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ORIGINAL PAPER

Effect of the Previous Storage of Ripe Olives on the Oil Composition of Fruits

Antonio López-López · Francisco Rodríguez-Gómez · Amparo Cortés-Delgado · Pedro García-García · Antonio Garrido-Fernández

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Abstract This work consists of a detailed study on the changes that occur in the oil of olives (Manzanilla and Hojiblanca cultivars) when subjected to previous storage before their processing as ripe olives. Storage significantly ($p < 0.05$) increased acidity (0.998 and 0.438 g oleic acid/kg oil, respectively), peroxide value (10.21 and 13.86 mequiv O_2/kg oil) and K_{270} (0.069 and 0.033) but decreased K_{232} (0.325 and 0.569). There was also a significant ($p < 0.05$) increment in polar compounds (3.17 and 0.78%, mainly due to the formation of diacylglycerols and fatty acids), OOO + PLP triacylglycerol (1.37% only in Manzanilla), and erythrodiol (with an increment of $\approx$20 mg/kg oil) but significantly ($p < 0.05$) decreased some triacylglycerols in Manzanilla (LLL, 0.022%; OLL, 0.243%; OOL + PoOO; 0.731%), polyunsaturated fatty acids (C18:2n-6, 0.879 and 0.051 g/100 g oil while C18:2t, C18:3n-6, practically disappeared) and Δ^5-avenasterol (9.16 and 9.75 mg/kg oil). In general, Hojiblanca cultivar was more resistant to fat deterioration than Manzanilla.

Keywords Diacylglycerols · Fatty acids · Monoacylglycerols · Oxidized triacylglycerols · Ripe table olives · Sterols · Storage · Triacylglycerols · Triterpenic dialcohols · Unsaponifiable matter

A. López-López (✉) · F. Rodríguez-Gómez · A. Cortés-Delgado · P. García-García · A. Garrido-Fernández
Departamento de Biotecnología de Alimentos, Instituto de la Grasa (CSIC), Avenida Padre García Tejero 4, 41012 Sevilla, Spain
e-mail: all@cica.es

Introduction

Ripe olive processing was introduced in California (USA) at the beginning of the 20th century. Nowadays, this style is widely spread. According to the latest data published by the International Olive Oil Council, ripe olives may account for around 30% of the word's production of table olives, which was 2,153,500 tons in the 2007/2008 season; this means that $\approx$630,000 tons a year may be prepared as ripe olives.

The style is named as olives darkened by oxidation in the Trade Standard for Table Olives issued by the International Olive Oil Council. However, they are commonly known by their original American name: ripe olives. Usually, fruits for producing this style are previously preserved in an aqueous solution (brine or acidic water) and darkened throughout the year according to demand [1].

It is likely that the fruits' composition may be affected by their processing as table olives. Sugars are used by the microorganisms and are practically exhausted in most of the styles; in addition, other water soluble components are also diluted. The contents of water, oil, and ash increase while the caloric value and the concentrations of protein, fiber and vitamins decrease during preparation as Spanish green and directly brined olives. However, the reuse of lye and the reduction in the number of washings did not produce any modification [1]. In wizened naturally black Oinotria olives (WOTO) a decrease in firmness and polyphenol content and an increase in the oil concentration were noticed [2]. Ünal and Nergiz [3] reported a fluctuation in the crude fiber and a decrease in the protein content in the Memecik cultivar when olives were processed according to Spanish, Kalamata and naturally black olive styles; higher decreases were also found in K, Fe and Zn. In general, changes expected in ripe olives may be higher than

those observed in any other style because the treatments they are subjected to are stronger.

Apart from moisture, oil is the major component in table olives [4]. However, changes produced in this nutrient during processing have rarely been studied. Ünal and Nergiz [3] attributed the main differences in olive oil contents to the diverse maturation stage of the products but variations during elaboration were small. No clear trend for fatty acid changes was observed in this work. These findings led them to conclude that, in general, the oil concentration did not change with respect to their original values as a result of processing methods and later bulk storage. Borzillo et al. [2] have reported that the fatty acid composition remained unaltered during the overall treatment (blanching, salting and drying) of WOTO; moreover, the extracted oil parameters indicated that oil quality was maintained without lipid deterioration as the WOTO treatment progressed. They attributed this behavior to the polyphenol content, which was sufficiently large to protect against autoxidation during shelf life. Recently, it has been shown that the diverse steps for producing ripe olives affected the sterols and fatty and triterpenic alcohols; there were significant effects of cultivars or processing steps (ps) on unsaponifiable matter, β-sitosterol, Δ^5-avenasterol, total sterols, 1-docosanol, 1-tetracosanol (ps), erythrodiol and percentage erythrodiol plus uvaol [5].

The aim of this work was to study the effect of the previous storage phase (in acidic conditions) on the characteristics and composition of the oil in fruits intended for ripe olive processing.

Materials and Methods

Cultivars

Olives were of the Hojiblanca and Manzanilla cultivars, which are the most popular for preparing ripe table olives in Spain. Fruits were harvested at the green maturation stage; those used for sterile acid storage were picked 15 days later.

Previous Storage

The common storage process in acidified brines, which mimics that applied at industrial scale, was used. A detailed description of the process can be found elsewhere [1]. In this case, the olives (15 kg of each cultivar) were stored in 25-L PVC fermenters for 7 months. Initial brines consisted of an acidified solution ($\approx$0.60%, w/v, initial acetic acid or $\approx$0.90% expressed as lactic acid) containing 5% (w/v) NaCl. Fermenters were left to evolve spontaneously during the storage period. Storage was followed by physico-chemical and microbiological controls but exhaustive descriptions of their changes are beyond the scope of this work. However, a concise description of their evolution follows. The average initial pH value was 3.0 $\pm$ 0.1 and increased during storage to equilibrate at 4.2 $\pm$ 0.1. Acidity decreased slightly and stabilized around 0.60 $\pm$ 0.10 (w/v), expressed as lactic acid. The salt concentration decreased with time and stabilized at about 3.5 $\pm$ 0.2% (w/v). Finally, combined acidity increased during storage and reached an average value of 50 $\pm$ 2 mequiv/l. The only microbiota found in the brine during storage consisted of yeast. When the storage ended, the olive fat was removed as described below and the resulting oil was analyzed. Two fermenters of each cultivar (replicates) were used for the experiment. The oils extracted from the four fermenters were also analyzed in duplicate or triplicate, depending on the parameter. The resulting total degree of freedom and those for the variables and the errors are included in the tables showing the results of the corresponding ANOVA of the experimental design.

Sterile Storage

The experiment was carried out with olives of the Manzanilla cultivar only, using acidified (HCl 6 N) brines (6% NaCl) buffered with a citrate–phosphate mixture at 3.75, 4.25 and 4.75 pH values in the equilibrium. These solutions were then sterilized through micro-filtration using a sterile filter unit with 0.2-μm porosity (Millipore Co., Bedford, Massachusetts, USA).

The olives used for the experiment were washed thoroughly, sterilized with a sodium hypochlorite solution (50 mg/L active chlorine) at 35 °C for 2 min and washed twice with sterilized water to remove chlorine. Subsequently, 175 g of fruits were placed in autoclaved bottles A-314, filled with the appropriate acid sterile brine and sealed and stored at room temperature for 7 months. Five bottles were prepared for each pH. All manipulations were carried out in a laminar flow cabinet. After a time period of 7 months, the glasses were opened and the sterile stored olives were then subjected to normal oil extraction.

Oil Extraction

Olives were pitted, mixed with a homogenizer Ultraturax T25 (IKA-Labortecnik, Staufen, Deutschland) and then boiling water (100 °C) was added to the paste. The resulting suspension was subjected to malaxation for 40 min at room temperature (22 °C $\pm$ 2) and the liquid was removed by centrifugation using ABENCOR equipment (Abengoa, Madrid, Spain). The process is similar to that used by Martínez, et al. [6] for the estimation of olive oil yield in olive mills. The liquid phase was allowed to decant and the oil was obtained, filtered and subjected to

analysis. This method was used to prevent changes in the oil quality as much as possible.

Determination of the Physicochemical Quality Parameters in the Extracted Oil

All determinations were carried out following the analytical procedures described in Regulations (EEC) No 2568/91 and (EC) No 79/2002 of the Commission of the European Union. A synthetic description of the methodology follows:

Free fatty acid, expressed as g oleic acid/100 g oil, was determined by the titration of a solution of oil dissolved in ethanol/ether (1:1) with 0.1 M potassium hydroxide in ethanol, using phenolphthalein as indicator.

The peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (mequiv O_2/kg oil), was determined by the reaction of a mixture of oil and chloroform/acetic acid with a solution of potassium iodine in the dark.

K_{270} and K_{232} extinction coefficients were calculated from absorption at 270 and 232 nm, respectively, with a UV spectrophotometer (Model Varian Cary 1E, Margrave, Vi, Australia), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Separation of Polar and Non-polar Compounds

The oils were fractioned using silica gel columns, according to the procedure developed by Dobarganes et al. [7].

Determination of Polar Compounds

The polar compounds (PCs) and diverse components triacylglycerol oligopolymers (TGP), oxidized triacylglycerols (ox-TG), diacylglycerols (DG), and free fatty acids (and other minor components) (FFA) were analyzed according to the method developed by Dobarganes et al. [7]. Conditions applied for HPSEC (high performance size exclusion chromatography) analysis were as follows: sample solutions of 10−15 mg of polar compounds/mL in tetrahydrofuran were used for the analysis. An HP1050 system, with a 10-μL sample loop and three 50, 100 and 500 Å Ultrastyragel columns (Waters Associates, Milford, MA, USA), 25 × 0.77 cm ID, packed with a porous, highly cross-linked styrene-divinylbenzene copolymer (<10 μm), connected in series and a refractive index detector (Hewlett-Packard, CA, USA) were used.

Triacylglycerol Composition

The analysis of triacylglycerols was performed, in the non-polar fraction, according to the official chromatographic methods of the EEC Regulations number 2568/91 and 2472/97. A Waters 2695 Separations Module, a Waters 2414 Refractive Index Detector, and a computer with Empower 2 software (Waters Assoc., Milford, MA) were employed using a Lichrospher/Superspher RP18 column (250 × 4.0 mm, 4 μm particle size; Phenomenex, Torrance, CA, USA), and the following settings were used: column oven, 30 °C; elution solvent, acetone/acetonitrile (1:1, v/v); flow rate, 1.5 mL/min. The standards used were trilinolein (LLL), triolein (OOO), tripalmitin (PPP), tristearin (SSS), trilinolein (LnLnLn), and tripalmitolein (PoPoPo) of purity greater than 98% and purchased from Sigma (St. Louis, MO). The abbreviations used for the fatty acids were Po for palmitoleic, L for linoleic, Ln for linolenic, O for oleic, P for palmitic and S for stearic.

Fatty Acid Composition

The analytical methods for the determination of fatty acid composition are described in the regulation EEC 2568/91. Fatty acids from the non-polar fraction were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2 g of oil and 3 mL of hexane with 0.4 mL of 2 N methanolic potassium hydroxide. The converted fatty acid methyl esters were analyzed with a Hewlett-Packard 5890 series II gas chromatograph, incorporating a fused silica capillary column Select FAME (100 m × 0.25 mm, 0.25 μm film thickness) (Varian, Bellefonte, PA) and a flame ionization detector was used for GC analysis. Hydrogen was used as the carrier gas at 1 mL/min. The injector (split 1:20) and detector temperatures were 250 °C. The operating conditions were as follows: oven temperature was held at 120 °C for 5 min and then increased by 4 °C/min to 240 °C and held for 20 min at 240 °C. Saturated and unsaturated methyl esters (C_4−C_{24}) (Sigma, St. Louis, MO) were used as reference standards as well as the linoleic and linolenic acid methyl ester isomers mix which was also purchased from Sigma.

Determination of the Unsaponifiable Fraction

The unsaponifiable matter was determined by saponification of the oil with potassium hydroxide in an ethanolic solution and extracted with diethyl ether, according to UNE 55004 issued by the *Asociación Española de Normalización y Racionalización* (AENOR).

Determination of Sterols and Triterpenic Dialcohols

This analysis was performed according to the method described in the regulation EEC 2568/91. The lipid with added α-cholestanol and betulin as internal standards was saponified and the unsaponifiable matter was extracted as

mentioned above. The bands corresponding to the sterols and triterpenic alcohol fractions were separated from the extract by TLC on a basic silica gel plate. The sterols and erythrodiol and uvaol recovered from the plate were transformed into trimethylsilyl ethers and the mixture was analyzed by GC using an HP 5890 Series II gas chromatograph (Hewlett Packard, Minnesota, USA) equipped with a flame ionization detector and a 30 m × 0.32 mm ID. Tracsil TRB-5 (95% dimethylpolysiloxane-5% diphenyl, film thickness 0.25 μm) capillary column (Teknokroma, Barcelona, Spain). The chromatographic conditions were: injector 300 °C, isothermal column 275 °C, and detector 300 °C. The split ratio was 1:50. Hydrogen carrier gas was used at 1.0 mL/min.

Determination of Fatty Alcohols

This analysis was performed according to the method described by Regulation (EC) N° 79/2002. The fatty substance, with 1-eicosanol added as internal standard, was treated as mentioned in the determination of lipid and unsaponifiable fraction section. The alcohol fraction was separated from the unsaponifiable matter by chromatography on a basic silica gel plate. The alcohols recovered from the silica gel were transformed into trimethylsilyl ethers and analyzed using capillary gas chromatography. The equipment and chromatographic conditions were the same as those mentioned above for sterols and triterpenic dialcohols, except that oven temperature was as follows: 215 °C (5 min); 3 °C/min increased to 290 °C and held for 2 min. All analyses were performed in duplicate.

Statistical Analyses

Changes in the different parameters analyzed were studied by the General Linear Model (GLM). The different groups of compounds were subjected to a nested ANOVA, considering the type of fruits (fresh and stored) as nested in cultivar in order to determine changes due to storage within cultivar. Significant changes due to processing were obtained from the results obtained from the univariate results for each variable. Later, a detail study of the significant effects was made by graphing (bar with errors) the changes in the significant parameters, using the corresponding option of the same statistical procedure, according to cultivar and type of fruit (fresh and stored) within cultivars. The different statistical techniques used in this work were implemented using STATISTICA, release 6.0.

Results and Discussion

Only those parameters for which significant ($p < 0.05$) changes during storage were observed by GLM analysis, nested design of type of fruit (fresh and stored) within cultivar (Manzanilla and Hojiblanca) will be discussed.

Oxidative Stability

Quality Characteristic of Oils in Fresh Olives

The quality characteristics of the oils in the fruits before being stored were determined. The values of acidity, peroxide value and coefficients of specific extinction (K_{232} and $K_{270,}$) are shown in Table 1. All values were lower than the limits set by EU Regulation (EC) 79/2002 for extra virgin olive oil.

Changes in Acidity

Triacylglycerol hydrolysis in table olives debittered with microbiological and chemical processes was studied by Ciafardini and Gomes [8]. In this experiment, acidity within the cultivar increased significantly ($p < 0.05$) during storage (Table 2) and the change was more pronounced in Manzanilla olives, which increased from 0.205 to 1.203 g oleic acid/100 g oil. This modification means a marked degradation in this parameter which transforms the oil quality from extra virgin olive oil in the raw material into only virgin olive oil in the stored fruits (Table 1). However, in the Hojiblanca cultivar, acidity in its oil changed from 0.280 to only 0.719 g oleic/100 g oil,

Table 1 Changes in the main physicochemical characteristics of the olive oil during the previous to oxidation storage of black ripe olives

Parameter	Manzanilla		Hojiblanca		Quality limits[a]	
	Fresh	Stored	Fresh	Stored	Extra virgin	Virgin
Free acidity (g oleic acid/100 g oil)	0.205 (0.005)	1.203 (0.031)	0.280 (0.005)	0.719 (0.011)	≤1.000	≤2.00
Peroxide value (mequiv O_2/kg oil)	15.36 (0.78)	25.57 (1.49)	12.92 (0.81)	26.87 (0.90)	≤20.00	≤20.00
K_{232}	1.841 (0.017)	1.515 (0.062)	1.897 (0.229)	1.328 (0.012)	≤2.500	≤2.60
K_{270}	0.184 (0.020)	0.254 (0.007)	0.120 (0.004)	0.168 (0.05)	≤0.200	≤0.20

Note: Standard error in parenthesis

[a] according to the EU Regulation 2568/91

Table 2 Results of the nested design ANOVA

Parameter	Cultivar (1 *df*)		Type of fruit (within cultivar) (2 *df*)	
	F value	*p* Value	*F* value	*p* Value
Acidity, peroxide value and spectrophotometric measurements (error = 8 *df*; total = 11 *df*)				
Acidity	70.74	<0.001	502.25	<0.001
Peroxide value	0.17	0.687	40.96	<0.001
K_{232}	0.60	0.462	14.96	0.002
K_{270}	68.64	<0.001	21.54	<0.001
Polar compounds and their components (error = 8 *df*; total = 11 *df*)				
Polar compounds	84.49	<0.001	215.45	<0.001
Oxidized triacylglycerols	9.38	0.015	10.37	0.006
Diacylglycerols	61.39	<0.001	151.11	<0.001
Free fatty acids (+others)	211.26	<0.001	773.58	<0.001
Triacylglycerols composition (error = 8 *df*; total = 11 *df*)				
LLL	103.65	<0.001	11.82	0.004
OLL	38.13	<0.001	21.46	<0.001
OLL + PoOO	52.57	<0.001	15.280	0.002
OOO + PLP	3074.10	<0.001	18.60	<0.001
Fatty acids (error = 20 *df*; total = 23 *df*)				
C16:0	230.07	<0.001	9.18	0.007
C18:0	48.79	<0.001	8.84	0.008
C23:0	8.02	<0.003	13.16	0.001
C16:1	2138.08	<0.001	9.17	0.007
C18:2n-6	31.81	<0.001	161.09	<0.001
C18:3n-6	3.00	0.072	2.85	0.107
C18:2t	3.11	0.067	2.66	0.118
Saturated fat (%)	180.66	<0.001	8.32	0.009
Polyunsaturated fat (%)	21.43	<0.001	131.95	<0.001
Sterols, fatty and triterpenic alcohols (error = 8 *df*; total = 11*df*)				
Δ^5-Avenasterol	5.54	0.046	7.83	0.013
Erythrodiol	148.85	<0.001	16.97	0.001

Only those parameters which showed significant changes during storage are included (*df*, degree of freedom)

increase that, although statistically significant ($p < 0.05$), still permits the classification of the stored fruits' oil as extra virgin olive oil when considering just this characteristic. Such behavior indicates that, although in the raw material, the initial acidity was higher in Hojiblanca, the oil in this cultivar was less affected by storage. Possibly, its stronger structure and higher polyphenol content was able to offer a better protection to the oil in the fruit [1] because, usually, the hydrolysis of triglycerides is higher as the free fatty acid increases [9].

The acidity increase may be due to enzymatic activity from the olives themselves or to lipases from the microorganisms present during storage. The increase in acidity due to the acetic acid absorption from the storage medium must be negligible because of its low partition coefficient

Table 3 Changes induced in the olive oil acidity (g oleic acid/100 g oil) due to the fruit itself and pH of the media (storage under aseptic conditions)

Type of olives	pH = 3.7	pH = 4.3	pH = 5.0
Fresh fruits	0.400 (<0.001)	0.400 (<0.001)	0.400 (<0.001
Aseptic stored fruits	0.645 (0.007)	0.595 (0.007)	0.680 (<0.001)

Note: Standard deviation in parenthesis

of acetic acid in olive oil. It is well documented that free fatty acid in olives intended for oil extraction may increase during the previous storage and along the extraction process [10]. However, in the case of the stored olives intended for ripe olives, the effect of the fruits themselves and pH storage was separately tested by aseptic storage of the Manzanilla cultivar (Table 3). Apart from the higher initial acidity due to a slightly higher maturation degree of the fruits, the storage in acid conditions of this cultivar, apparently the most sensitive to hydrolysis, showed that the participation of the olives' own enzymes or the pH of the media was very limited and accounted for about 0.200 g oleic acid/100 g olive oil regardless of the pH of the samples (Table 3). Thus, the hydrolysis may be due to enzymatic activity from the microbial population in the storage media. Only yeasts were isolated and identified during this phase. Two of the species were not lipase producers (*Saccharomyces cerevisiae* and *Pichia membranaefaciens*); however, the other two were positive lipase producers: *Candida boidinii* and *Pichia membranifaciens* (weak). A detailed description of the characteristics of these species is outside the scope of this work but their presence points out that they can play an essential role in the hydrolysis of olive oil during storage.

Primary Oxidation

Lipid oxidation is considered to be the predominant cause of deterioration in various fat-containing foods during storage. It involves the interaction between unsaturated lipids and oxygen-active species. Oxidation may be responsible for the alterations in the quality characteristics of foods and lead to the development of undesirable off-flavor [11]. The initial peroxide value in the raw material was slightly, non significantly, higher in Manzanilla than in Hojiblanca. Storage increased the peroxide value similarly in both cases (Table 1) and the changes were significant at $p < 0.05$ (Table 2). The final values ($\approx$25 mequiv O_2/kg oil) indicated that, regardless of the cultivar, the oil suffered a marked oxidative deterioration in the storage and the resulting oil at the end of this phase could not be classified as extra virgin olive oil or even virgin olive oil. There are numerous studies dealing with the oxidation

mechanism in bulk oil; however, when it is still in the fruits, the cells are intact and the oil is dispersed, forming droplets in the interior of the cells, the reaction should follow a very different mechanism. In some aspects, the system could be assimilated to emulsions. In this case, the lipid susceptibility to oxidation is influenced by various factors (chemical structure, oxygen concentration, antioxidants, droplet characteristics), interactions with the aqueous phase components (sugars, salts, polysaccharides, proteins) and the presence of prooxidants (e.g., transition metal impurities) [12]. It is likely that lipid oxidation takes place at the surface of the oil droplets. Many studies suggest that the interaction between lipid hydroperoxides, the first products formed by oxidation, located at the droplet surface and transition metals, from the surrounding aqueous phase, is the most common cause of oxidative instability [13], although other factors such as the presence of chlorophyll pigments, vitamin E content and light can also have an affect. The most likely mechanism is the decomposition of lipid hydroperoxides (RCOOH) by the prooxidants into highly reactive peroxyl (RCOO*) and alkoxyl (RO*) radicals, which react with unsaturated lipids within the droplets, leading to the formation of lipid radicals. The lipid oxidation chain reaction propagates as these lipid radicals react with other lipids in their immediate vicinity [14]. The necessary fatty acid may be easily available in these olives in which there is always a certain residual acidity in addition to that due to the hydrolysis of the triacylglycerols produced by lipolytic enzymes from the fruits or from the environmental microflora [15]. Lipoxygenase activity, which also requires the presence of unsaturated free fatty acids, may also be responsible for this oxidation because some of these enzymes are capable of oxidizing the lipids in the absence of oxygen [16].

Oxidation Products

During lipid oxidation, a number of decomposition reactions occur simultaneously that in turn result in the generation of a wide variety of different molecules including aldehydes, ketones, alcohols, and hydrocarbons. These oxidation products are responsible for the characteristic physicochemical and sensory properties of oxidized oils since they are more surface-active than the initial lipids and some of them are water soluble. It is known that the oxidation products of oils and fats, which may result from their decomposition, display characteristic spectra in the ultraviolet region (at about 232 nm). Therefore, a determination of the absorbance at this wavelength is an indication of the state of oxidation of oil, and particularly of the level of dienes present in it.

The K_{232} coefficients (Table 1) were similar in the oil from the fresh fruits (Manzanilla and Hojiblanca cultivars), having values below the limits established by Regulation (EC) n° 79/2002 for virgin olive oil. Storage decreased significantly ($p < 0.05$) the values of this index, indicating that, apparently, the storage period in acid conditions has produced a decrease in the presence of dienes. Consequently, there was no deterioration with respect to this parameter, which always remained far below 2.5 units (EU regulation limit).

On the contrary, the values of K_{270} (related to the presence of trienes), which was significantly higher in the oils from fresh Manzanilla fruits, increased (Table 1) significantly during storage in both cultivars (Table 2), while maintaining approximately the same proportion between them as in the raw material (Table 1). Only in the stored fruits from Manzanilla olives did their final values exceed the limits established by Regulation (EC) 79/2002 for extra or virgin olive oil ($\leq$0.2). This change is a new sign of the higher sensitivity of the Manzanilla cultivar to oxidative deterioration with respect to Hojiblanca during storage. However, the increase in ΔK was moderate (data not shown) and always led to values below the limits set by Regulation (EC) 79/2002.

Overall changes observed in the evolution of oil oxidation during the storage of fruits showed that, apparently, regardless of the cultivar, the hydroperoxides formed do not develop into dienes but are transformed into non-absorbing in the ultraviolet spectrum compounds or trienes. In addition, new trienes are formed during this storage phase.

In any case, transformations observed in the oil of the stored olives, means that there is a degradation of some of the oil parameters (acidity, peroxide value, and K_{270}) which modifies its classification from extra virgin olive oil in the fresh fruits into lampante olive oil which, if extracted, will require refining to be edible.

Changes in the Olive Oil Components

In this section, changes in the main constituents of the olive oil are reported. They were studied separately for polar and non polar fractions as well as for unsaponifiable.

Polar Compounds

The PCs comprise all the substances with higher polarity with respect to those of the unaltered triacylglycerols and in particular triacylglycerol polymers (TGP), ox-TG, diacylglycerols (DG) and FFA (including free sterols, triterpene diols and other compounds). The polar compounds in the fresh fruits were fairly similar in Manzanilla and Hojiblanca ($\approx$ 2.0% oil) and markedly lower than values found by Caponio et al. [17] at crusher and paste temperatures between 12 and 20 °C or 20 and 24 °C, respectively,

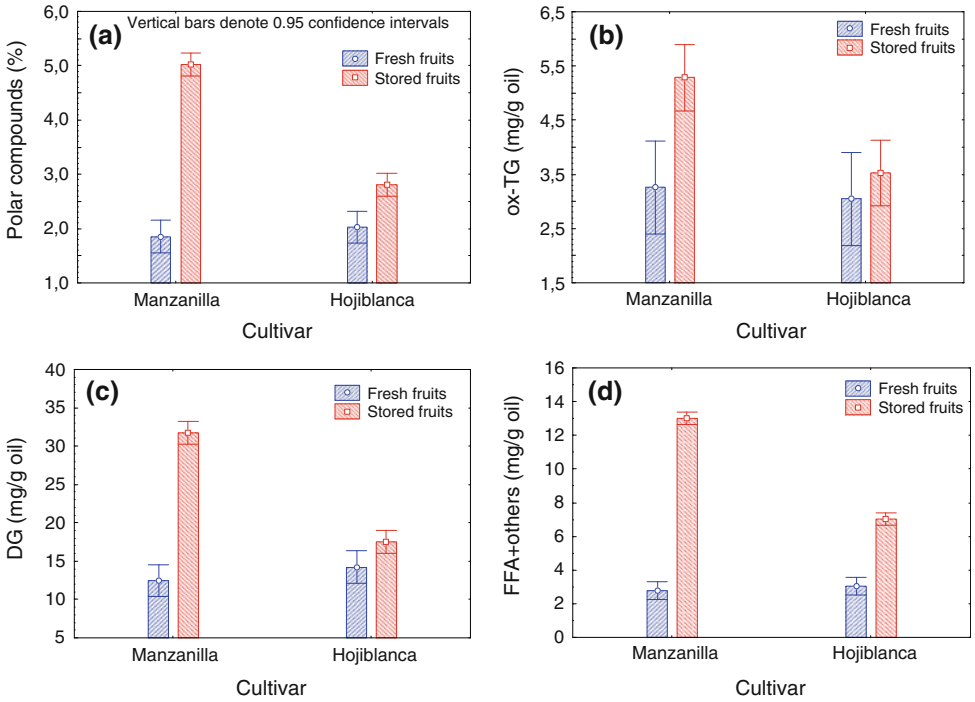

Fig. 1 Changes, according to cultivars, in total polar compounds and their components during the previous storage of fruits intended for ripe olive processing

which were around 3.5%. The storage always produced a significant ($p < 0.05$) increase in polar compounds (Table 2) and there was no overlapping in the corresponding confidence limits of the respective bars between the contents in fresh and stored fruits within cultivars (Fig. 1a). However, the increment was markedly higher in Manzanilla olives, where it reached about 5%, while in Hojiblanca the rise was up to only slightly below 3% oil (Fig. 1a). This is another indication of the higher susceptibility to oxidation of the Manzanilla oil during storage. This behavior is consistent with most of the parameters studied and is in agreement with the higher level of acidity and K_{270} values reached in Manzanilla olives at the end of storage. No polymerized triacylglycerols were found. The oxidized triacylglycerols, comprising all the oxidized forms of triacylglycerols, which is an index of the primary step of oxidative degradation had a fairly low value in the fresh fruits of both cultivars, but increased markedly (significant at $p < 0.05$) after storage within cultivars (Table 2). This effect was mainly due to the increment in Manzanilla (up to ≈0.55%); however, in Hojiblanca, they remained at around 0.30−0.35% and the confidence limits for fresh and stored fruits from this cultivar overlapped (Fig. 1b). This level is similar to those found by Caponio et al. [17] regardless of the crushing temperature (up to 24 °C) after 6 months' storage of extra virgin olive oil but lower than the values reached by the same oil after 12 months' storage [17]. Diacylglycerols in the raw material of both cultivars were about 1.2−1.5% and also increased significantly ($p < 0.05$) (Table 2) but the confidence limits between fresh and stored fruits did not overlap only in Manzanilla (≈3.2%) (Fig. 1c). The values in the fresh fruits were lower than those reported by Caponio et al. [17] in oils extracted at different temperatures. On the contrary, in Manzanilla, the levels after storage were markedly higher. Free fatty acids (including other minor components) had significantly (Table 2) higher values at the end of storage, regardless of cultivar, although the increase was markedly higher in Manzanilla (Fig. 1d), as a result of its more fragile sensitivity to alteration. The value of this parameter increased from 0.2−0.3% to about 1.3 and 0.8% in Manzanilla and Hojiblanca cultivars, respectively. These data are in agreement with the free fatty acid determined by titration. Overall, the formation of polar compounds turned out to be a good index of oil deterioration. Especially noticeable was the formation of diacylglycerols and free fatty acids as a result of the oil hydrolysis, changes that were especially high in Manzanilla due to the fact that, as indicated above, the oil in Hojiblanca is more resistant to degradation.

Triacylglycerols

More than half of the proportion of triacylglycerols in olive oils is OOO, followed by SOL + POO (≈21%), OLO + LnPP (8%), and OLA + SOO (≈7%) while those including mainly polyunsaturated fatty acids are in low proportions (POL + SLL, 2.5%; LLL, ≈0.06%; OLL + PoOL, ≈0.7%; OLLn + PoLL, 0.1%, etc.) [18]. Changes in triacylglycerol composition due to olive oil

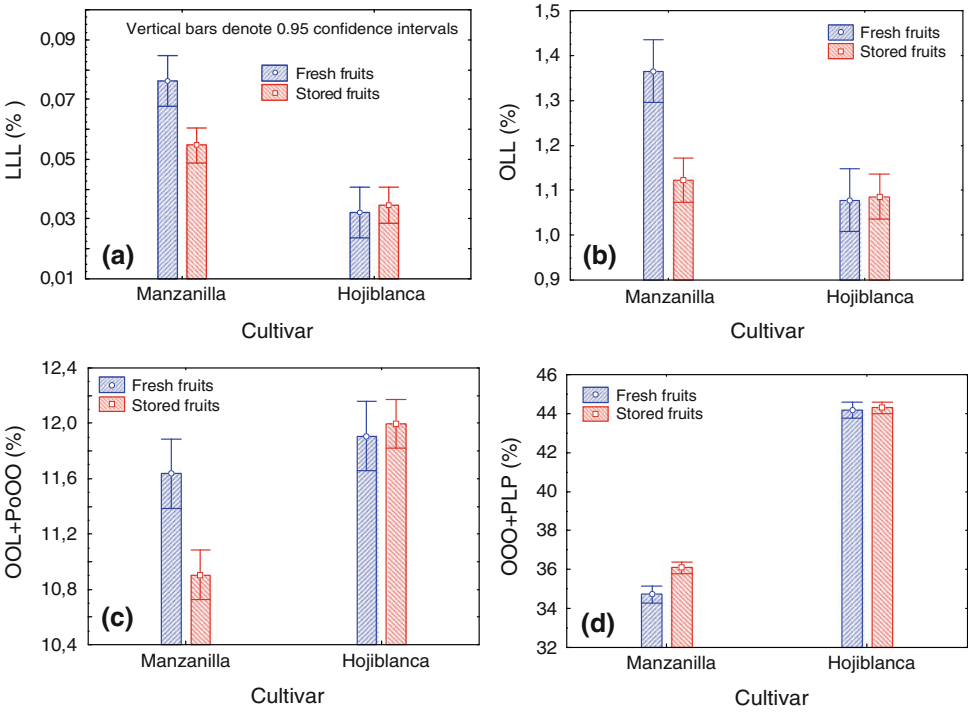

Fig. 2 Triacylglycerol components which showed significant changes, according to cultivars, during the previous storage of fruits intended for ripe olive processing

oxidation are scarcely studied. However, Mateos et al. [19] established that olive oil stability mainly dependeds on the triacylglycerol composition. In the storage phase of ripe olives, only four triacylglycerols showed significant changes within cultivars (Table 2). However, this effect was due to changes in the Manzanilla cultivar but no modification of these compounds was observed in Hojiblanca (usually less sensitive) (Fig. 2). In three of them (LLL, OLL, and OOL + PoOO) the proportion decreased while in OOO + PLP an increase was observed. Apparently, the more sensitive triacylglycerols were those containing three, two, or one linoleic acid in their structure. It could be possible that OOO + PLP could also have decreased but, due to the marked decrease of the other three, its overall balance was positive. Thus, in general, the sensitivity of the triacylglycerols to degradation decreased as the unsaturation degree decreased and none of those with high proportions of saturated fatty acids were affected.

Fatty Acids

In virgin olive oils, the most abundant fatty acid is oleic (including all forms of C18:1) which accounts for 70–80%, followed by palmitic (C16:0) (10–20%), and linoleic (C18:2n-6) (3–20%) [20]. In general, no significant changes in monounsaturated fat were observed (Table 2). The unsaturated fatty acids that showed significant ($p < 0.001$) changes in content due to storage (within cultivars) were C16:0, C18:2, C23:0 and, as result, the proportion of saturated fat was reduced (Fig. 3), although the decrease was only observed in Hojiblanca. The meaning of these changes is not clear because the high stability of saturated fat but might be related to the structure and composition of Hojiblanca. There was also a significant ($p < 0.005$) decrease in the palmitoleic (C16:1) concentration in Manzanilla (Table 2, Fig. 3e) but its impact was reduced and not sufficient to induce overall significant differences in the monounsaturated fat.

On the contrary, there was a significant decrease in polyunsaturated fat and the effect was consistent in both cultivars, although the difference between the contents in the fresh fruits and at the end of storage was also markedly higher in Manzanilla (Fig. 4). Among the polyunsaturated fatty acids, three of them showed a significant decrease in Manzanilla: linoleic acid (C18:2n-6), *trans*-linoleic acid (C18:2t, including all *trans* forms), and linolenic acid (C18:3n-6) (Fig. 4). In Hojiblanca, only C18:2n-6 decreased significantly but in a lower proportion than in Manzanilla (Fig. 4a).

These transformations are in agreement with the observed changes in triacylglycerols and demonstrate that oleic acid is fairly stable in acidic storage conditions while the polyunsaturated fatty acids are the most sensitive to transformation and oxidative deterioration. In spite of this, the destruction of C18:2n-6 is relatively low and its decrease was about 1%; however, in the case of C18:2t or C18:3n-6, the decrease in Manzanilla practically removed the acids from the oil composition (Fig. 4b, c). Results correspond to the relative susceptibility of the respective

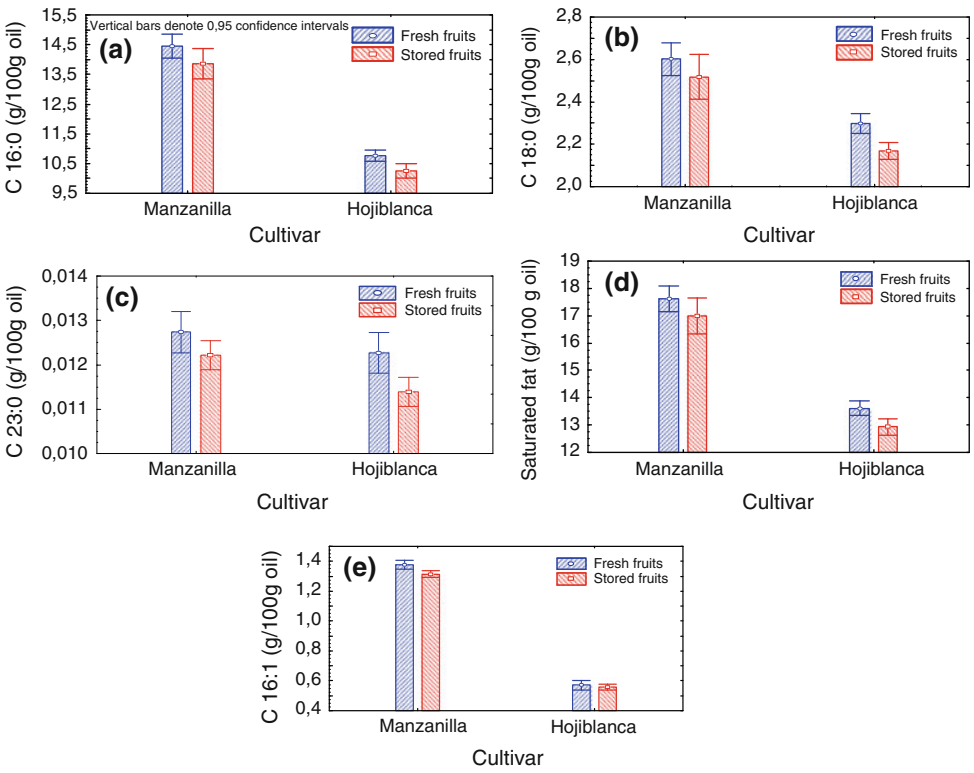

Fig. 3 Saturated and monounsaturated fatty acid components which showed significant changes, according to cultivars, during the previous storage of fruits intended for ripe olive processing

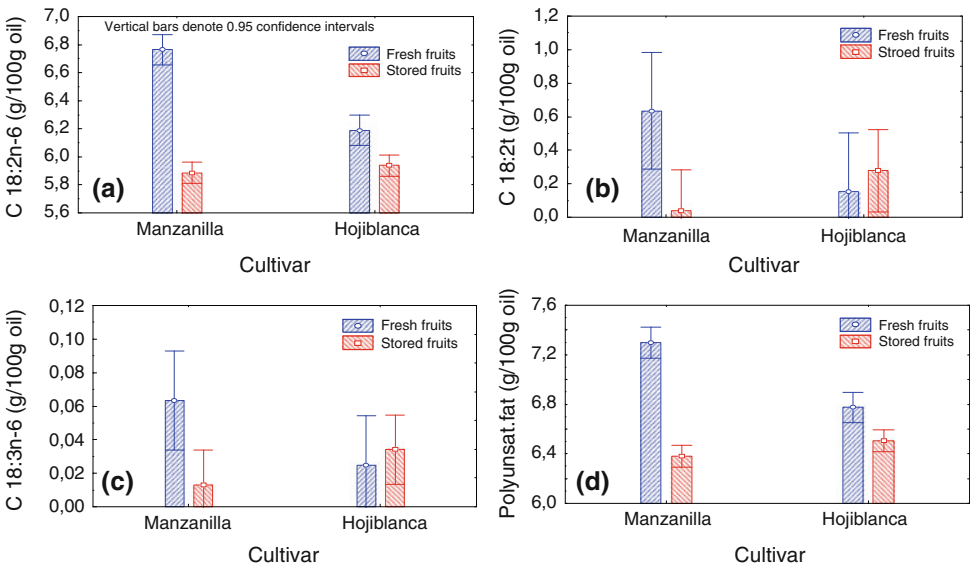

Fig. 4 Polyunsaturated fatty acid components which showed significant changes, according to cultivars, during the previous storage of fruits intended for ripe olive processing

acids; linoleate is 40 times more reactive than oleate and linolenate 2.4 times more reactive than linoleate [21].

Sterols, Fatty Alcohols and Triterpenic Dialcohols

The unsaponifiable matter was not significantly affected by the storage phase of ripe olives and just a reduced number of its components showed significant transformations. With respect to sterols, only Δ^5-avenasterol showed a significant decrease in Manzanilla, which changed from 59.20 ($\pm$ 2.10) mg/kg oil in the raw material to 40.05 ($\pm$ 1.98) mg/kg oil, a decrease of about 20 mg/kg oil; in Hojiblanca, it followed the same trend but the difference was insignificant (63.54 $\pm$ 1.98 mg/kg oil in the raw material and 53.80 $\pm$ 4.57 mg/kg oil in the stored olives). The proportion of this sterol is not included in Regulation (EC)

79/2002. No aliphatic alcohol was affected by storage but there was a significant ($p < 0.05$) (Table 2) increase in the proportion (about 20 mg/kg oil) of erythrodiol during storage regardless of the cultivar, although the initial (54.22 ± 2.15 mg/kg oil) and final (76.25 ± 1.96 mg/kg oil) levels in Manzanilla were markedly higher than in Hojiblanca (6.30 ± 1.30 and 29.48 ± 4.04 mg/kg oil, respectively). Overall, changes in sterols and triterpenic dialcohol can be considered of limited significance, although their absolute values were higher than in some Spanish olive oils [10].

In summary, this work has studied, in detail, the changes that occur in the oil when olives are stored before their processing as ripe olives. The investigation has shown that the oil may suffer degradation, which depends on the cultivar. Hojiblanca olives have shown to be markedly less sensitive to oil degradation than Manzanilla. In fact, regarding their oxidative characteristics, the oil from the Hojiblanca cultivar could be classified as extra virgin olive oil, except for the peroxide value. Most of the changes observed were related to the formation of polar compounds, especially diacylglycerols and fatty acids, and degradation (disappearance) of polyunsaturated fatty acids, mainly linoleic and linolenic (only in Manzanilla) acids but in Hojiblanca a consistent significant decrease of unknown origin in some saturated fatty acids was also observed. On the contrary, the unsaponifiable matter was slightly affected and only Δ^5-avenasterol (decrease) and erythrodiol (increase) showed significant changes. Results clearly show that processing can produce noticeable changes in table olive fats which may affect the quality of the final products. Apparently, such modifications have been underestimated in the past.

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