Cold storage of ‘Manzanilla de Sevilla’ and ‘Manzanilla Cacereña’ mill olives from super-high density orchards

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ABSTRACT

The suitability of the cold storage (2 °C) of fruit to maintain the quality of ‘Manzanilla de Sevilla’ and ‘Manzanilla Cacereña intended for virgin olive oil extraction was investigated. This temperature was effective in keeping the best commercial category of oil quality in both manually harvested olives and in mechanically harvested Manzanilla Cacereña fruits for 11 days. Mechanical harvesting induced significant decreases in oxidative stability, phenolic compounds content in the oils during cold storage and, only initially, in the total volatiles, regardless of the cultivar considered. However, the contents of volatile esters, associated to fruity flavor, were always higher in the oils from mechanically harvested fruits. Manzanilla de Sevilla oils exhibited higher total volatiles during fruit cold storage, regardless of the harvesting system used.

Keywords: Olea europaea L., grape straddle harvester, ethylene production, oxidative stability, phenolic compounds, volatiles

Chemical compounds studied in this article

Tyrosol (PubChem CID: 10393); Hydroxytyrosol (PubChem CID: 82755); (Z)-hex-3-enal (PubChem CID: 643139); (E)-hex-2-enal (PubChem CID: 5281168); hexanal (PubChem CID: 6184); hexan-1-ol (PubChem CID: 8103); (E)-hex-2-enol (PubChem CID: 54670067); hexyl acetate (PubChem CID:8908); (E)-pent-2-en-1-ol (PubChem CID: 15306); pentan-3-one (PubChem CID:7288)
1. Introduction

The cold storage of mill olives has been proposed as an alternative to prevent the deterioration of virgin olive oil (VOO) quality, when the available machinery is not able to process the harvested fruit before the extracted oil loses its level of initial quality. Thus, hand-harvested ‘Picual’ (García, Gutiérrez, Castellano, Perdigüero, Morilla & Albi, 1996), ‘Villalonga’ and ‘Blanqueta’ (Garcia, Gutiérrez, Barrera & Albi, 1996), ‘Coratina’ (Clodoveo, Delcuratolo, Gomes & Colelli, 2007) and ‘Picual’, ‘Manzanilla de Sevilla’ and ‘Verdial’ (Yousfi, Cayuela & García, 2008) olive varieties stored at 5ºC for 30 days offered oils with the same level of quality as those extracted from immediately harvested fruits.

Regarding minor compounds, the contents of natural antioxidants (phenolic compounds, tocopherols and carotenoids) decreased with storage time. This may occur at different speeds depending on the olive variety and the selected temperature (Yousfi et al., 2008; Inarejos-García, Gómez-Rico, Desamparados Salvador & Fregapane, 2010). An increase in the storage temperature of the fruit before processing causes a deterioration in both the oxidative stability and the sensory quality of extracted oils, since both factors depend on the presence of these compounds (Tena, Wang, Aparicio-Ruiz, García-González & 2015). Furthermore, fruit cold storage has offered different results with respect to the volatile development according to the variety or the degree of maturation. Hachicha-Hbaieb, García-Rodríguez, Sanz & (2015) found an increase in the content of C6 compounds in the oil extracted from ‘Arbequina’ hand-harvested green fruits, stored at 4 ºC for 4 weeks, whereas the oils from fruits stored at 20 ºC exhibited a significant decrease in these compounds. These results agreed with those found in oils from ‘Cornicabra’ fruits stored at 10 ºC (Inarejos-García et al., 2010).
Nevertheless, no significant variations were observed in terms of C6 compounds in the oils obtained from ‘Chetoui’ fruits, stored in similar conditions (Hachicha-Hbaieb, Kotti, Gargouri, Msallem, & Vichi, 2016).

Super-high density olive orchards, which are narrow hedgerows (≥ 1500 trees h⁻¹), emerged in the 90’s due to the need to reduce production costs, as a way to ensure early production and easy, fast and economical mechanization of all agronomical practices, in particular the harvest. This is done by means of grape straddle harvesters with a high efficiency (Connor, Gómez del Campo, Rousseaux & Searles, 2014). However, mechanical harvest negatively affects quality of the oil from ‘Arbequina’ olives, which suffer a rapid decay. The phenolic and tocopherol contents in the extracted oils decrease, and, consequently, the oxidative stability as well (Yousfi, Weiland & García, 2012; 2013). Therefore, the production of extra VOO in super high density orchards seems to be possible as long as the harvest is well synchronized with the extraction process. However, given the constant increase in olive cultivation areas grown under this system, this is expected to not always be possible. In this sense, a fruit storage temperature of 2 ºC preserved the best level of VOO quality for 12 days (Yousfi et al., 2013).

The adaptation to super-high density cultivation of other traditional olive cultivars such as ‘Manzanilla de Sevilla’ and ‘Manzanilla Cacereña has been recently studied (Morales-Sillero, Rallo, Jiménez, Casanova & Suárez, 2014; Morales-Sillero & Garcia, 2015). Both cultivars are mainly cultivated for table olive production, but the quality of the oils is highly appreciated in the market, in particular for their sensory attributes (fruity, bitter and pungent) and their high stability against oxidation. In fact, ‘Manzanilla de Sevilla’ oil is often mixed with that of ‘Arbequina’ to produce a ‘coupage’ which is well-balanced in flavor and stability. Morales-Sillero and Garcia
(2015) have found that oils from ‘Manzanilla de Sevilla’ and ‘Manzanilla Cacereña’ fruits processed immediately after mechanically harvesting exhibited the best level of commercial quality. However, as for ‘Arbequina’, in comparison to hand-picked fruits, mechanical harvesting caused a decrease in the overall grading of its sensory quality, in the phenolic composition and in the oxidative stability of the oils, but it does not determine the loss of the ‘Extra’ quality. It is not yet known how long these fruits can be cold stored without suffering a significant deterioration of its initial commercial quality.

The aim of this study is to evaluate the suitability of cold storage (2ºC) of ‘Manzanilla de Sevilla’ and ‘Manzanilla Cacereña’ fruits from super-high density orchards, manually and mechanically harvested to maintain the quality of the oils obtained. Therefore, not only the physiological response of the olive fruits was studied but also the most important oil quality parameters, as well as those compounds specifically related to the sensory quality of VOO such as phenolic and volatile compounds. This comparison makes sense to evaluate if the mechanical harvesting determined deterioration in the physiology of the fruit of both varieties, which could result in a decrease in their post-harvest viability and accelerate the deterioration of its oil. The results of this study will form the basis for the selection of new varieties for super-intensive cultivation and will provide relevant data on the appropriateness of reserving part of the production to obtain superior quality of extra VOOs through fruit cold storage and even hand-harvesting.

2. Materials and methods

2.1 Plant material, mechanical harvest and storage conditions
Fruits of ‘Manzanilla de Sevilla’ (MS) and ‘Manzanilla Cacereña’ (MC) from olive hedgerows (*Olea europaea* L.) were harvested in 2012 in a commercial orchard near Elvas (Portugal) (38\(^{\circ}\)56’N,7\(^{\circ}\)02’O, 201 m above sea level). Hedgerows were planted in 2007 with a frame of 3.75 m x 1.35 m (1,975 trees ha\(^{-1}\)) in a north-south orientation. Fruit production was ca. 10,000 kg ha\(^{-1}\) for ‘Manzanilla de Sevilla’ and ca. 18,000 kg ha\(^{-1}\) for ‘Manzanilla Cacereña’. Climate characteristics and orchard management are described in Morales-Sillero and García (2015).

Fruits with a ripening index between 3 and 4 (García et al., 1996a) were harvested on December 10\(^{th}\) in four random rows of trees (ca. 90) per cultivar (‘Manzanilla de Sevilla’ and ‘Manzanilla Cacereña’). Prior to the mechanical harvest, fruit samples (5 kg per row) were hand-picked around the canopy of 25 randomly selected trees. Subsequently, the rest of the fruits of the same replicate were mechanically harvested with a grape straddle harvester (New Holland VX 7090 Olive, CNH Global, Belgium) and samples of 5 kg per experimental unit (each one of the four rows) were also randomly taken from the total amount.

All samples were transported in ≤ 3 h to the Institute de la Grasa in Seville (Spain). Upon arrival they were placed in perforated plastic boxes and randomly distributed in a room under cold storage (2.0±0.5 °C and 95% relative humidity) for 11 days. Sampling dates for oil extraction were made at 1, 6 and 11 days.

2.2 Fruit decay and physiology

From the 5 kg samples of each experimental unit, 100 fruits were randomly taken and packed in plastic boxes which were placed in the same cool-room. The incidence of
decay was tested in these fruits at periods of 1, 3, 7, 10 and 11 days of cold storage, considering the fruit that showed any sign of infection as decayed, with the values expressed in percentages. At the same time, and in the same cool-room, 150 g of fruits were randomly taken and packed in plastic boxes previously tarred to assess changes in weight. Fruit weight (0.01 g precision) was measured on the same sampling dates and the results were expressed as percentage of weight loss, referring to the initial weight.

Respiration rate (mL CO$_2$kg$^{-1}$ h$^{-1}$) and ethylene production (µL Ethylene kg$^{-1}$ h$^{-1}$) were determined in replicate in fruit samples of 500 g which had been previously placed into 2.0 L glass jars and stored at 20 ºC for 4 hours. A G100 portable gas analyzer (Geotechnical Instrument Ltd. Leamington Spa, UK) and an ICA portable ethylene analyzer (International Controlled Atmosphere Ltd., Paddock Wood, UK) were used to measure the CO$_2$ and ethylene contents of the head space, respectively. These parameters were tested at 1, 3, 7 and 11 days of storage.

2.3 Oil extraction, oil content and oil analysis

Olive oil was physically extracted in an Abencor analyzer (Comercial Abengoa S.A., Seville, Spain). 1 kg of olive fruits was extracted per experimental unit. The Abencor analyzer was used to determine the production of VOO and the physical extractability. Oil content was also determined by the Soxhlet method on a fresh weight basis. Analyses related to free acidity, peroxide index value, and coefficients of specific extinction $K_{232}$ and $K_{270}$, were carried out in the oil samples of each extraction date, while the sensory quality was only measured in the samples corresponding to the beginning and the end of the storage period. All of them were measured following the European Union Standard Methods (Annexes II and IX 153 in European Community Regulation
The sensory intensities of the positive (fruity, bitter and pungent) and negative (rancid, fusty, winey, musty, etc.) attributes, were graded by a panel of eight trained tasters using a non-structured 10 cm line scale, and the results calculated into a continuous numerical score by measuring the placed mark along the line. The results are presented by the medians among taster sensory scores. The overall grading of the sensory quality of each oil sample was graded by the same panel according to a structured scale of nine points, “1” being the minimal value of quality and “9” for the best possible, being “6.5” the lowest limit value for the best level of sensory quality (named “Extra”) for VOOs. The results are presented by the means among sensory scores.

Oxidative stability was calculated following the Rancimat method, which measures the resistance to oxidation (h) of 3 g oil samples exposed to streams of dry air (20 Lh⁻¹) heated to 100 °C (Laübli & Bruttel, 1986).

The presence of chlorophyll and carotenoid in the oils were estimated by their absorbances at 470 and 670 nm, respectively, and the results expressed as mg kg⁻¹ (Mínguez-Mosquera, Rejano-Navarro, Gándul-Rojas, Sánchez-Gómez and Garrido-Fernández, 1991).

Tocopherol composition was evaluated by HPLC using the IUPAC method (1992). The phenolic fraction was analyzed by HPLC according to Mateos, Espartero, Trujillo, Ríos, León-Camacho et al. (2001). The quantification of the main phenolic compounds was carried out at 280 nm using p-hydroxyphenylacetic acid as internal standard, while flavones (luteoline and apigenine) and ferulic acid were quantified at 335 nm using o-coumaric acid as internal standard. The results were expressed in mg kg⁻¹.

Fatty acid composition was determined by gas chromatographic analysis of methyl esters for each oil sample (Cert, Moreda & Pérez-Camino, 2000). This was performed
on an Agilent 6890 with a flame ionization detector, fitted with a capillary column (SP-
2380, 60 m / 0.25 mm I.D.) coated with cyano-propyl-silicone (0.20 mm film
thickness). Hydrogen was employed as carrier gas at flow of 1mL min\(^{-1}\). The oven
temperature was maintained at 185 °C and the injector (split 1:20) and detector at 225
°C.

Extraction and analysis of VOO volatile compounds were performed according to
Pérez, de la Rosa, Pascual, Sánchez-Ortiz, Romero-Segura, León and Sanz (2016). The
quantification of volatile components and the clustering of compounds into different
subgroups related to their biochemical origin were carried out according to Sánchez-
Ortiz, Pérez and Sanz (2013).

2.4 Statistical analysis

The data were subjected to statistical analysis using variance analysis to determine
the effect of three factors (cold storage time, harvesting method and cultivar) and the
interactions among them. Tukey’s test (P < 0.05) was used to discriminate among the
mean values. All analyses were made using Stat Graphics Plus 5.1 (Stat graphics
Software, Inc., La Jolla, California, USA).

3. Results and discussion

3.1 Changes in fruit characteristics

Mechanically harvested MS olives were especially sensitive to decay, exhibiting a
faster deterioration than the other treatment (Fig. 1A). Mechanically harvested MC
olives showed a slight, but statistically significant higher decay incidence than the hand-harvested olives. Cold storage at 2 °C for 11 days resulted in an effective system for controlling the increase in the decay incidence in the MC olives, regardless of the harvesting method used. The positive effect of the refrigeration of hand-picked MS fruits was previously reported by Yousfi et al. (2008), who did not find fruit decay when they stored green fruits at 5 °C for 8 weeks. The higher incidence of fruit decay in mechanically harvested MS fruits could be due to the damage caused by the harvester. Morales-Sillero et al. (2014) found a high proportion of fruits with cuts (18%) in green MS fruits harvested with the grape straddle harvester in the same orchard of this study, while the proportion in MC was only 2%. Therefore, the damage caused by the harvester could favor microbial infection in MS fruits so that storage at 2 °C was not enough to stop its progress. The decay incidence in ‘Arbequina’ fruits from super-high density conditions, harvested with a grape straddle harvester, also increased significantly up to 20% when they were stored at 2 and 3 °C for 10 and 12 days, respectively. Moreover, the decay incidence increased substantially with the increase in temperature (Yousfi et al., 2012; 2013).

The percentage of fruit weight loss increased progressively throughout storage for all fruits regardless of cultivar or harvest treatment (Fig.1B). Significant differences were found between hand-picked and mechanically harvested fruits from day 3 in MC and day 7 in MS. Differences were more appreciable in the MS cultivar, for which mechanically harvested fruits had the highest percentage of weight loss after 11 days, which could also be related to fruit damage caused by the harvester.

The respiration rate was always significantly higher in MC fruits regardless of harvest treatment (Fig.1C). Moreover, mechanically harvested fruits showed higher values throughout the storage period in both cultivars. MS hand-picked fruits
maintained the same minimum respiration rate throughout the storage period, while MS mechanically harvested and MC hand-picked fruits maintained the initial value only for 7 days, point from which a significant decrease was found. Mechanically harvested MC fruits showed a continuous decrease since the beginning of the cold storage. The results partially agree with those of Yousfi et al. (2013) for mechanically harvested ‘Arbequina’ fruits in a super-high density orchard; they found that a temperature of 2 °C preserved the same respiration rate for 15 days. Rinaldi, Amodio, Colelli & Clodoveo, (2010), previously, observed a reduction of the respiration of ‘Coratina’, ‘Leccino’ and ‘Ogliarola Leccese’ hand-picked olives stored at 5°C in relation to the ones kept at ambient conditions (15-20 ºC) and, consequently, a slowing down of its metabolic activity, which is determinant to improve the postharvest viability of the olives.

As for respiration, mechanically harvested fruits showed a significantly higher ethylene production than hand-picked ones (Fig.1D), probably as a consequence of the damage caused by the harvester, as reported by Morales-Sillero and García (2015). In addition, for each type of harvest, ethylene production was always higher in the MC fruits than in the MS ones. MC olives of each treatment showed the same value of ethylene production for 7 days of storage. Subsequently, they exhibited a similar increase on the last storage day. In contrast, the MS olives kept their respective initial values throughout the storage period. This behavior was observed by Yousfi et al. (2013) in mechanically harvested ‘Arbequina’ fruits.

3.2 Oil content, VOO yield and extractability

The total oil content exhibited by both olive varieties reached relatively low values (< 15%), even taking into account that both of them are considered table olives (Fig.
MC olives presented slightly higher oil contents than MS fruits. These differences became more apparent after 6 days of storage. With the exception of the first olive MS sampling, the mechanically harvested olives systematically showed higher values for this parameter than the ones harvested by hand. Theoretically this parameter should not vary or, in any case, should increase in line with the weight loss experienced by the fruit throughout its cold storage. This fact may explain the higher values presented by the mechanically harvested fruit, which also presented higher values of weight loss.

MS olives exhibited a higher VOO yield than MC fruits, regardless of the harvesting system used (Fig. 2B). However, from an absolute point of view, even these first olives achieved a low value (< 10%). Yousfi et al. (2012) obtained slightly higher values for this parameter using ‘Arbequina’ olives also from hedgerow, hand or mechanically harvested and stored at 3 ºC. Perhaps, the high irrigation of the super-intensive cultivation causes an increase in the production of fruit, but, also, a reduction in the total content of oil or in its physical extractability. The VOO yield repeated the same profile of behavior presented during cold storage for the total oil content, since the olives harvested mechanically systematically presented higher values than the ones collected by hand, with the same exception for the initial samples of MS.

The physical extractability of an olive is measured in percentage by the ratio between the amount VOO physically extracted and the total oil content of the same fruit. Consequently, as MS olives presented, at the same time, greater production of virgin olive oil and lower total oil content. They obtained values for this parameter which were considerably higher than the MC fruits (Fig. 2C). Systematically, the olives harvested mechanically showed a better physical extractability during the period of cold storage, though only in the last sampling made with MC olives were the differences found statistically significant. This fact cannot be explained as solely due to chance, any non-
parametric statistic test would confirm, by analyzing the entire process altogether, that the harvest system exerted a highly significant effect on this parameter. Most likely, mechanical harvesting caused damage to the internal cellular structure of the fruit, facilitating the release of VOO and the increase in its physical extractability. Our results confirm those obtained by Yousfi et al. (2012), who previously also found higher values of physical extractability in mechanically harvested ‘Arbequina’ olives than in the hand-picked ones, in a systematic way, during 14 days of storage at different temperatures.

3.3. VOO quality indices

Cold storage prevented the increase in free acidity in oils from hand-picked fruits in both cultivars (Fig. 2D). Surprisingly the free acidity in oils from mechanically harvested MS fruits increased steadily throughout the storage causing the loss of the extra virgin category at day 6 (> 0.8%). The high acidity of these oils was probably due to the high incidence of fruit decay observed (Fig. 1A), similar to those previously reported for other cultivars (García et al., 1996b; Yousfi et al., 2012; 2013). In MC oils from mechanically harvested fruits, free acidity only increased slightly at the end of the storage period without affecting the “Extra Virgin” category. This increase in free acidity with cold storage has been observed in other olive cultivars. Yousfi et al. (2013) found that Arbequina oils from mechanically harvested fruits also reached the limit value of 0.8 when they were stored at 2 ºC for 15 days. Previously, Dag, Boim, Sobotin & Zipori (2012) studied the cold storage of ‘Barnea’ and ‘Picual’ olives collected with a commercial linear-vibrating trunk shaker and ‘Koroneiki’ olives collected with an overhead mechanical harvester. They observed that cultivars responded differently to
the storage at 4 °C for periods of up to 8, 15 and 22 days, respectively. However, free
acidity values were always ≤ 0.8 % oleic acid.

The oils from mechanically harvested olives exhibited only slightly higher average
peroxide values than the ones from hand-harvested fruits (5.6 and 4.8 mEqO₂ kg⁻¹,
respectively, for those coming from MS and MC olives in front of 4.8 and 4.1 mEq O₂
kg⁻¹), but during all the period of cold storage the differences between the treatments on
this parameter were not significant (data not shown). These results agree with those
obtained by Dag, Boim, Sobotin & Zipori (2012), who did not observe significant
changes in this parameter during a period of preservation at 4 °C for 22 days. On the
other hand, the absorbance at 232 and 270 nm, which evaluates, respectively, the
presence of conjugated fatty acids, in a previous step to the hydro peroxide formation,
and the late oxidation by the formation of carbonyl compounds, was not affected by any
of the three factors studied: variety, type of harvesting and storage time (data not
shown). These results also coincide with those presented by Yousfi et al. (2012) with
oils from ‘Arbequina’ olives whether hand or mechanically harvested and stored at 3 °C.
The increase in the peroxide value shown by the oil extracted from mechanically
harvested olives may be related with the probable internal damage that these fruits
suffered due to the action of the grape straddle harvester. The rupture of the internal cell
structures would facilitate the contact of the accumulated oil with the atmospheric
oxygen, facilitating its initial oxidation. However, neither the change in position of the
double bond in the carbonated acyl chain nor the formation of carbonyl compounds has
any reason to be accelerated or delayed due to this cause. The formation of carbonyl
compounds would suppose an advanced step of fatty acid oxidation. Eleven days at 2 °
C is a very short time for the simple contact of oil with atmospheric oxygen, which
determines the formation of these oxidized compounds related to the development of
tocidity. For this reason, the ultraviolet absorbance was not altered in this period.
Negative sensory attributes were not identified in any of the oils tested and all of
them presented an appreciable intensity of the ‘fruity’ attribute. In consequence,
according exclusively to the sensory analysis, all of them maintained the best
commercial category for VOO (Extra) (Table 1). Nevertheless, the three factors studied
(cold storage, harvesting system and variety) seem to exert effects on the sensory
variables evaluated. Thus, systematically, the intensity of the positive sensory attributes
(fruity, bitter and pungent), and the overall grading of the sensory quality decreased in
the oils extracted at the end of the cold storage, regardless of the system of harvesting or
the variety considered. The decline observed in these sensory variables was small, but
its systematic character rules out the possibility that they could be exclusively random.
The oils extracted from hand-picked fruits presented better evaluations of these sensory
variables than their counterparts which were extracted from mechanically harvested
fruits. Yousfi et al. (2012) did not find a decrease in the grading of the sensory quality
evaluated in the VOO extracted from ‘Arbequina’ olives stored during 14 days at 3 ºC,
but observed a significant reduction in this parameter in those obtained from
mechanically harvested fruits. The decrease in this parameter observed in our
experiment for all the treatment coincided with these results. Finally, the oils extracted
from hand-picked MS olives show higher values of these positive sensory attributes
than those obtained from hand-picked MC fruits on the first sampling date.
Subsequently, at the end of the cold storage, the evaluations of the oils from both
cultivars decreased, but MS oils continued to be systematically better evaluated.
Initially the MS oils obtained from mechanically harvested fruits were also better
evaluated in terms of fruity and bitterness than the MC oils from mechanical harvesting,
but at the end of the fruit storage both oils exhibited very similar intensities for these two attributes. Yousfi et al. (2013) observed a similar reduction in these sensory attributes in oils extracted from mechanically harvested ‘Arbequina’ after a storage period of 12 days at 2 °C. The results obtained seem to suggest that the cold storage helped to maintain the initial level of commercial quality of the olive oil, but did not totally prevent a certain loss in its original quality, given that cold temperature delays, but does not prevent the progress of fruit ripening.

3.4. Oxidative stability, photosynthetic pigments and natural antioxidants

In general, the oxidative stability of the oils decreased during cold storage in all the treatments tested (Fig.3A). MS oils systematically exhibited higher values of this parameter than the MC oils from each complementary treatment. The oils from mechanically harvested fruits showed the most appreciable decrease in oxidative stability in the second sampling date, after 6 days of cold storage, while those from hand-picked olives presented a similar effect 5 days later on the last sampling date, regardless of the variety tested. Yousfi et al. (2008) found a non-significant reduction in oxidative stability (< 10%) in oils from MS hand-picked fruits stored at 5 °C for four weeks, which was probably due to the olive ripening progress. Our results coincided with those of Yousfi et al. (2012), who found that oils from mechanically harvested ‘Arbequina’ fruits always showed the lowest values for resistance to oxidation. Similarly, a significant reduction in this parameter was subsequently found by Yousfi et al. (2013) in oils from ‘Arbequina’ fruits which were mechanically harvested and stored for 15 days at 2 °C.
The α-tocopherol content declined progressively during cold storage in all the treatments (Fig. 3B). The difference between the values found in the oils of both MS treatments after the first day of cold storage was remarkable. These values were then equalized from the second test sample (6 days) until the end of the storage period (11 days). Morales and Garcia (2015) observed similar differences in oils extracted from immediately harvested olives. The grape straddle harvester seems to exert a negative effect on the content of this natural antioxidant in this variety. This fact is also likely to be related with the internal damage caused in these fruits during mechanical harvesting as well, which would favor the oxidizing action of the atmospheric oxygen, resulting in a more rapid decrease in tocopherols.

The oil of both cultivars showed a different profile of changes in carotenoid contents during fruit cold storage. While in MS oils this parameter was not affected by the harvesting system, MC oils from mechanically harvested fruit systematically exhibited higher carotenoid contents than those obtained from hand-picked olives (Fig. 3C). In contrast, the chlorophyll content changed erratically, without following a logical trend, regardless of the variety or the harvesting system (Fig. 3D). The results shown by MS oils coincided with those obtained by Yousfi et al. (2012) who did not observe appreciable changes in the carotenoid contents of oils extracted from ‘Arbequina’ fruits stored at 3 ºC for up to 14 days, regardless of the harvesting system used. The contents of these pigments in VOO depend on their presence in the chloroplasts of the fruit cells, the ease with which these are broken and released during olive milling, and the action of enzyme activities that can destroy them during the oil extraction process. This multiplicity of determinant factors explains the scarce consistency of the results obtained for these parameters.
Olive cold storage was not able to maintain the initial values of the total phenolic compounds content, especially in the mechanically harvested fruits. The profiles exhibited by both the total phenolic (Fig. 3E) and o-diphenol (Fig. 3F) contents were very similar to those exhibited by the oxidative stability in both varieties (Fig. 3A).

Again, the MS oils systematically exhibited higher values of these parameters than the MC ones. Hand-picked fruit oils also systematically presented the highest contents of these natural antioxidants in both cultivars; and the oils from mechanically harvested olives, from both varieties, exhibited the most appreciable decrease in these natural antioxidants after 6 days of cold storage, while those from hand-harvested MC fruits presented a similar decrease in content on the last sampling date. The profiles only differed in the oils from hand-picked MS fruits, which showed content reductions up to 6 storage days and subsequently maintained these values, while oxidative stability showed a progressive decrease in its value. These coincidences supported that these molecules really act as natural antioxidants, delaying the oil rancidity. The other possible antioxidant (tocopherols and carotenoids) exhibited profiles which coincided to a lesser degree with that of the oxidative stability, which suggests their poor connection with this parameter. Furthermore, as a general rule, as it has been noted by the authors who have studied the refrigerated preservation of olives harvested mechanically, as the storage time progressed, decreased the total content of phenolic compounds (Yousfi, Weiland and García 2012, 2013). However, Dag, Boim, Sobotin & Zipori (2012) found different responses among cultivars fruits. Thus, the total content of phenolic compounds descended abruptly in ‘Koroneiki’ oils and moderately in ‘Barnea’ oils, while in ‘Picual’ oils remained constant during the 22 days of storage.

3.5. Phenolic composition
During cold storage the losses in oils from hand-picked fruits were mainly due to those of the dialdehydic form of the decarboxymethyl oleuropein aglycone (3,4-DHPEA-EDA) and of the dialdehydic form of the decarboxymethyl ligstroside aglycone (p-HPEA-EDA) which declined by almost 50% (Fig. 4A and B). The losses in oils from mechanically harvested fruits were related with changes in most phenolic compounds, but primarily p-HPEA-EDA and hydroxytyrosyl elenolate (3,4-DHPEA-EA). In fact, the contents of those phenolic compounds declined by 87%, up to 6.3 mg kg\(^{-1}\) in the case of p-HPEA-EDA, and by 61% 3,4-DHPEA-EA. Concerning MC, cold storage contributed to preserving the initial contents of total phenolic compounds, total \(o\)-diphenols and total secoiridoid in oils from hand-picked fruits up to day 6, from which point they declined significantly by 47, 62 and 43%, respectively. The differences were related to changes in most phenolic compounds, but mainly 3,4-DHPEA-EDA, p-HPEA-EDA and 3,4-DHPEA-EA (Fig. 4C), in which the contents dropped by 80, 73 and 54%, respectively. On the contrary, progressive and significant decreases in total phenolic compounds and total secoiridoids were found over the storage period in oils from mechanically harvested fruits of up to 44 and 46%, respectively, while the total \(\sigma\)-diphenol content decreased by 53% in the first seven days of storage, although it remained constant later. The results therefore demonstrate that storage at 2 °C of MS and MC fruits causes a strong and negative effect on the phenolic compound contents of the oils, particularly in those from mechanically harvested fruits. Losses in these oils were even higher than those reported by Yousfi et al. (2013) for Arbequina oils extracted from fruits stored at the same temperature and for the same time. A large number of studies demonstrate that phenolic compounds, even though they are components of the VOO minor fraction, have health and sensory properties.
Total phenolic compounds are correlated to VOO shelf life due to their antioxidant activity and the strongest antioxidant effects have been associated in particular to hydroxytyrosol and its derivatives (3,4-DHPEA-EDA and 3,4-DHPEA-EA), which were strongly affected in our study. Moreover, certain phenolic compounds play a key role in anti-inflammatory processes and prevent and/or reduce chronic-generative diseases as well as several types of cancer. This is the case of 3,4-DHPEA-EDA and also of the p-HPEA-EDA, also named oleocanthal, which has recently received great interest as a potential therapeutic agent (Servili, 2014; Parkinson & Keast, 2014). VOO sensory attributes are also associated with certain compounds. In fact, p-HPEA-EDA is related to the pungent attribute, whereas 3,4-DHPEA-EA and tyrosyl elenolate (p-HPEA-EA), and to a lesser extent 3,4-DHPEA-EDA, are associated with the bitter attribute (Servili, Esposto, Fabiani, Urbani, Taticchi, Mariucci, Selvaggini & Montedoro, 2009). Changes in these compounds therefore explain the lower perception of pungent and bitter attributes by the panel test at the end of the storage period in oils from mechanically harvested fruits, in particular those of MS. A decrease in the phenolic composition in oils from hand-picked fruits of this cultivar when stored at 5 °C for five weeks was previously reported by Yousfi et al. (2008), who suggested this post-harvest technology as an alternative to modulate the typical bitterness attribute of MS oils in order to encourage consumer acceptance.

### 3.6. Fatty acid composition

Cold storage and harvest system determined non-significant effects on the fatty acid composition of the oils from both cultivars, which, on the other hand, differed among themselves (data not shown). MC oils showed higher contents of oleic and linolenic acids (79.0 and 0.4 %, respectively), lower contents of palmitic, stearic and linoleic
acids (12.6, 1.5 and 4.4%, respectively) than those extracted from MS fruits (74.9, 0.2, 14.1, 2.9 and 5.4%, respectively), whereas both of them did not differ in the contents of palmitoleic, arachidic, gondoic and behenic acids (1.3, 0.4, 0.7 and 0.1%, respectively). Previously, Pereira, Casal, Bento and Oliveira (2002) observed no changes in the fatty acid composition of oils extracted from three Portuguese cultivars stored for 14 days at 5 ºC. Similarly, Yousfi et al. (2012) found no differences between the fatty acid composition of oils extracted from manually or mechanically harvested ‘Arbequina’ olives stored at 3 ºC for 14 days. This fact was expected because these compositions are consequences of the action of multiple types of enzymes (transacylases, dehydrases, reductases, synthetases, elongases and desaturases) which are responsible for the fatty acid biosynthesis. These enzymes act from the pit hardening, to the end of fruit maturation (Garcia & Mancha, 1992). Then, the presence of the different fatty acids in the oil does not depend on the punctual moment of the VOO extraction, but of the full development of the oil biosynthesis process. In consequence, the genetic factor is the most relevant for these variables.

3.7. Volatile compounds

The oils obtained from MS had significantly higher levels of volatile compounds than those extracted from MC during fruit cold storage, regardless of the harvesting system used (Fig. 5). It is well known that the lipoxygenase (LOX) pathway is the main biochemical pathway involved in the biosynthesis of VOO aroma. It is induced by plant-tissue damage, so that most of the volatile compounds found in the VOO are formed during the milling of the olives (Olías, Pérez, Rios & Sanz, 1993). In this sense, the fruit damage caused by mechanical harvesting probably induces the LOX pathway
triggering the formation and emission of volatile compounds during the storage of the fruits. This premature activation of the LOX pathway alters the biochemical status of the fruit and limits the amount of volatiles produced at the milling step during the oil extraction process which determines the aroma of VOO. For this reason, in both cultivars, mechanical harvesting caused a decrease in the amount of total volatiles found in the oils. Thus, after 24h of storage, the oils obtained from mechanically harvested fruits had significantly lower contents of six carbon compounds and five carbon compounds (C6 and C5-compounds), with a reduction of 48% for C6 and 40% for C5 in MS and 40% and 60% in MC (Fig. 5C and D). However, these initial amounts were minimized throughout cold storage, with very similar contents of total volatiles and C6-compounds found in the oils obtained on day 11. In this sense, while a certain reduction in the volatile content was observed throughout cold storage, for both cultivars and harvesting systems, this effect was less pronounced in mechanically harvested fruits, so that at the end of the storage period, they showed slightly higher contents in total. However, the changes observed in some groups of volatile compounds exhibited a different pattern from that described above. Thus, the contents of alcohols with 6 carbon atoms from linoleic and linolenic acids (C6/LA and C6/LNA/ alcohols), and the contents of esters formed in the lipoxygenase pathway (LOX esters) (Fig. 5B, D and F) were higher in the oils of mechanically harvested fruits, and both C6/LNA/ alcohols and LOX esters increased during cold storage. It is important to point out that at any point of the storage period, the contents of volatile esters in the oils obtained from mechanically harvested MS and MC are five times higher than those of hand-picked. Given that volatile esters are associated to fruity-odor notes, the higher content of these compounds could impart fruity notes to these oils which in turn would increase the complexity of their aroma.
4. Conclusions

This study demonstrates that storage at 2 °C for 11 days is an effective treatment to delay deterioration in MC fruits from super-high density orchards, which contributes to maintaining VOO quality as “Extra”. However, cold storage was not effective in maintaining the quality of mechanically harvested MS fruits. In these fruits, the grape straddle harvester causes greater damage which favours an increase in decay incidence from the beginning of storage; Consequently, free acidity also increases in the VOO extracted to such an extent that it rapidly loses the “Extra” category. Furthermore, this oil is characterized by a greater loss of antioxidant compounds (mainly α-tocopherol and phenolic compounds). The phenolic and volatile contents of the oils obtained from mechanically harvested fruits was significantly lower than those of fruits which were manually harvested. Regardless of the cultivar, the results also show a progressive decrease in oxidative stability and the intensity of positive sensory attributes of VOO (fruity, pungent and bitter) through the storage period, which were more pronounced in oils from mechanically harvested fruits. However, the oils from mechanically harvested MC and MS fruits showed a lesser decrease throughout storage in terms of volatile content and exhibited a more complex aroma profile than those manually harvested due to their higher content of volatile esters.

Acknowledgments

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References


Cacereña”) for mechanical harvesting in super-high density


Table 1.- Sensory analysis of virgin oils extracted from Manzanilla de Sevilla (MS) and Manzanilla Cacereña (MC) olives cultivated in hedgerow, manually or mechanically harvested, during cold-storage at 2 °C. Overal grading: From 1.0 (the worst possible) to 9.0 (the best possible). Intensity of attributes: From 0.0 (absence) to 10.0 (the strongest possible). No negative attributes were detected. Each value corresponds to a tetra-plicate evaluated by 8 trained tasters.

<table>
<thead>
<tr>
<th>Sensory analysis</th>
<th>Variety</th>
<th>Hand</th>
<th>Mechanical</th>
<th>Effect of different factors (three way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Harvesting system</td>
<td>Storage time (days)</td>
<td></td>
<td>Variety (V) Harvesting (H) Storage (S) V × H</td>
</tr>
<tr>
<td>Overall grading^a</td>
<td>Manzanilla de Sevilla</td>
<td>7.5 ± 0.2 7.0 ± 0.1 7.0 ± 0.2 6.9 ± 0.1</td>
<td>7.2 ± 0.2 6.8 ± 0.1 7.0 ± 0.2 6.9 ± 0.1</td>
<td>*** *** *** ns ns *** **</td>
</tr>
<tr>
<td></td>
<td>Manzanilla Cacereña</td>
<td>7.5 ± 0.2 7.0 ± 0.1 7.0 ± 0.2 6.9 ± 0.1</td>
<td>7.2 ± 0.2 6.8 ± 0.1 7.0 ± 0.2 6.9 ± 0.1</td>
<td>*** *** *** ns ns *** **</td>
</tr>
<tr>
<td>Fruity^b</td>
<td>Manzanilla de Sevilla</td>
<td>4.4 ± 0.7 2.4 ± 0.4 3.4 ± 1.2 2.2 ± 0.7</td>
<td>3.5 ± 0.5 1.6 ± 0.6 2.5 ± 0.7 2.2 ± 0.7</td>
<td>** * *** ns ns ** ns</td>
</tr>
<tr>
<td></td>
<td>Manzanilla Cacereña</td>
<td>4.4 ± 0.7 2.4 ± 0.4 3.4 ± 1.2 2.2 ± 0.7</td>
<td>3.5 ± 0.5 1.6 ± 0.6 2.5 ± 0.7 2.2 ± 0.7</td>
<td>** * *** ns ns ** ns</td>
</tr>
<tr>
<td>Bitter^b</td>
<td>Manzanilla de Sevilla</td>
<td>3.0 ± 0.5 1.4 ± 0.3 2.2 ± 1.0 0.9 ± 0.4</td>
<td>2.5 ± 0.6 0.5 ± 0.5 1.7 ± 0.5 0.8 ± 0.5</td>
<td>** ** *** ns ns * ns</td>
</tr>
<tr>
<td></td>
<td>Manzanilla Cacereña</td>
<td>3.0 ± 0.5 1.4 ± 0.3 2.2 ± 1.0 0.9 ± 0.4</td>
<td>2.5 ± 0.6 0.5 ± 0.5 1.7 ± 0.5 0.8 ± 0.5</td>
<td>** ** *** ns ns * ns</td>
</tr>
<tr>
<td>Pungent^b</td>
<td>Manzanilla de Sevilla</td>
<td>3.3 ± 0.5 2.6 ± 0.7 2.6 ± 0.6 1.0 ± 0.1</td>
<td>2.5 ± 0.3 0.8 ± 0.8 1.5 ± 0.5 1.3 ± 0.3</td>
<td>*** *** *** * ns ns ***</td>
</tr>
<tr>
<td></td>
<td>Manzanilla Cacereña</td>
<td>3.3 ± 0.5 2.6 ± 0.7 2.6 ± 0.6 1.0 ± 0.1</td>
<td>2.5 ± 0.3 0.8 ± 0.8 1.5 ± 0.5 1.3 ± 0.3</td>
<td>*** *** *** * ns ns ***</td>
</tr>
</tbody>
</table>

ns: no significant; *: P ≤ 0.05; **: P ≤ 0.01 and ***: P ≤ 0.001; P: Probability of no effect.

^aMean value ± SD
^bMedian value ± SD
Figure 1.- Incidence of decay (A), weight loss (B), respiration rate (C) and production of ethylene (D) of Manzanilla de Sevilla (MS) and Manzanilla Cacereña (MC) olives cultivated in hedgerow, manually or mechanically harvested, during cold-storage at 2°C.
Figure 2.- Total oil content (A), virgin oil yield (B), physical extractability (C) and acidity of the virgin oil extracted (D) of Manzanilla de Sevilla (MS) and Manzanilla Cacereña (MC) olives cultivated in hedgerow, manually or mechanically harvested, during cold-storage at 2 °C.
Fig. 3.- Oxidative stability (A), α-tocopherol content (B), carotenoid content (C), chlorophyll content (D), total phenol content (E) and total o-diphenol content (F) of virgin oils extracted from Manzanilla de Sevilla (MS) and Manzanilla Cacereña (MC) olives cultivated in hedgerow, manually or mechanically harvested, during cold-storage at 2 °C.
Fig. 4.- Contents of the main secoiridoid derivatives: Dialdehydic form of the decarboxymethyl oleuropein aglycon (A), dialdehydic form of the decarboxymethyl ligstroside aglycone (B), hydroxytyrosyl elenolate, (C) and tyrosyl elenolate (D) of virgin oils extracted from Manzanilla de Sevilla (MS) and Manzanilla Cacereña (MC) olives cultivated in hedgerow, manually or mechanically harvested, during cold-storage at 2 ºC.
Fig. 5.- Presence of the main groups of volatile compounds: Total volatiles (A), total alcohols with 6 carbon atoms from linolenic acid (B), total volatiles with 6 carbon atoms (C), total alcohols with 6 carbon atoms from linoleic acid (D), total volatiles with 5 carbon atoms (E) and total esters from lipoxygenase pathway (F) in the head space of virgin oils extracted from Manzanilla de Sevilla (MS) and Manzanilla Cacereña (MC) olives cultivated in hedgerow, manually or mechanically harvested, during cold-storage at 2 ºC.