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Effect of Harvesting System and Fruit Cold Storage on Virgin Olive Oil Chemical Composition and Quality of Superintensive Cultivated 'Arbequina' Olives

Khaled Yousfi,[†] Carlos M. Weiland,[†] and José M. García*[,§]

[†]Departamento de Ciencias Agroforestales, Universidad de Huelva, Carretera de Palos de La Frontera s/n, 21819 Huelva, Spain

[§]Departamento de Fisiología y Tecnología de Productos Vegetales, Instituto de la Grasa (CSIC), Avenida Padre García Tejero 4, 41012 Seville, Spain

ABSTRACT: Storage at 3 and 18 °C of 'Arbequina' olives (*Olea europaea* L.) cultivated in hedgerows and harvested manually or mechanically (wine grape harvester) was tested. Fruit characteristics and oil quality were monitored. Mechanical harvesting caused internal fruit damage that induced its rapid softening and decay, but also facilitated obtaining higher amounts of oil, which suffered a rapid deterioration during fruit storage. This oil presented lower tocopherol and phenol contents and lower oxidative stability than the oil extracted from manually harvested olives, but showed similar fatty acid composition. Cold storage (3 °C) delayed all of these deterioration processes. It allowed maintaining the best commercial level of quality ("extra") in the oil from mechanically harvested olives for 10 days. This cold storage could be considered as an alternative to the increase in machinery for processing the growing olive production, due to both hedgerow cultivation and mechanized harvesting.

KEYWORDS: *acidity, Olea europaea, olive hedgerow cultivation, phenols, stability, tocopherols*

INTRODUCTION

The olive oil industry has been historically conditioned by the high costs of the fruit harvesting, which has been estimated to be around 60% of the total costs of oil production.[1] In the next decade, most of olive groves will show the dimensions and arrangement suited to modern harvesting techniques.[2] A new cultivation system has been developed, which involves densities of >1000 trees/ha and, for this reason, has been termed "hedgerow" or "superintensive".[3,4] This system allows for the use of the harvester already used for grape gathering in vineyards, reducing labor costs as well as the period of harvesting.[5] Due to its small size, precocity, high oil yield, oil quality, branch flexibility, and easy fruit abscission, the 'Arbequina' variety ranks among the traditional Spanish oil varieties best suited to hedgerow cultivation and mechanical harvesting.[6] Virgin olive oil from 'Arbequina' is highly acclaimed on the international market due to its excellent sensorial quality. However, this appreciated quality depends on the maturity of the fruit. Advanced maturation results in a clear reduction of sensory positive attributes and oxidative stability due to the reduction of contents on photosynthetic pigments (chlorophylls and carotenoids) and phenolic compounds.[7] This circumstance makes it necessary that the 'Arbequina' fruit be harvested at an early stage of maturation and during a short period.[8] Because the practice of hedgerow cultivation results in a yield increase of 3−5 times as compared with traditional cultivation, and due to the fact that modern practice is spreading, it is to be expected that the milling industries will, in the near future, face an extreme concentration of production, which might surpass their processing capacity. Because olive mills lack suitable facilities for storing fruit to be processed, this leads to a rapid deterioration.[1] As the oil quality depends on the physiology of the fruit from which it is extracted, the oil from deteriorated fruits will not reach the quality level required to be commercialized as extra virgin olive oil.[9] Furthermore, Dag et al.[10] verified that the use of mechanical harvesting with hand vibrating combs was responsible for a slight loss of quality in the oil subsequently extracted. The effect of the use of a grape harvester on olive oil quality has, to date, not been tested, but knowing that its action may be more aggressive for fruit than with vibrating combs, it is easy to assume that the time margin in which an oil of optimal quality may be obtained from fruit harvested using this system should be very small.

Cold storage may be an alternative to the increase of the milling capacity to preserve the soundness of the fruit prior to its processing. Storage of 'Picual' olives at 5 °C delayed the deterioration of free acidity, peroxide value, ultraviolet absorbance, and sensory quality of the resulting oil for up to 45 days of fruit storage, keeping their values within the limit admitted for "extra" quality.[11] García et al.[12] and Canet and García[13] demonstrated the viability of cold storage (5 °C) of olive fruits ('Blanqueta' and 'Villalonga') on an industrial scale. Recently, Vichi et al.[14] noted no development of negative sensorial attributes in oils extracted from 'Arbequina' olives until after 15 days of storage at temperatures of 5 ± 3 °C (diurnal) and 8 ± 3 °C (nocturnal). Despite the rather vague storage conditions used in this work, as it was not the aim of the investigation to maintain fruit quality, this is precedent that offers a good starting point to choose assay conditions to obtain optimal conservation of fruits avoiding the deterioration of the extracted oil, because 15 days could entail sufficient delay for

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fruit processing. The aim of this paper is to study the viability of the cold storage of the 'Arbequina' fruit cultivated in hedgerow and mechanically harvested to maintain the quality of virgin oil.

MATERIALS AND METHODS

Cultivation Conditions and Plant Material. The experiments were made in a commercial olive orchard near Seville, southwestern Spain (37° 30′ N, 5° 44′ W, ca. 60 m asl). The trees (*Olea europaea* L. cv. 'Arbequina'), cultivated in a north−south oriented hedgerow (1667 trees/ha), were 4 years old in 2009, when measurements were made. They were planted at 4 m × 1.5 m and had a single trunk with three or four main branches from 1.0 to 1.2 m above ground. The canopy was of ca. 1.4 m diameter and ca. 2.2 m height. Fruit productions in 2008 and 2009 were ca. 6600 and 7000 kg ha^{-1}, respectively. The soil is a stony sandy-loam with 15% clay, 13% silt, and 72% sand. The upper drained soil water content limit is 0.24 m^3 m^{-3}, and the lower soil water content limit is 0.08 m^3 m^{-3}. Common crop management practices in the area were followed. The soil was kept free of weeds by applying herbicides during the growing season (March−October). Climate in the area is Mediterranean, with a mild, wet season from October to April and a hot, dry season for the rest of the year. For the 2002−2009 period, the yearly averages of rainfall and potential evapotranspiration (ET_o) were 500.1 mm and 1580.9 mm, respectively. The orchard was irrigated from mid-May with an irrigation strategy consisting of two or three irrigation events per week aimed to supply two-thirds of ET_c estimated.

'Arbequina' olive fruits were hand harvested from 100 trees of four hedgerows (2 kg per tree) and randomly placed in eight perforated plastic boxes holding 20 kg of olives. Subsequently, the rest of the fruit of the same olives was mechanically harvested, using a VX680 wine grape harvester (New Holland España, Madrid, Spain). From the total amount of mechanically harvested fruit, 160 kg of olives was also randomly taken and distributed in a further eight similar perforated plastic boxes. All of the boxes were then transported to the Instituto de la Grasa in Seville. Samples of 100 healthy fruits were taken from each box to evaluate the initial level of fruit maturity from the ripening index (RI), commonly used in the virgin olive oil industry, based on the visual evaluation of skin and flesh color.[15]

Storage Treatments and Measurements of Fruit Characteristics. Each group of eight boxes was randomly distributed in two different storage rooms, respectively, under ambient conditions (18 ± 3 °C and 80% relative humidity (RH)) and under cold storage (3 ± 1 °C and 95% RH) for 20 days. Sampling dates were programmed at 0, 4, 7, 10, 14, and 21 days, but storage was interrupted when the oil extracted lost the "extra" commercial level of quality. To evaluate the changes in incidence of fruit decay during fruit storage, two samples of 100 olives were randomly taken from each box and placed in small plastic jars, which were stored in the same room as their respective original box. On each sampling date, the number of fruit with visible signs of decay was evaluated in each of these samples and expressed as a percentage, as the mean value of eight replicates. Two samples of 100 healthy olives were also randomly taken from each box, weighed with a 0.1 g precision, and also placed in small plastic jars, which were stored in the same room as their respective original box, for monitoring the changes on fruit weight during storage, using eight replicates for each different treatment. Other groups of one sample of 25 healthy fruits randomly taken from each 20 kg box were similarly placed in small plastic jars and stored to control the changes in skin color and fruit firmness, respectively, during storage. The color was determined on the equatorial zone of these 100 fruits (4 samples of 25 fruits), 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°. The color index (CI) was calculated according to the formula

$$CI = L^*(b^* - a^*)/100 \quad (1)$$

This equation has been previously used to monitor the changes in skin color during olive cold storage.[16] Fruit firmness was also evaluated on the equatorial zone of the same fruits, using a Zwick 3300 hand densimeter (Zwick GmbH & Co., Ulm, Germany). The consistency of the fruit was measured without rupture by the pressure of a 5 mm diameter disk. The results were expressed in N/cm^2. Each point of these two variables in each sampling date expressed the mean value of 100 determinations.

Virgin Oil Yield, Total Oil Content, and Physical Extractability. One kilogram of olives was randomly taken from each 20 kg box and separately milled. From the resulting paste in each sample, 800 g was taken and extracted separately, constituting four replicates of each treatment, using an "Abencor" extractor (Comercial Abengoa S.A., Seville, Spain). This unit simulates the industrial process of virgin olive oil production at laboratory scale.[17] After centrifuging, the oil was decanted into a graduated tube to measure the volume obtained to calculate the virgin oil yield, which was expressed as the percentage of fresh weight, considering 0.915 kg L^{-1} the olive oil density at ambient temperature. Subsequently, the extracted oil was filtered with filter paper (1320 qualitative filter paper, Filtros Anoia S.A., Barcelona, Spain) and stored at −20 °C under a N_2 atmosphere until analysis. From each replicate of each treatment, a 50 g sample of surplus paste was separately weighed in previously weighed capsules and dried at 105 °C to constant weight. The oil from the dried paste was solvent extracted with hexane, by using the Soxhlet method, to determine the total oil content of the paste as a percentage of the paste fresh weight. The extractability obtained by the different treatments tested was calculated on the basis of the mean value of the percentages of oil physically extracted from the total oil contents.

Oil Analysis. In each oil sample, replicate free acidity, peroxide index value, coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}), and the overall grading of the sensory quality of the oils were evaluated according to European Union Standard Methods (Annexes II, III and IX in European Community Regulation EEC/2568/91). Each oil sample was sensory graded by a panel of eight trained tasters (with at least 6 years of experience) according to a scale of nine points, 1 being the value for the poorest quality possible and 9 for the best, considering that the presence of any negative attribute (rancid, fusty, winey, musty, etc.) determines that the oil is evaluated below 6.5, the limit value established for the best commercial category ("extra"). Oxidative stability was measured by the Rancimat method, which evaluates the time (h) of resistance to oxidation of 3 g of oil sample exposed to a stream of dry air at a temperature of 100 °C.[18] The content of pigments in the oils was evaluated by their absorbance at 470 and 670 nm for carotenoids and chlorophylls, respectively, and the results were expressed as mg/kg of lutein and pheophytin *a*, respectively.[19] The tocopherol content of the oil samples was measured by HPLC using the IUPAC method.[20] The compositions on phenolic compounds and fatty acids were determined only in the oils extracted from the samples stored at 3 °C. The composition of fatty acids was determined by gas chromatographic analysis of methyl esters. This was performed on a Varian Aerograph equipped with a flame ionization detector (FID), fitted with a column (2 m, $^1/_8$ in. i.d.) packed with 12% EGS on a Chromosorb G, 80/100 mesh. The oven temperature was maintained at 185 °C, and the injector and detector were maintained at 225 °C. The flow rate of N_2 carrier gas was 30 mL/min. The phenolic fraction was isolated by solid-phase extraction and analyzed by reversed-phase HPLC using a diode array UV detector.[21] The results were expressed in milligrams of phenol compound per kilogram of oil.

Statistical Analysis. An analysis of variance (ANOVA) was carried out on all data. A 5% level of least significant difference (lsd), calculated by Duncan's multiple-range test, was used to establish differences between the mean values when ANOVA detected a significant ($p \leq 0.05$) effect due to each factor studied independently (harvesting system, storage temperature, and storage time).

RESULTS AND DISCUSSION

Fruit Characteristics. No significant differences were found between the mean values of RI shown by the olives manually or mechanically harvested (2.6 and 2.8, respectively). Mechanically harvested olives decayed more quickly than the hand

Table 1. Some Parameters of Quality Showed by 'Arbequina' Olive Fruits, Grown in Hedgerow Cultivation and Hand or Machine Harvested, during Storage at 18 or 3 °C[a]

storage (T, °C; days)	decay incidence[b] (%)	color index[c] ($L^*(b^* - a^*)/100$)	firmness[c] (N cm^{-2})	wt loss[b] (%)	virgin oil yield[d] (%)	extractability[d] (%)
			Hand Harvested			
18; 0	2.0 ± 0.2 γ	8.15 ± 0.32 α	40.2 ± 1.9 α	0.0 ± 0.0 γ	8.7 ± 0.4 bβ	64.2 ± 2.8 β
18; 4	10.2 ± 0.4 bAβ	7.04 ± 0.45 β	37.2 ± 1.5 $\alpha\beta$	0.7 ± 0.1 bAβ	9.9 ± 0.5 bAα	68.5 ± 3.2 $\alpha\beta$
18; 7	15.4 ± 0.8 Aα	6.92 ± 0.52 β	35.8 ± 1.3 Bβ	1.2 ± 0.3 Aα	10.5 ± 0.4Aα	70.2 ± 3.1 α
3; 0	2.0 ± 0.2 δ	8.15 ± 0.32 α	40.2 ± 1.8 α	0.0 ± 0.0	8.7 ± 0.4 b	64.2 ± 2.8
3; 4	2.2 ± 0.4 bBδ	7.96 ± 0.42 $\alpha\beta$	39.8 ± 1.7 α	0.1 ± 0.0 bB	8.9 ± 0.3 bB	65.3 ± 2.6 b
3; 7	2.4 ± 0.3 bB$\alpha\delta$	7.72 ± 0.38 $\alpha\beta$	39.7 ± 1.8 Aα	0.2 ± 0.1 bB	9.0 ± 0.4 bB	67.0 ± 2.9 b
3; 10	3.2 ± 0.4 bγ	7.21 ± 0.40 $\beta\gamma$	40.1 ± 2.3 aα	0.2 ± 0.1 b	9.1 ± 0.3 b	68.9 ± 2.8 b
3; 14	3.8 ± 0.4 b$\beta\gamma$	6.74 ± 0.46 γ	40.0 ± 2.3 aα	0.3 ± 0.1 b	9.2 ± 0.4 b	69.5 ± 3.2 b
3; 17	4.4 ± 0.5 β	5.43 ± 0.52 δ	36.8 ± 1.8$\alpha\beta$	0.3 ± 0.1	8.8 ± 0.3	68.8 ± 2.9
3; 21	6.0 ± 0.4 α	4.76 ± 0.51 δ	34.2 ± 1.6 β	0.4 ± 0.1	8.8 ± 0.3	68.6 ± 3.0
			Machine Harvested			
18; 0	2.3 ± 0.3β	8.07 ± 0.38 α	39.9 ± 2.1 α	0.0 ± 0.0 β	10.0 ± 0.5 aβ	68.4 ± 2.8
18; 4	28.6 ± 1.2 aAα	6.59 ± 0.43 β	35.2 ± 1.6 Bβ	2.4 ± 0.3 aAα	12.0 ± 0.7 aAα	72.1 ± 3.6
3; 0	2.3 ± 0.3ε	8.07 ± 0.38 α	39.9 ± 2.1α	0.0 ± 0.0 γ	10.0 ± 0.5 a	68.4 ± 2.8 γ
3; 4	6.4 ± 0.9 aBδ	7.44 ± 0.41 $\alpha\beta$	38.8 ± 1.5 Aα	1.5 ± 0.3 aBβ	10.2 ± 0.6 aB	70.6 ± 2.7 a$\beta\gamma$
3; 7	12.0 ± 1.0 aγ	7.19 ± 0.42 β	38.3 ± 1.7 α	2.5 ± 0.3 aα	10.4 ± 0.4 a	74.0 ± 2.8 a$\alpha\beta$
3; 10	20.8 ± 1.6 aβ	6.95 ± 0.44 β	36.9 ± 1.7 b$\alpha\beta$	2.7 ± 0.4 aα	10.5 ± 0.5 a	75.4 ± 3.0 a$\alpha\beta$
3; 14	31.2 ± 2.2 aα	6.79 ± 0.46 β	35.0 ± 1.4 bβ	3.0 ± 0.4 aα	10.7 ± 0.6 a	76.4 ± 3.2 aα

[a]A 5% level of least significant difference (lsd), calculated by Duncan's multiple-range test, was used to establish differences between the mean values when ANOVA detected a significant ($p \leq 0.05$) effect due to each factor studied independently. Thus, in each column, two values of the same temperature and time of storage, but differently harvested, followed by different lower case letters are significantly different; two values of the same harvesting system and time of storage, but different storage temperature, followed by different upper case letters are significantly different; and two values of the same harvesting system and storage temperature, but different storage time, followed by different Greek letters are significantly different. [b]Each value of this parameter is the mean ± SD of 8 replicates. [c]Each value of this parameter is the mean ± SD of 100 replicates. [d]Each value of this parameter is the mean ± SD of 4 replicates.

harvested fruits during storage, regardless of the temperature used (Table 1). Similarly, independent of the method of harvesting, fruits stored at 3 °C showed a significantly lower decay incidence than the olives stored at 18 °C. The level of decay incidence reached by the mechanically harvested olives after 4 days of storage at 18 °C (28.6%) was not surpassed by the same olives if they are stored at 3 °C even after 10 days (20.8%). It seems clear that this kind of mechanical harvesting favored the infection by microbial parasites of the stored olives, and this phenomenon is accelerated if a cold temperature is not used in the storage room. This fact confirms that this harvesting system induced mechanical damages in the fruits that allowed their rapid decay. Despite this, visually, it was not easy to note any indication of fruit damage. However, at the bottom of the boxes, where the mechanically harvested olives were kept, an appreciable amount of juice was always found, but this was never observed in the boxes of the hand-harvested fruit.

The method of harvesting exerted no effect on the skin color of the stored fruit, evaluated by its CI. No significant effect on this parameter was observed due to the temperature during the first storage week. However, during the cold storage time, this parameter was clearly decreasing, indicating the ripening progress of the stored fruit. This finding coincides with the decreasing of this parameter found by Castellano et al.[16] on 'Picual' olives stored at 5 °C.

Although during the first week of storage no significant differences in fruit firmness were found between the fruits mechanically harvested and the ones hand harvested, since 10 days of storage at 3 °C the olives mechanically harvested showed a significant reduction of this parameter. In contrast, hand-harvested olives stored at 3 °C presented a significant decrease of this parameter in relation to its initial value only after 21 days of storage. The storage temperature also affected the fruit firmness, but in a different way, depending on the harvesting system used. Whereas hand-harvested olives showed a significant decrease of this parameter after 7 days of storage at 3 °C, those picked mechanically had already presented the same effect after 4 days, indicating a faster fruit softening. This fact may be due to an internal partial breakage of the cellulose walls of the cells of the olive mesocarp, induced by mechanical harvesting. This fact would not be initially detected, but it would favor fruit softening during storage, especially at ambient temperature. In contrast, hand-harvested fruit softened more slowly, in line with the progress of its ripening and the temperature of storage. García et al.[11] monitored the effect of the storage temperature (5, 8, 12 °C) on the softening of 'Picual' olives, and they observed that the increase of storage temperature induced a faster fruit softening.

In coherence with the remaining juice found in the boxes used to store the mechanically harvested fruits, these systematically presented significantly higher weight losses than those harvested by hand. This fact may be also due to the possible internal breakage of the olive cells, cited above.

Mechanical harvesting favored a significantly better physical oil extraction of the fruits regardless of the temperature of storage used, even in the extraction carried out immediately after harvesting. This fact also supports the hypothesis that mechanical harvesting provokes internal breakages in the olive tissues, which would facilitate the subsequent physical extraction of the virgin olive oil. Olive storage at 18 °C induced the physical extraction of a significantly higher yield of virgin oil than storage at 3 °C, independent of the harvesting system used. This fact should be related to the higher weight losses experienced by the fruit stored at this temperature,

Table 2. Some Quality Parameters Noted in 'Arbequina' Virgin Oils Extracted from Fruits, Grown in Hedgerow Cultivation and Hand or Machine Harvested, during Storage at 18 or 3 °C[a]

storage (T, °C; days)	free acidity (% oleic acid)	peroxide value (mequiv O_2 kg^{-1})	K_{232}	K_{270}	panel test
		Hand Harvested			
18; 0	0.2 ± 0.1 γ	5.6 ± 1.6 b	1.54 ± 0.12 b	0.11 ± 0.01β	7.3 ± 0.2
18; 4	0.5 ± 0.1 bAβ	6.8 ± 1.8 b	1.62 ± 0.14 b	0.13 ± 0.02A$\alpha\beta$	7.4 ± 0.3 a
18; 7	0.9 ± 0.2 Aα	8.7 ± 1.5 A	1.78 ± 0.18 A	0.14 ± 0.02Aα	7.0 ± 0.4
3; 0	0.2 ± 0.1β	5.6 ± 1.6 b	1.54 ± 0.12 b	0.11 ± 0.01 β	7.3 ± 0.2
3; 4	0.2 ± 0.1 Bβ	5.8 ± 1.7 b	1.48 ± 0.14 b	0.10 ± 0.01 bBβ	7.4 ± 0.3
3; 7	0.2 ± 0.1 bBβ	5.8 ± 1.6 bB	1.46 ± 0.14 bB	0.11 ± 0.02 bBβ	7.3 ± 0.4 a
3; 10	0.3 ± 0.1 bβ	6.1 ± 1.8 b	1.42 ± 0.15 b	0.11 ± 0.01 bβ	7.4 ± 0.3 a
3; 14	0.3 ± 0.2 bβ	6.2 ± 2.0 b	1.47 ± 0.15 b	0.10 ± 0.01 bβ	7.4 ± 0.4 a
3; 17	0.5 ± 0.2 α	6.3 ± 1.9	1.60 ± 0.20	0.14 ± 0.01 α	7.2 ± 0.3
3; 21	0.6 ± 0.2 α	6.6 ± 2.1	1.73 ± 0.22	0.15 ± 0.02 α	7.0 ± 0.3
		Machine Harvested			
18; 0	0.3 ± 0.1β	12.2 ± 2.5 a	1.78 ± 0.15 a	0.13 ± 0.02	7.4 ± 0.3 α
18; 4	1.0 ± 0.3 aAα	14.1 ± 2.4 a	2.10 ± 0.22 aA	0.15 ± 0.03	6.6 ± 0.2 bBβ
3; 0	0.3 ± 0.1 δ	12.2 ± 2.5 a	1.78 ± 0.15 a	0.13 ± 0.02	7.4 ± 0.3 α
3; 4	0.4 ± 0.1 B$\gamma\delta$	12.8 ± 2.6 a	1.75 ± 0.17 aB	0.14 ± 0.02 a	7.2 ± 0.5A $\alpha\beta$
3; 7	0.6 ± 0.2 a$\beta\gamma$	14.9 ± 2.3 a	1.74 ± 0.18 a	0.15 ± 0.03 a	6.8 ± 0.3 b β
3; 10	0.8 ± 0.2 a$\alpha\beta$	15.1 ± 2.4 a	1.82 ± 0.22 a	0.15 ± 0.02 a	6.6 ± 0.3 b β
3; 14	1.1 ± 0.3 aα	15.7 ± 2.3 a	1.96 ± 0.21 a	0.16 ± 0.03 a	5.2 ± 0.4 b γ

[a]Each value is the mean ± SD of four replicates. A 5% level of least significant difference (lsd), calculated by Duncan's multiple-range test, was used to establish differences between the mean values when ANOVA detected a significant ($p \leq 0.05$) effect due to each factor studied independently. Thus, in each column, two values for the same temperature and time of storage, but differently harvested, followed by different lower case letters are significantly different; two values of the same harvesting system and time of storage, but different storage temperature, followed by different upper case letters are significantly different; and two values of the same harvesting system and storage temperature, but different storage time, followed by different Greek letters are significantly different.

basically due to the loss of water. As the total oil content evaluated by the chemical oil extraction of the fruit did not significantly change as a result of the different treatments (data not shown), the extractability of the samples showed a profile similar to the virgin oil yield.

Oil Quality. Regardless of the temperature used, after 4 days of storage, free acidity was significantly higher in oil from mechanically harvested in comparison with manually harvested fruit (Table 2). Also, after 4 days of storage, the temperature used played an important role in the changes of free acidity recorded in the oil samples, regardless of the harvesting system used. Systematically, at 18 °C the values of this parameter increased significantly more rapidly than in the oils from fruit stored at 3 °C. At 18 °C, virgin oil extracted from hand-harvested fruit surpassed the limit (0.8%) established for the best commercial category of quality (named "extra") after 7 days of storage, whereas the oils from mechanically harvested olives had already lost this quality level after 4 days. Similarly, at 3 °C, the oils from hand-harvested fruit maintained the "extra" quality after 21 days of storage, whereas the oils from mechanically harvested olives did so for 10 days only. As García et al.[11] found, the free acidity of the oils increased in terms of the storage temperature and decay incidence. The higher increase of free acidity of the virgin oils should be associated with the lipolytic activity of the parasitical micro-organisms that infect the stored fruit.[1] Dag et al.[10] observed that the oil extracted from olives harvested by vibration showed significantly higher free acidity than the oils from hand-harvested olives in samples processed immediately after harvesting. Apparently, this fact would not be explained by a fruit infection, because there was not enough time for this. However, the action of the vibrating combs would favor the harvesting of a higher proportion of mature and rotten fruits than the harvesting by hand, due to their lower resistance to abscission.

Systematically, the peroxide values of the oils obtained from mechanically harvested olives were significantly higher than the ones obtained from the fruits harvested by hand, regardless of the temperature of storage used (Table 2). Even this fact was observed in the samples immediately processed after harvesting, which supports the hypothesis of an initial internal breakdown of the olives as a consequence of mechanical harvesting. This breakdown would favor the contact of the olive oil with the atmosphere oxygen and, thus, the increase of its peroxide value. Dag et al.[10] previously found the same effect with 'Souri' olives harvested by mechanical vibration.

As occurred with peroxide values, mechanical fruit harvesting significantly increased the K_{232} values of the subsequently extracted oils. This parameter evaluates the presence of conjugated fatty acid in the oil, being the conjugation of the double bonds in the polyunsaturated fatty acid carbon chain a previous step of its peroxidation. This fact confirms that mechanical harvesting leads to an increase in the primary steps of the oxidation process, the level of which is evaluated with both parameters. Storage at 18 °C also significantly accelerated the increase of this parameter, especially when mechanical harvesting was used. However, the limiting value of 2.50 for "extra" quality category was not exceeded by the oils, irrespective of the fruits from which they were extracted. Previously, García et al.[12] had found that the K_{232} value of oils extracted from hand-harvested 'Blanqueta' and 'Villalonga' olives remained the same under refrigeration at 5 °C during 21 days of storage, whereas the oils obtained from olives stored at ambient temperature showed significantly higher values.

The K_{270} values evaluate the content of the oils on carbonylic compounds, which appear in the advanced stages of oil

Table 3. Photosynthetic Pigment Contents, Total Tocopherol Content, and Stability against Oxidation Exhibited by 'Arbequina' Virgin Oils Extracted from Fruits, Grown in Hedgerow Cultivation and Hand or Machine Harvested, during Storage at 18 or 3 °C[a]

storage (T, °C; days)	carotenoids (mg/kg)	chlorophyls (mg/kg)	tocopherols (mg/kg)	stability (h)
		Hand Harvested		
18; 0	2.9 ± 0.2	5.0 ± 0.6	423.2 ± 5.6 α	29.8 ± 2.4 aα
18; 4	3.0 ± 0.3	5.0 ± 0.7	368.4 ± 6.6 aBβ	25.2 ± 2.5 aβ
18; 7	3.0 ± 0.2	5.2 ± 0.7	346.2 ± 7.4 Bγ	20.3 ± 2.0 Bγ
3; 0	2.9 ± 0.2 β	5.0 ± 0.6 β	423.2 ± 5.6 α	29.8 ± 2.4 aα
3; 4	2.9 ± 0.4 β	5.4 ± 0.8 β	402.6 ± 8.1 aAβ	28.0 ± 2.4 a$\alpha\beta$
3; 7	3.4 ± 0.2 β	5.6 ± 0.7 β	387.3 ± 6.5 aAγ	26.3 ± 2.3 aA$\alpha\beta$
3; 10	3.2 ± 0.4 β	5.2 ± 0.7 β	384.6 ± 6.2 aγ	26.2 ± 2.7 a$\alpha\beta$
3; 14	2.8 ± 0.3 β	5.0 ± 0.8 β	385.1 ± 5.6 aγ	25.1 ± 2.0 β
3; 17	3.0 ± 0.2 β	6.3 ± 0.9 $\alpha\beta$	376.5 ± 5.6$\gamma\delta$	25.4 ± 2.4 β
3; 21	4.0 ± 0.2 α	7.1 ± 0.7 α	369.8 ± 5.6δ	24.7 ± 2.4 β
		Machine Harvested		
18; 0	3.1 ± 0.3	4.7 ± 0.5	421.3 ± 5.6 α	20.6 ± 2.4 b
18; 4	3.1 ± 0.2	5.3 ± 0.6	344.2 ± 7.6 bBβ	18.6 ± 3.2 b
3; 0	3.1 ± 0.3	4.7 ± 0.5	421.3 ± 5.6 α	20.6 ± 3.5 b
3; 4	3.2 ± 0.2	4.7 ± 0.6	388.1 ± 5.6 bAβ	20.4 ± 2.9 b
3; 7	3.4 ± 0.2	4.7 ± 0.8	362.5 ± 6.9 bγ	19.8 ± 3.1 b
3; 10	3.4 ± 0.2	4.8 ± 0.7	370.8 ± 5.8 bγ	18.4 ± 2.9 b
3; 14	3.3 ± 0.2	5.1 ± 0.7	370.7 ± 5.6 bγ	17.4 ± 3.2 b

[a]Each value is the mean ± SD of four replicates. A 5% level of least significant difference (lsd), calculated by Duncan's multiple-range test, was used to establish differences between the mean values when ANOVA detected a significant ($p \leq 0.05$) effect due to each factor studied independently. Thus, in each column, two values of the same temperature and time of storage, but differently harvested, followed by different lower case letters are significantly different; two values of the same harvesting system and time of storage, but different storage temperature, followed by different upper case letters are significantly different; and two values of the same harvesting system and storage temperature, but different storage time, followed by different Greek letters are significantly different.

oxidation. Mechanical harvesting induced a significant increase of this parameter in the oils during a period between 4 and 14 days of storage at 3 °C in comparison to the oils extracted from hand-harvested fruit. Initially, no significant differences were found in this parameter between the oils extracted from olives harvested by both systems tested. This means that immediately after harvesting the oil contained in the fruit has not yet undergone an advanced level of oxidation. Up to 17 days of fruit storage at 3 °C, the oils extracted from hand-harvested olives did not show significantly higher K_{270} values than the initial ones. In any case, the values of this parameter did not exceed the limit established for "extra" quality category (0.22), regardless of the harvest method or the temperature and time of storage tested. Previously, different authors, García et al.,[11] Kiritsakis et al.,[22] Clodoveo et al.,[23] and Kalua et al.,[24] found no significant increases of K_{232} and K_{270} in the olive oils subsequently extracted during cold storage at 5 °C for hand-harvested olives.

Sensory analysis of the overall grading of quality of the oils extracted from hand-harvested fruit found no negative sensory attributes in all of the samples tested, which maintained the initial level of quality. In contrast, those extracted from mechanically harvested fruits showed significantly lower values of this parameter after 4 days of storage at 18 °C or after 7 days of storage at 3 °C. After 14 days at 3 °C, the oils from these olives presented a slight level of negative musty attribute that determined the loss of the best commercial category of quality (≥6.5). This fact may be strongly related to the high values of decay incidence presented by these olives (Table 1).

The contents of photosynthetic pigments (carotenoid and chlorophyll) of the oils were not affected by the harvesting system and the storage conditions applied to the fruits from which they were extracted (Table 3). Only after 21 days of fruit storage at 3 °C did the oils extracted from manually harvested fruit show higher values of these parameters. This fact may be related to a possible decrease in the consistency of the chloroplast wall that would facilitate the release of these pigments in the olive oil. This coincided with a remarkable decrease in fruit firmness (Table 1) that would support this hypothesis.

Tocopherol content of the oil extracted during fruit storage was significantly affected by the system used for fruit harvesting, the storage temperature, and the time of storage (Table 3). Systematically, since the fourth day of storage, when the first sampling was carried out, the oils extracted from hand-harvested olives showed significantly higher contents of this natural antioxidant, regardless the temperature of storage applied. In parallel, since this date the tocopherol contents of the oils extracted from olives cold stored at 3 °C were higher than the ones from olives stored at 18 °C, independent of the system of how they were harvested. The time of storage was also a factor that determined changes in tocopherol content during storage. Therefore, this parameter decreased significantly during storage, independent of the storage temperature or the harvesting system used. It seems that mechanical harvesting and the use of 18 °C favored the subsequent degradation of these compounds during fruit storage. The use of cold storage at 3 °C would delay this process, but does not avoid it. Pereira et al.[25] described a marked decrease in tocopherol content of the oils extracted from three Portuguese olive varieties ('Cobrançosa', 'Madural', and 'Verdeal Transmontana') during fruit storage at 5 °C.

The stability of the oils, which evaluates the time (hours) of their resistance to oxidation, was always significantly higher in

Table 4. Phenolic Compound Composition (Milligrams per Kilogram) Exhibited by 'Arbequina' Virgin Oils Extracted from Fruits, Grown in Hedgerow Cultivation and Hand or Machine Harvested, during Storage at 3 °C[a]

	hand harvested, stored for				machine harvested, stored for		
phenolic compound	0 days	7 days	14 days	21 days	0 days	7 days	14 days
hydroxytyrosol	1.64	1.90	1.64	1.35	1.59	1.81	1.64
tyrosol	2.61 B	2.78 A	2.82 A	2.06 C	2.52 C	2.73 B	2.98 A
vanillic acid	0.56	0.54	0.46	0.40	0.64	0.58	0.49
vanillin	0.30 A	0.34 A	0.30 A	0.16 B	0.30	0.24	0.20
p-coumaric acid	0.30 B	0.36 B	0.44 B	0.69 A	0.39	0.33	0.46
hydroxytyrosol acetate	44.56 aA	44.56 aA	38.16 aB	38.81B	33.46 b A	20.16 bB	13.61 bC
3,4 DHPA-EDA[b]	21.86 aA	18.45 aA	10.86 aB	11.30B	14.31 bA	8.26 bB	4.24 bB
tyrosol acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p-HPEA-EDA[c]	9.04B	11.98 aA	7.16 aC	6.12C	9.99 A	5.13 bB	3.90 bB
pinoresinol	2.49	2.56	2.45 a	2.48	2.44 A	2.20 B	1.90 bC
cinamic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acetoxypinoresinol	25.17	26.46 a	26.00 a	23.96	24.90A	21.58 bB	18.76 bC
3,4-DHPA-EA[d]	7.51	10.19 a	8.48 a	8.85	7.90 A	4.95 bB	2.83 bB
p-HPEA-EA[e]	11.94	9.18	10.68	9.13	9.36	8.35	10.01
ferulic acid	26.93 A	13.36 bB	10.44 bB	10.55 B	27.51	25.40 a	33.90 a
luteoline	4.46	5.18	4.78	4.26	4.12	4.08	4.36
apigenine	1.18	1.45 a	1.32 a	1.35	1.14	0.90 b	1.08 b
total flavones	5.64	6.63 a	6.10 a	5.61	5.26	4.98 b	5.44 b
total *o*-diphenols	80.03 aA	80.28 aA	63.92 aB	53.27 C	67.25 bA	39.26 bB	26.68 bC
total secoiridoids	50.35 aA	49.80 aA	37.12 aB	35.40 B	41.56 bA	26.69 bB	20.98 bC
total phenols	160.55 aA	149.29 aB	125.99 aC	121.47 C	140.57 bA	106.70 bB	100.36 bC

[a]Each value is the mean of four replicates. A 5% level of least significant difference (lsd), calculated by Duncan's multiple range test, was used to establish differences between the mean values when ANOVA detected a significant ($p \leq 0.05$) effect due to each factor studied independently. Thus, in each row, two values of time of storage, but differently harvested, followed by different lower case letters are significantly different; and two values of the same harvesting system, but different storage time, followed by different upper case letters are significantly different. [b]Dialdehydic form of the decarboxymethyl oleuropein aglycone. [c]Dialdehydic form of the decarboxymethyl ligstroside aglycone. [d]Hydroxytyrosyl elenolate. [e]Tyrosyl elenolate.

oils extracted from hand-harvested olives, even immediately after harvesting. This parameter was also significantly affected by the temperature and the time of fruit storage. As in the case of the tocopherol content, cold storage at 3 °C delayed decrease of oxidative stability, but could not stop it. The decrease in oil stability during the time of fruit cold storage at 5 °C had been previously described by García et al.,[11,12] using 'Picual' and 'Blanqueta' and 'Villalonga' olives, respectively. This reduction could be associated with the progress of ripening experienced by fruit during storage.[16]

The different systems used for olive harvesting did not induce significant changes in the fatty acid composition of the oils subsequently extracted during fruit storage at 3 °C (data not shown). This composition did not significantly vary throughout storage time. Previously, Pereira et al.[25] had observed that fatty acid compositions of oils extracted from three Portuguese olives stored at 5 °C did not change for 14 days. As habitually found in olive oils, oleic acid was the main monounsaturated fatty acid (58%, on average) and palmitic acid was the main saturated fatty acid (18%, on average). The maximum limits legally established for linolenic, eicosenoic, and behenic acids (≤1.0, ≤ 0.4 and ≤0.2%, respectively) were not surpassed in any case.

The total phenol content, the content on the more relevant groups of phenolic molecules (flavones, *o*-diphenols, and secoiridoids), and the content of different phenolic molecules, individually considered in the oils extracted, were significantly affected by the harvesting system of the fruit (Table 4). From the moment of harvesting, the oil from mechanically harvested fruit contained significantly lower amounts of total phenols, secoiridoid derivatives, and *o*-diphenols. This result confirms the findings of Dag et al.,[10] who had observed that oil from hand-picked 'Souri' olives had higher total polyphenol content than the oil extracted from fruit harvested using vibrating combs. In our work, these initial differences were due to the higher contents of hydroxytyrosol acetate (*o*-diphenol compound) and of the dialdehydic form of the decarboxymethyl oleuropein aglycone (3,4-DHPA-EDA, *o*-diphenol and secoiridoid derivative compound) in the oils from manually harvested olives. Both compounds are the result of oleuropein breakage, which occurs during the process of the physical extraction of the virgin olive oils.[26] This process could be delayed as a consequence of the internal damage caused by mechanical harvesting. A further possibility could be that this damage favored the action of other enzymatic activities (polyphenoloxidases and/or peroxidases) that could destroy these intermediate metabolites.[27] This hypothesis would also explain the results found in oil stability, because these molecules have antioxidant activity.[28,29] During the storage period, the changes in concentration in different phenolic compounds varied according to the harvesting system used. Thus, the ferulic acid content decreased significantly after 7 days of storage at 3 °C in the oils from olives manually harvested, whereas the oils extracted from mechanically harvested fruits maintained their initial value during the 14 days of storage at 3 °C tested for this treatment. Acetoxypinoresinol and pinoresinol (lignan compounds) contents of oils from hand-harvested olives maintained their initial values without significant changes during the 21 days of storage at 3 °C tested for this treatment, whereas oils from mechanically harvested fruit showed a progressive

decrease in these contents after 7 and 14 days of cold storage. In contrast, Kalua et al.[24] have described in 'Frantoio' olives an increase in acetoxypinoresinol content induced by cold storage temperature at 4 °C. The hydroxytyrosyl elenolate (3,4-DHPA-EA) content of the oils from manually harvested olives showed no significant change during 21 days of cold storage, whereas the content of this secoiridoid derivative decreased in the oils from mechanically harvested fruits. The time of storage at 3 °C determined a significant decrease of the total phenol, *o*-diphenol, and secoiridoid derivatives contents of all the oils extracted, regardless of the harvesting system used. This fact supported the idea that the enzymatic activities responsible for the release of oil soluble phenolic compounds would decrease during cold storage and could be associated with the progress of fruit ripening, as evidenced by the changes in CI and firmness experienced by the fruits during this period (Table 1). Previously, Yousfi et al.[30] and Kalua et al.[24] had observed a decrease of these groups of phenolic compounds in oils extracted from 'Manzanilla', 'Picual', and 'Verdial' olives stored at 5 °C and 'Frantoio' olives stored at 4 °C, respectively.

In summary, mechanical harvesting by an adapted wine grape harvester led to internal damage in the fruits that determined a more rapid decay, softening, and considerable higher weight losses of the fruit during storage. As a consequence of this, the legally established parameters to evaluate the level of commercial quality of the virgin olive oils were clearly deteriorated in these oils. The chemical composition of the oils extracted was also modified during fruit storage as a consequence of the harvesting system. Thus, the oils from mechanically harvested olives presented lower contents of tocopherols and phenolic compounds. This caused the stability against oxidation of these oils to be significantly lower in these oils. Cold storage at 3 °C delayed postharvest decay of olives during storage in comparison with storage at 18 °C, which simulated ambient conditions. This fact determined a delay in the deterioration of the fruit characteristics and in the quality parameters of the oils extracted from them. Cold storage at 3 °C efficiently delayed more fruit and oil deteriorations in manually harvested olives. After 21 days of storage at 3 °C, these oils maintained their initial level of "extra" quality. In contrast, the oil extracted from mechanically harvested olives maintained this level of quality for a period of only 10 days of storage. Actually, a delay of this time could be enough so that the available processing machinery could extract the oil from the surplus fruit, avoiding its deterioration. The use of cold storage could be considered as a more versatile alternative to the increase of the processing capacity when faced with the challenge involved in olive hedgerow cultivation and its massive mechanical harvesting.

AUTHOR INFORMATION

Corresponding Author

*Phone: +34 954 611550. Fax: +34 954 616790. E-mail: jmgarcia@cica.es.

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