally by 'Manzanilla' oils with 32%. Curiously, the oils that exhibited a higher content in any one of these compounds were those that presented systematically lower values of stability. Uceda and Hermoso (*25*) explained this phenomenon by the dependency of oil stability to factors other than phenolic compound content, such as fatty acid composition or tocopherol content.

In summary, during the cold storage of green mature olives, the bitterness intensity of the oils subsequently extracted gradually diminished. The time of refrigeration necessary to achieve a middle level of this attribute, which can be acceptable for an extensive sector of consumers, was strongly related to the olive variety used. The commercial quality of the oils, measured by the parameters legally established by European Community (EC) regulations, did not deteriorate as a consequence of fruit storage, and the oil yield was significantly increased in mill olives. Although the use of green mature olives may require a more prolonged storage time and a more difficult harvesting, because they are attached to the tree more firmly than ripe olives, it would allow for a better postharvest handling of the fruits, which are more resistant to physical damages or fungal infections than the riper ones. Furthermore, an earlier harvesting would also have other additional advantages, such as a better tree recuperation for the next season, a minor exposition of the fruit to olive fly (*Bractocera oleae*) or to climatic injuries, and the better use of the olive mill machinery, which could be used for a longer period each season.

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