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Responses of fruit physiology and virgin oil quality to cold storage of mechanically harvested 'Arbequina' olives cultivated in hedgerow

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RESUMEN

Respuestas de la fisiología del fruto y de la calidad del aceite virgen al almacenamiento en frío de aceitunas 'Arbequina' de seto cosechadas mecánicamente

El aumento de la producción de aceituna (*Olea europaea* L. cv. 'Arbequina'), debido al uso creciente del cultivo super-intensivo y la necesidad de un rápido procesamiento del fruto forzará a la industria a hacer una considerable inversión en maquinaria para el procesado, para mantener el nivel de calidad del aceite de oliva virgen (AOV). Este trabajo pretende estudiar cómo la temperatura de almacenamiento afecta a la fisiología de la aceituna y a la calidad del aceite, en orden de usar la conservación del fruto como una alternativa más barata y versátil al aumento de la capacidad de procesamiento. La aceituna 'Arbequina' no presentó síntomas de daños por frío durante 15 días de frigoconservación. La podredumbre, el desverdizado, el ablandamiento, la respiración y la producción de etileno de la aceituna aumentaron en relación directa a como aumentaba la temperatura de conservación. Estos hechos determinaron un deterioro proporcional de la acidez libre y de la calidad sensorial de los AOVs. Además, los contenidos de tocoferoles y de los principales grupos de compuestos fenólicos en los AOVs experimentaron una reducción durante la conservación del fruto en razón directa a la temperatura de almacenamiento utilizada. La frigoconservación a 2 °C preservó la integridad del fruto para mantener el nivel «Extra» de calidad durante un periodo de 12 días.

PALABRAS CLAVE: Ablandamiento del fruto – Color del fruto – Compuestos fenólicos – Etileno – Olea europea – Poscosecha de aceituna – Respiración – Tocoferoles.

SUMMARY

Responses of fruit physiology and virgin oil quality to cold storage of mechanically harvested 'Arbequina' olives cultivated in hedgerow

The increase in olive fruit production (*Olea europaea* L. cv. 'Arbequina'), due to the increasing use of super-intensive cultivation and the need for a rapid fruit processing will force the industry to make a considerable investment in machinery for processing in order to maintain the level of quality of virgin olive oil (VOO). This work aims to study how the storage temperature affects the physiology of the olive and the quality of the oil, in order to use fruit storage as a cheaper and more versatile alternative to the increase in processing capacity. 'Arbequina' fruit did not present symptoms of chilling injury during 15 days of cold-storage. Postharvest decay, de-greening, softening, respiration and ethylene production of the olive fruit increased in direct relationship as the storage temperature increased. These facts determined a proportional deterioration of the free acidity and the sensory quality of the VOOs. Furthermore, the contents of tocopherols and of the main phenolic compounds in the VOO exhibited a reduction during fruit storage according to the increase in the temperature used. Storage at 2 °C preserved the integrity of the olive to maintain the best "Extra" level of VOO quality for a period of 12 days.

*KEY-WORDS***:** *Ethylene – Fruit color – Fruit softening – Olea europaea – Olive postharvest – Phenolic compounds – Respiration – Tocopherols.*

1. INTRODUCTION

Over the last decade, super-intensive cultivation of the olive tree (>1000 trees/ha) is becoming increasingly important as it facilitates mechanized harvesting, leads to greater yields and reduces the effect of the typical olive tree alternation of production (Pastor-Vega *et al*., 2005; Gómez del Campo *et al*., 2009). Among the Spanish olive varieties used for the production of Virgin olive oil (VOO), only the cultivar 'Arbequina' is conveniently adapted to this type of cultivation. Factors such as its relatively small size, its precocity in the production of fruit, its high virgin oil yield and the excellent quality of its virgin oil have determined its almost exclusive use in this type of cultivation (Tous and Romero, 1992; Ferguson, 2006). However, the predictable increase in production by the use of this cultivation will pose a serious challenge for the olive industry, which will be struggling to process this quantity of fruit quickly enough before it deteriorates and, accordingly, before the quality of its oil is altered (García and Yousfi, 2006). In addition, the period in which the olive 'Arbequina' produces optimum quality VOO is relatively short (15-20 days). As its degree of maturation increases, the oil from this cultivar loses intensity in the sensory attributes that make it appealing to the consumer. Its initial golden-green color becomes transparent, its flavor becomes tasteless and its characteristic green almond smell fades as the skin of the fruit

darkens (Morello *et al*., 2004). Therefore, to achieve a relevant level of VOO quality, total fruit production must be processed in a reduced period, which would involve the olive industry increasing its processing capacity, since super-intensive cultivation could multiply the production four-fold relative to the traditional cultivation of this tree (< 300 trees/ha).

Dag *et al*. (2012) tested the effect of the temperature and storage time of mechanically harvested 'Barnea', 'Koroneiki' and 'Picual' olives from the intensive density of cultivation on the VOO quality parameters. They found that these cultivars showed different responses to storage. Mainly, they differed in the presence of free fatty acids and phenols. They conclude that fruit originating from modern orchards (irrigated and mechanically harvested) are not necessarily more sensitive to storage than those from traditional orchards (rain-fed and hand harvested).

The first reference about handpicked 'Arbequina' olive storage was published by Vichi *et al*. (2009), who observed no significant deterioration of the oils extracted from 'Arbequina' olives until after 15 days of storage at temperatures of 5 ± 3 °C (diurnal) and 8 ± 3 °C (nocturnal). More recently, our research team (Yousfi *et al*., 2012) compared the cold storage of 'Arbequina' olives from hedgerow cultivation, either hand or mechanically harvested, for extending the period of delaying processing at the olive mill. Mechanical harvesting with a grape harvester machine significantly reduced the feasibility of the fruit stored at 3 °C, which only maintained the initial level of the commercial quality of the VOO extracted for a period of 10 days, compared to that noted for the olives harvested by hand, which maintained their best level for a period of at least 21 days. Since the mechanically harvested fruit is free of external visible damage, it is assumed that the cause of this loss in postharvest viability is due to inner damage, which makes the oil more susceptible to deterioration. However, a more in-depth study on the fruit cold storage is necessary before the olive industry can be able to incorporate this postharvest treatment into their lines of work. The industry requires getting to know the range of storage temperatures that can be applied without taking the risk of chilling injury or fruit decay. Furthermore, studies on the physiological behaviour of olive fruit during its postharvest period are scarce, especially those focusing on oil production (Fernández-Bolaños *et al*., 1997; Ranalli *et al*., 1998). The physiological response of hedgerow olives to a full range of cold storage temperatures (2 to 6 °C) would be of great interest because it would explain changes in the chemical composition of the oil extracted.

The main objective of this paper is to evaluate the changes in the most significant parameters of metabolic activity (softening, color of skin, respiration and ethylene production) during the time between harvesting and processing, studying how the virgin olive oil, subsequently extracted, responds in its chemical composition and commercial quality to these physiological changes, in order to evaluate, with a better knowledge basis, the use of cold storage to preserve the integrity of fruit physiology and maintain the original VOO quality.

2. MATERIALS AND METHODS

2.1. Cultivation Conditions and Plant Material

Experiments were conducted on a commercial olive orchard near Seville (37° 30' N, 5° 44' W, ca. 60 m a.s.l.). The trees (*Olea europaea* L. cv. 'Arbequina') cultivated in a North-South orientated hedgerow (1667 trees/ha) were 6 years old in 2011, when measurements were made. They were planted at 4 m × 1.5 m, and had a single trunk with 3-4 main branches from 1.0-1.2 m above ground. The canopy was of ca. 1.4 m diameter and ca. 2.2 m height.

In October (2011) 'Arbequina' olive fruits were mechanically harvested at the mature green stage of ripening, using a VX680 wine grape harvester (New Holland España, Madrid, Spain) from 100 trees of four hedgerows and randomly placed in 16 perforated plastic boxes holding 20 kg of olives each. All the boxes were then transported to the Instituto de la Grasa in Seville on the same day.

2.2. Storage Treatments and Measurements of Fruit Characteristics

The boxes were randomly distributed into four different storage rooms, respectively, under ambient conditions (18 ± 3 °C and RH 80%) or under three different cold storage conditions at 2, 4 and 6 °C (RH 95%) for 15 days. Sampling dates were programmed at 0, 1, 6, 9, 12, and 15 days. To evaluate the changes in incidence of fruit decay during fruit storage, on each sampling date, 2 samples of 100 olives were randomly taken from each box, and the number of fruit with visible signs of decay was evaluated and expressed in percentage as the mean value of 8 replicates. A decay incidence of 100% in a treatment determined the removal of the samples and the end of the experiment for this treatment. Previously, before the time of storage, 2 samples of 100 healthy olives were randomly taken from each box, weighed to a precision of 0.1 g, and placed in small plastic jars in the same room as their respective original box, to evaluate the changes in fruit weight during storage. Simultaneously, other groups of 25 healthy fruits randomly taken from each 20 kg box were similarly placed in small plastic jars and stored in the same room as their respective original box, to monitor the changes in skin color and fruit firmness during storage. The color was determined on the equatorial zone of these 100 fruits per treatment, 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 0º. The International Commission on Illumination's color notation system (ICI L*a*b*) was applied to determine the parameters L*, a*, and b*; where L* indicates the lightness, a* means the color axis from green to red, and b* means the color axis from blue to yellow. By means of these parameters a color index (CI) was calculated according to the formula:

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

This equation was used to evaluate the natural changes in fruit skin color during olive cold storage (Castellano *et al*., 1993). Fruit firmness was also evaluated in the same place on each fruit, using a Zwick 3300 hand densimeter (Zwick GmbH & Co., Ulm, Germany). The firmness of the fruit was measured without rupture by the pressure of a 5 mm diameter disk. The results were expressed in N cm^{-2}. On each sampling date, each point of these two variables expressed the mean value of 100 determinations, using the same fruits every time (four samples of 25 fruits).

2.3. Respiration Rate and Ethylene Production of Olive Fruits

On each sampling date, 1 kg olives was taken from each 20 kg box and placed into 2.0 L glass jars which were hermetically sealed during 3 h. The CO_2 contents of the head space of these jars were determined with a G100 portable gas analyzer (Geotechnical Instrument Ltd., Leamington Spa, UK) and the ethylene content was subsequently evaluated using an ICA portable ethylene analyzer (International Controlled Atmosphere Ltd., Paddock Wood, UK) one hour later.

2.4. Virgin Oil Yield, Total Oil Content and Physical Extractability

From each box, a sample of 1 kg olives were randomly taken and milled separately, constituting 4 replicates for each treatment. From the resulting paste in each sample, 800 g were taken and extracted separately, using an 'Abencor' extractor (Comercial Abengoa S.A., Seville, Spain) (Martínez *et al*., 1975). After centrifuging, the oil was decanted into a graduated tube to measure the volume of the oil obtained, in order to evaluate the virgin oil yield, which was calculated as the percentage of fresh weight, considering 0.915 kg L^{-1} the olive oil density at ambient temperature. The extracted oil was then filtered and stored at –20 °C under N_2 atmosphere until analysis. From each replicate, a 50 g sample of the surplus fresh paste (about 200 g) was separately weighed a precision of 0.1 g in previously similarly weighed capsules, and dried at 105 °C to constant weight, to estimate in percentage the dry weight and the humidity of each sample, compared to its initial fresh weight (50 g). The oil from this dried paste was solvent extracted with hexane using the Soxhlet method to determine the total oil content, expressing it as a percentage of the fresh weight of the original paste. The physical extractability of the fruits was calculated in each sample as the percentage of total oil content that represents the oil physically extracted.

2.5. Oil Analysis

Free acidity, peroxide 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 in each oil sample replicate according to the European Union Standard Methods (Annexes II and IX 153 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 structured 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).

The photosynthetic pigment contents in the oils were estimated by their absorbance at 470 and 670 nm for carotenoids and chlorophylls, respectively, and the results were expressed as mg kg^{-1} (Minguez-Mosquera *et al*., 1991). Stability against oxidation was evaluated using the Rancimat method, according to the method proposed by Laübli and Bruttel (1986).

To determinate the composition of phenolic compounds, previously the phenolic fraction of each oil sample was isolated by solid-phase extraction and analyzed by reverse-phase HPLC using a diode array UV detector (Mateos *et al*., 2001). Subsequently, the quantification was firstly carried out at 280 nm using *p*-hydroxyphenylacetic acid as internal standard, whereas the quantification of flavones and ferulic acid was secondly conducted at 335 nm using *o*-coumaric acid as internal standard. The results were expressed in mg of phenol compound per kilogram of oil.

2.6. Statistical Analysis

All the studied variables were analyzed by ANOVA. When ANOVA detected a significant ($p \leq 0.05$) effect due to each factor studied independently (temperature and time of storage), a 5% level of least significant difference (lsd), calculated by Duncan's multiple range test, was used to establish differences between the mean values.

3. RESULTS AND DISCUSSION

3.1. Fruit Decay

Cold storage produced a delay in the progress of the decay incidence from the first day of conservation in comparison to storage at ambient

conditions, becoming statistically significant from the second sampling date (6 days) (Fig. 1). The different cooling temperatures tested determined different speeds of fruit decay, which increased as the temperature used was increased. Thus, after 6 days of storage this incidence was already significantly higher in the fruits stored at 6 °C than in the olives maintained at 2 °C. In the following sample, the differences in this parameter between the three treatments of cold storage progressively increased. In a recent work carried out by our research team (Yousfi *et al*., 2012), using the same kind of olives, a significant difference in fruit decay incidence was found between cold (3 °C) and ambient storages from the first sampling date (4 days). Previously, Garcia *et al*. (1996) observed a similarly rapid decay of hand-picked green 'Picual' olives from a traditional olive orchard (≤ 300 trees/ha) maintained at 12 ± 2 °C in comparison to cold stored olives at 5 and 8 °C, which did not show significantly different decay incidence until 14 storage days. The decay incidence of the 'Picual' olives stored at 5 °C did not exceed 20% at up to 30 storage days, whereas in our experiment, 'Arbequina' olives stored at 2 °C already achieved this value after 15 days. It seems clear that cold storage is more effective in avoiding post-harvest decay for hand-harvested 'Picual' olives than for machine-harvested 'Arbequina' olives. This negative effect on decay incidence during cold storage of hedgerow 'Arbequina' olives due to mechanical harvesting has already been reported by Yousfi *et al*. (2012), who compared the behavior of handpicked or machine harvested olives during storage. The handpicked olives stored at 3 °C showed 6% decay incidence after 21 days, whereas the machine-harvested fruits already surpassed this value after 4 storage days. Despite the fact that these olives showed no external injury due to mechanical harvesting, it seems that this system provoked internal damage which favoured their rapid decay. The results obtained in this study confirm this fact, showing a similar progression in the decay of the fruits during cold storage.

3.2. Weight Loss

Olives maintained at ambient conditions already exhibited a significantly higher loss in weight after only 1 storage day (Figure 2). The temperature of refrigeration determined differences in this parameter among the stored olives. The weight losses in the olives increased as the storage temperature used also increased, and the differences in this parameter among treatments increased with the progression of storage time. Thus, the weight losses in the fruit stored at 6 °C and the other two treatments of cold storage (2 and 4 °C) significantly differed in a period between 2 and 6 days, whereas these two latter treatments still showed no significantly different values in this parameter after 12 storage days. The weight loss values presented in this experiment confirm the results obtained by Yousfi *et al.* (2012) with hedgerow 'Arbequina' olives which were also harvested and maintained at 18 or 3 °C, but considerably differ from the ones published by Castellano *et al*. (1993) or Agar *et al.* (1999), who found lower values for this parameter, respectively working with handpicked 'Picual' and 'Manzanilla' olives, stored at ambient or 5 °C. Yousfi *et al*. (2012) also tested the changes in this parameter in handpicked 'Arbequina' olives during their storage, obtaining significantly lower values than those harvested by machine. However, these values were also notably higher than those noted for 'Picual' or 'Manzanilla' olives. It seems that the small size of the 'Arbequina' olive determines a higher surface/volume ratio, which determines a more rapid loss in water by transpiration. Furthermore, the use of mechanical harvesting accelerated this process.

3.3. Fruit Color

Olives stored at ambient conditions already experienced a significantly higher de-greening (lower CI value) than the fruits maintained in cold storage after the first day of conservation

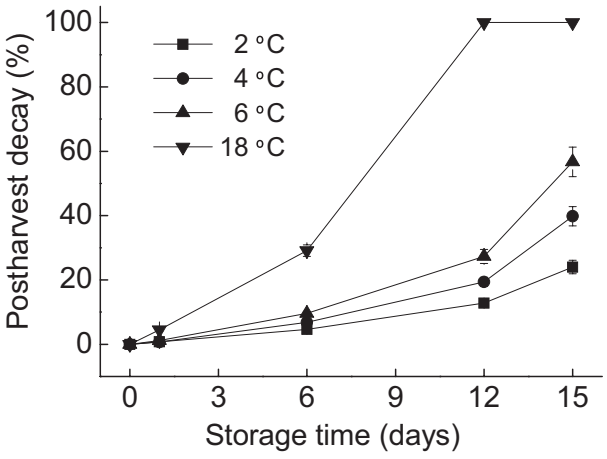

Figure 1
Changes in post-harvest decay of 'Arbequina' mechanically harvested olives from hedgerow cultivation during storage at different temperatures.

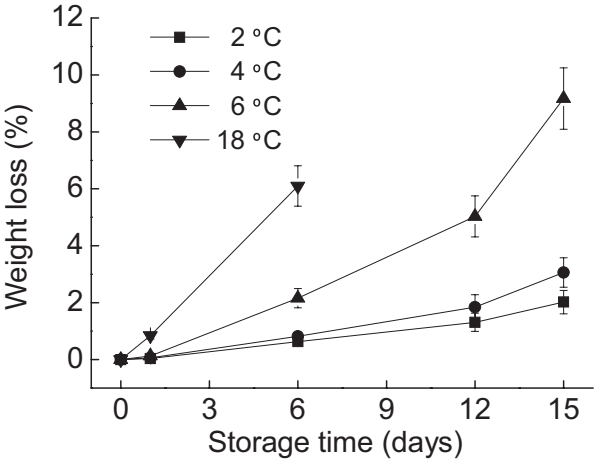

Figure 2
Changes in weight loss of 'Arbequina' mechanically harvested olives from hedgerow cultivation during storage at different temperatures.

(Figure 3). Similarly as in the case of decay incidence, although exhibiting an inverse behavior, the CI values decreased as the cold storage temperature increased, indicating that the use of lower temperatures induced a more effective delay of fruit skin de-greening.

Castellano *et al*. (1993) observed how 'Picual' olives stored at 12 °C suffered a significantly faster skin de-greening than those stored at 5 °C. However, the decrease in CI values noted for 'Arbequina' olives under cold storage (2, 4 or 6 °C) in our experiment was faster than those noted for 'Picual' olives at 5 °C, indicating that cold storage is more effective in delaying the ripening progress of this second variety. It is also interesting to note that, regardless of the temperature applied, the fruit developed visible bruising on the skin after the third day of storage as a result of machine harvesting since the fruit that was hand harvested at the same time did not, at any time, show this type of damage. However, no symptoms of chilling injury were detected in the fruits.

3.4. Fruit Firmness

Storage temperature was a determinant factor for fruit softening during its conservation awaiting oil extraction (Figure 4). As temperature increased, fruit firmness decreased, with the values noted for the olives stored at 18 °C being significantly lower than those noted for the fruits maintained under refrigeration after one storage day. Subsequently, the differences between the treatments progressively increased. After 6 days, the fruit stored at 6 °C showed a significantly higher softening rate than the fruits maintained at 2 or 4 °C, and after 12 days these two treatments also differed significantly. These results confirmed those obtained by García *et al.* (1996), who found a temperature-related fruit softening in 'Picual' olives during storage. However, the softening speed of the machine harvested 'Arbequina' olives was considerably faster, despite the low temperatures of refrigeration. This fact supports the notion that mechanical harvesting causes internal damage in the fruits.

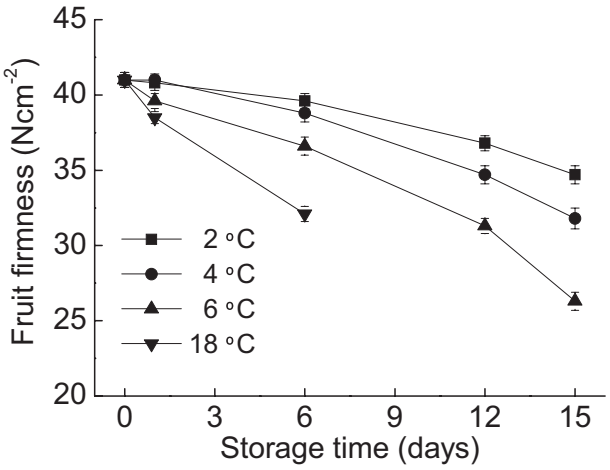

Figure 4
Changes in fruit firmness of 'Arbequina' mechanically harvested olives from hedgerow cultivation during storage at different temperatures.

3.5. Respiration Rate and Ethylene Production

As storage temperature increased, the CO_2 production of the fruit also increased (Figure 5). Olives stored under ambient conditions exhibited significantly higher respiration rates than the refrigerated ones. These fruits showed the values of this parameter systematically ordered according to the cold storage temperature used. However, no significant differences were found among the three treatments tested at up to 12 days of storage. Previously, only the respiration rates of the fruits stored at 2 and 6 °C differed significantly, whereas the olives stored at 4 °C showed no significantly different intermediate values. Only fruit stored at 2 °C maintained the initial value of CO_2 production

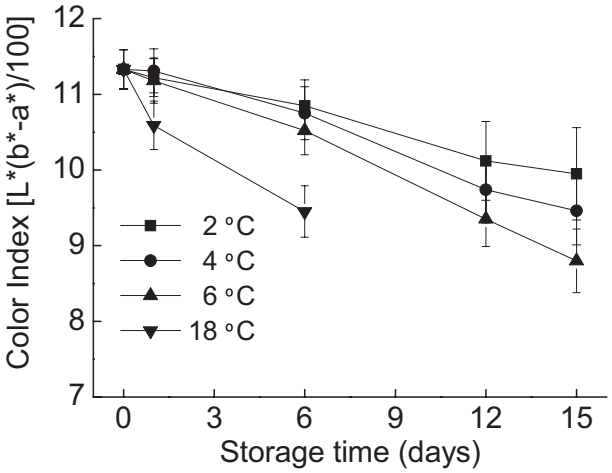

Figure 3
Changes in skin colour of 'Arbequina' mechanically harvested olives from hedgerow cultivation during storage at different temperatures.

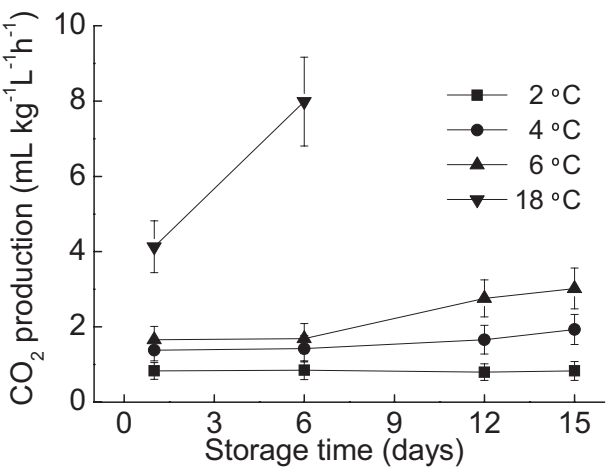

Figure 5
Changes in CO2 production of 'Arbequina' mechanically harvested olives from hedgerow cultivation during storage at different temperatures.

throughout the storage period tested (15 days). These results agree with the ones obtained by Rinaldi *et al*. (2010), who observed that cold storage at 5 °C had a significant effect on slowing down the respiration rate of 'Coratina', 'Leccino' and 'Ogliarola Leccese' olives. Previously, Nanos *et al.* (2002) had found no significant differences in respiration rates between mature-green 'Conservolea' olives stored at 0 and 5 °C at up to 12 days of cold storage. The respiration rates observed in mature-green 'Arbequina' olives are notably lower than those noted for mature green 'Hojiblanca' olives stored at 20 °C (Fernández-Bolaños *et al.,* 1997) and 'Conservolea' olives stored at 0, 5, or 20 °C (Nanos *et al*., 2002) and are closer to those found by Garcia *et al.* (1995), who kept mature green 'Manzanilla' and 'Gordal' olives for 46 hours at temperatures of 10, or 20 °C. Such differences were probably due to the physiological characteristics of each cultivar in different seasons.

Cold storage reduced the ethylene production of the olives in comparison to the fruits maintained at 18 °C. Already, from the first storage day these olives released significantly higher amounts of ethylene (Fig. 6). Similarly, Agar *et al.* (1998) found lower values of ethylene production in black-ripe 'Ascolano', 'Manzanilla', 'Mission', and 'Sevillana' olives stored at 5 °C. Among the fruits kept at 2, 4 or 6 °C, no significant differences in ethylene production were found after 6 days of storage. However, after 12 and 15 days, this parameter significantly increased in the olives stored at 4 and 6 °C, whereas the ones stored at 2 °C maintained the same initial value. The olives that were mechanically harvested produced significantly higher amounts of ethylene than manually harvested fruits, which were kept at the same cold storage temperatures (data not shown). This result supports the idea of Yousfi *et al.* (2012), who proposed that mechanical harvesting induced internal damage in 'Arbequina' olives. This damage would cause stress to these fruits, and the general response of higher plants to stress is the increase of ethylene biosynthesis. As chlorophyll degradation, cell wall decomposition, or the increase of respiratory rate are ethylene-dependent processes, as well as other events associated with fruit ripening and senescence, will exhibit different behavior according to how well the the synthesis of this phytohormone is inhibited by cold storage.

3.6. Physical Extractability

Physical extractability of the olive fruit was proportional to the temperature of storage (Figure 7). Fruits stored at 18 °C showed significantly higher values of this parameter from the first day of storage, showing a spectacular rise in the second sampling date, after 6 days of storage. Among fruit kept in cold storage, no significant differences in this parameter were detected at up to 6 days of storage, when these three treatments significantly differed in order according to the temperature of storage used. Subsequently, these differences were increasing because the physical extractability of the fruit stored at 6 °C clearly increased, whereas in the olives stored at 4 °C, this parameter only slightly increased, and in the olives stored at 2 °C, it even slightly decreased. This result may be related with both the changes in weight loss and in firmness suffered by the olives during the storage period (Fig. 2 and 4). Weight loss is basically a loss in fruit water content, meaning that the fruit becomes drier with increasing storage temperature or time, and it is well known that the physical extraction of oil decreases with the level of fruit humidity (Ben-David *et al*., 2010). In the same manner, the main factor responsible for oil loss during the physical extraction of VOO is the emulsifying effect of the cell wall and the lipoproteic membrane remains that are present in the olive paste (Martínez-Moreno *et al.,* 1964; Petursson *et al*., 2004). Since fruit firmness is linked to the rigidity of the cell walls and its turgidity, it is logical to consider that an increase

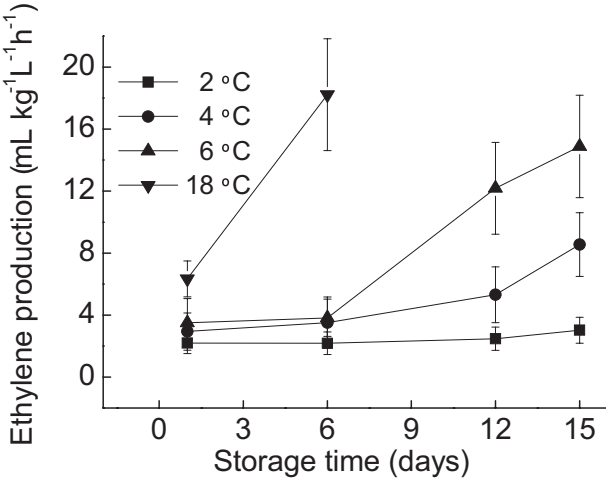

Figure 6
Changes in ethylene production of 'Arbequina' mechanically harvested olives from hedgerow cultivation during storage at different temperatures.

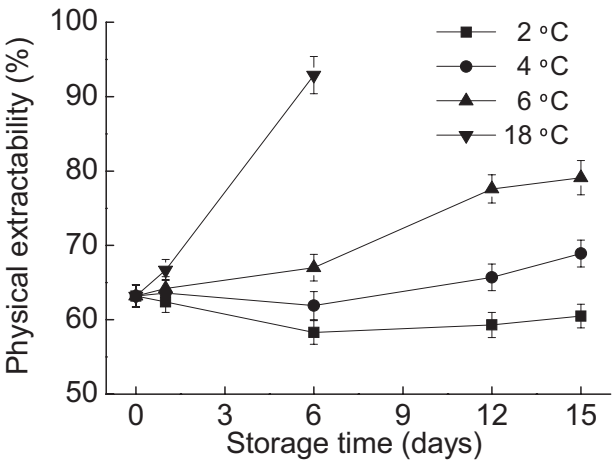

Figure 7
Changes in physical extractability of 'Arbequina' mechanically harvested olives from hedgerow cultivation during storage at different temperatures.

in the physical extractability of the fruit must be related to its softening. Furthermore, the collapse of the cell walls is a process with high-sensitivity to ethylene. This fact would explain the coincidence between the profiles of the ethylene production and the physical extractability of the oils during fruit storage at different temperatures (Figures 6 and 7).

3.7. Oil Quality

The level of quality achieved by the extracted VOOs responds to the incidence of decay presented by the fruits (Table 1, Figure 1). A decay incidence near to 20% meant the loss of the best level of quality ("Extra virgin") due to the development of a negative sensory attribute named "musty" and/or due to exceeding the limit value of free acidity established (0.8 %) for being classified as "Extra virgin". Similarly, a decay incidence near 30% in the stored fruits coincided with the loss of the second level of quality named "Virgin" in the oil extracted from these fruits. As decay incidence is strongly related to the temperature and the time of storage, the quality level of the oils is also related to these variables. Thus, the oil extracted from olives stored at 18°C showed "Lampante" category of quality, which cannot be marked for human consumption, after 6 days of storage, and the oil extracted from

Table 1
Changes in physical, chemical and sensorial quality parameters in oils extracted from 'Arbequina' mechanically harvested olives from hedgerow cultivation stored at different temperatures

Variable[a,b]	Storage time (days)	Storage temperature (°C)			
		2	4	6	18
Free acidity (% oleic acid)	0	0.2 ± 0.1C	0.2 ± 0.1 B	0.2 ± 0.1 C	0.2 ± 0.1 C
	1	0.2 ± 0.1 Cb	0.3 ± 0.2 Bb	0.4 ± 0.2 Ca	0.7 ± 0.2 Ba
	6	0.3 ± 0.1 BCc	0.4 ± 0.2 Bc	0.7 ± 0.1 Bb	6.8 ± 0.3 Aa
	12	0.5 ± 0.2 Ba	2.0 ± 0.3 Ab	3.2 ± 0.3 Ac	–
	15	0.8 ± 0.2A	–	–	–
Peroxide value (meq O_2 kg^{-1})	0	3.5 ± 0.5	3.5 ± 0.5	3.5 ± 0.5	2.5 ± 0.5 B
	1	3.4 ± 0.6	3.6 ± 0.7	3.6 ± 0.6	2.5 ± 0.7 B
	6	3.1 ± 0.8	3.0 ± 0.7 b	3.6 ± 0.8	5.1 ± 0.9 Aa
	12	3.3 ± 0.7	3.7 ± 0.8 b	4.3 ± 0.8	–
	15	4.2 ± 0.9	–	–	–
K_{232}	0	1.85 ± 0.16	1.85 ± 0.16	1.85 ± 0.16	1.85 ± 0.16
	1	1.82 ± 0.13	1.96 ± 0.12	1.86 ± 0.13	1.81 ± 0.12
	6	1.84 ± 0.14	2.03 ± 0.19	2.16 ± 0.18	2.22 ± 0.21
	12	1.88 ± 0.12	1.95 ± 0.21	2.13 ± 0.22	–
	15	1.90 ± 0.12	–	–	–
K_{270}	0	0.08 ± 0.01	0.08 ± 0.01 B	0.08 ± 0.01 B	0.08 ± 0.01 B
	1	0.08 ± 0.01	0.08 ± 0.01 B	0.09 ± 0.01 B	0.08 ± 0.01 B
	6	0.08 ± 0.01 b	0.08 ± 0.01 Bb	0.10 ± 0.01 ABb	0.20 ± 0.03 Aa
	12	0.08 ± 0.01 b	0.13 ± 0.02 Aa	0.12 ± 0.03 Aa	–
	15	0.09 ± 0.01	–	–	–
Panel test	0	7.5 ± 0.3 A	7.5 ± 0.3 A	7.5 ± 0.3 A	7.5 ± 0.3 A
	1	7.5 ± 0.4 A	7.5 ± 0.4 A	7.5 ± 0.4 A	7.2 ± 0.5 A
	6	7.3 ± 0.4 Aa	7.2 ± 0.5 ABa	6.0 ± 0.4 Bb	3.2 ± 0.6 Bc
	12	7.0 ± 0.4 Aa	6.8 ± 0.3 Ba	3.4 ± 0.5 Cb	–
	15	6.0 ± 0.5 B	–	–	–

[a] Each value is the mean ± SD of 4 replicates. [b] A 5% level of least significant difference (lsd), calculated by Duncan's multiple range test, was used to establish differences among 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 storage temperature, but different storage time followed by different uppercase letters are significantly different; and in each row two values of the same time of storage, but different storage temperature followed by different lowercase letters are significantly different. Absence of letters means no effect.

olives stored at 6 °C showed this same worst level of quality after 12 storage days, both of them due to the high level of acidity and for exhibiting a notable presence of the "musty" attribute. At this time the oil obtained from fruits stored at 4 °C showed the second level of quality ("Virgin") due to the high level of free acidity, and the oils extracted from the olives stored at 2 °C still presented the original level of quality ("Extra virgin"). Finally, in the final assessment carried out after 15 days of storage these last oils showed the limit value of free acidity, but also exhibited a perceptible level of the musty attribute which determined their classification into the second category of commercial quality ("Virgin"). Yousfi *et al.* (2012) observed a similar rapid deterioration of free fatty acid and of the overall grading of sensory quality in the oils from mechanically harvested 'Abequina' olives in comparison to those harvested by hand during storage at 3 °C. Among the parameters used to evaluate oil oxidation, only K_{270} showed significant changes due to the different temperature or time of fruit storage. Thus, after 6 days of storage the oils from fruit stored at 18 °C exhibited a significantly higher value of this parameter than those of the oils from olives stored at lower temperatures and also higher than those of the oils from the previous sampling of the same treatment. In the following sampling the oils from fruit stored at 4 and 6 °C also suffered a significant increase in K_{270}, whereas the oils extracted from fruit stored at 2 °C maintained the original values of this parameter even after 15 days of fruit storage. This result contrasts with those obtained previously in other works that did not find any effect on K_{270} due to the time of cold storage (Clodoveo *et al.*, 2007; Kalua *et al.*, 2008) and, on the other hand, with those obtained by Ben Yahia *et al.* (2012), who did not find any increase in this parameter as a consequence of cold storage in Chétoui olives.

The carotenoid and chlorophyll contents of the oils were not significantly affected by the time or the temperature of fruit storage (Table 2). However, these parameters exhibited a tendency to increase with storage time, especially when the fruit was

Table 2
Changes in carotenoid, chlorophyll and tocopherol contents and oxidative stability in oils extracted from 'Arbequina' mechanically harvested olives from hedgerow cultivation stored at different temperatures

Variable[a,b]	Storage time (days)	Storage temperature (°C)			
		2	4	6	18
Carotenids (mg kg^{-1})	0	3.5 ± 0.5	3.5 ± 0.5	3.5 ± 0.5	3.5 ± 0.5
	1	3.5 ± 0.5	3.6 ± 0.7	3.6 ± 0.6	3.5 ± 0.6
	6	3.5 ± 0.6	3.6 ± 0.7	3.6 ± 0.6	3.8 ± 0.6
	12	3.6 ± 0.5	3.7 ± 0.8	3.6 ± 0.7	–
	15	3.6 ± 0.6	–	–	–
Chlorophyll (mg kg^{-1})	0	5.2 ± 0.4	5.2 ± 0.4	5.2 ± 0.4	5.2± 0.4
	1	5.2 ± 0.5	5.3 ± 0.4	5.4 ± 0.4	5.7 ± 0.5
	6	5.3 ± 0.5	5.4 ± 0.5	5.6 ± 0.5	6.1 ± 0.6
	12	5.4 ± 0.6	5.5 ± 0.6	5.5 ± 0.6	–
	15	5.5 ± 0.6	–	–	–
Tocopherols (mg kg^{-1})	0	385.3 ± 8.4 A	385.3 ± 8.4 A	385.3 ± 7.4 A	385.3 ± 8.4 A
	1	377.8 ± 7.2 Aa	376.2 ± 7.2 Aa	370.3 ± 7.5 Ba	352.8 ± 8.2 Bb
	6	362.3 ± 7.7 Ba	352.0 ± 7.7 Bab	346.3 ± 8.1 Cb	328.8 ± 7.6 Cc
	12	338.9 ± 7.9 Ca	329.2 ± 8.3 Cb	318.9 ± 7.9 Db	–
	15	336.2 ± 8.2 C	–	–	–
Stability (hours)	0	28.4 ± 2,2 A	28.4 ± 2.2 A	28.4 ± 2,2 A	28.4 ± 2,2 A
	1	26.8 ± 2.4 A	25.9 ± 2.1 AB	25.6 ± 3.0 AB	24,2 ± 2.7 AB
	6	24.7 ± 2.3 AB	23.6 ± 2.5 B	22.9 ± 2.3 BC	21.1 ± 2.6 B
	12	23.5 ± 2.1 Ba	23.0 ± 2.6 Ba	19.8 ± 2,6 Cb	–
	15	21.8 ± 2.4 B	–	–	–

[a] Each value is the mean ± SD of 4 replicates. [b] 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 storage temperature, but different storage time followed by different uppercase letters are significantly different; and in each row two values of the same time of storage, but different storage temperature followed by different lowercase letters are significantly different. Absence of letters means no effect.

kept at 18 °C. Yousfi *et al.* (2012), who found a similar trend in oils extracted from stored hand-picked 'Arbequina' olives, explained this fact by the release of these pigments in the olive oil due to a decrease in the chloroplast consistency. The tocopherol contents and the oxidative stability of the oils exhibited a similar behavior during fruit storage. During this period, both parameters suffered a progressive reduction, which was accelerated by the increase in storage temperature. This fact can be considered normal, since the tocopherols play an antioxidant role in the oils (Marmesat *et al.,* 2010).

The contents in the oils of phenolic compounds such as hydroxytyrosol (1.8 mg kg^{-1}), tyrosol (2,0 mg kg^{-1}), vanillic acid (0.7 mg kg^{-1}), Vanillin (0.2 mg kg^{-1}), ρ-coumaric acid (0.4 mg kg^{-1}), pinoresinol (2.2 mg kg^{-1}), acetoxy-pinoresinol (22.7 mg kg^{-1}), tyrosyl elenolate (10.0 mg kg^{-1}), ferulic acid (4.8 mg kg^{-1}), luteoline (4.0 mg kg^{-1}) and apigenine (1.6 mg kg^{-1}) did not suffer significant changes due to the temperature and /or the time of fruit storage (data not shown). Nevertheless, the contents in the oil of hydroxytyrosol acetate, the dialdehydic form of the decarboxymethyl oleuropein aglycone (3,4 DHPA-EDA), tyrosol acetate, the dialdehydic form of the decarboxymethyl ligstroside aglycone (ρ-HPEA-EDA) and the hydroxytyrosyl elenolate (3,4 DHPA-EA) decreased with an increase in both storage factors, determining the consequent reduction of the contents in the oil of the main groups of phenolic molecules: *o*-diphenols, secoiridoids and total phenol compounds (Table 3). As these molecules are natural antioxidants (Marmesat *et al*., 2010), the loss in stability exhibited by the oils coincided with the reduction in these phenolic compounds. These results agree with those of different authors (Clodoveo *et al*., 2007; Kalua *et al*., 2008), who found a similar reduction in these compounds in oils extracted from different mill olives during their storage and confirm those obtained by Yousfi *et al*. (2012) with 'Arbequina' olives at 3 °C. However, Dag *et al.* (2012) reported

Table 3
Changes in relevant groups of phenol compounds in oils extracted from 'Arbequina' mechanically harvested olives from hedgerow cultivation stored at different temperatures

Phenol type[a,b] (mg kg^{-1})	Storage time (days)	Storage temperature (°C)			
		2	4	6	18
Flavones	0	4.8 ± 0.7	4.8 ± 0.7	4.8 ± 0.7	4.8 ± 0.7
	1	5.0 ± 0.6	4.6 ± 0.5	4.4 ± 0.6	3.9 ± 0.6
	6	4.4 ± 0.5	4.3 ± 0.5	4.4 ± 0.5	3.9 ± 0.5
	12	4.8 ± 0.4	5.2 ± 0.6	5.0 ± 0.5	–
	15	4.4 ± 0.4	–	–	–
o-Diphenols	0	67.1 ± 5.5 A	67.1 ± 5.5 A	67.1 ± 5.5 A	67.1 ± 5.5 A
	1	66.6 ± 7.1 Aa	44.9 ± 6.8 Bb	40.2 ± 6.2 Bb	35.7 ± 5.8 Bb
	6	50.1 ± 5.4 Ba	41.2 ± 6.6 Bab	35.4 ± 6.3 Bbc	26.9 ± 5.4 Bc
	12	41.1 ± 5.3 BC	38.8 ± 6.2 B	34.5 ± 6.0 B	–
	15	35.2 ± 6.0 C	–	–	–
Secoiridoids	0	82.4 ± 6.4 A	82.4 ± 6.4 A	82.4 ± 6.4 A	82.4 ± 6.4 A
	1	75.1 ± 7.0 ABa	67.8 ± 7.1 Bab	64.9 ± 6.7 Bab	60,7 ± 6.2 Bb
	6	63.7 ± 6.9 BCa	60.2 ± 7.4 Bab	53.2 ± 6.5 BCab	50.0 ± 6.5 Bb
	12	59.3 ± 7.3 Ca	57.9 ± 7.2 Ba	44.8 ± 6.6 Cb	–
	15	50.3 ± 7.5 C	–	–	–
Total phenols	0	144,8 ± 12.2 A	144,8 ± 12.2 A	144,8 ± 12.2 A	144,8 ± 12.2 A
	1	136.1 ± 11.4 A	129.1 ± 10.9 A	126.0 ± 11.2 A	118,6 ± 11.6 B
	6	114.4 ± 12.1 Ba	97.0 ± 11.4 Bab	93.0 ± 12.4 Bab	77.3 ± 12.0 Cb
	12	95.0 ± 13,5 Ca	83.2 ± 13.5 Bab	70.8 ± 11.5 Cb	–
	15	77.4 ± 12.6 D	–	–	–

[a] Each value is the mean ± SD of 4 replicates. [b] 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 storage temperature, but different storage time followed by different uppercase letters are significantly different; and in each row two values of the same time of storage, but different storage temperature followed by different lowercase letters are significantly different. Absence of letters means no effect.

that during fruit storage at 4 °C polyphenol content of then VOO behaved differently among cultivars: in 'Picual' it was relatively stable, in 'Barnea' it decreased moderately, and in 'Koroneiki' it decreased sharply, and Ben Yahia *et al*. (2012) even found higher phenol contents in oils extracted from Chetoui olives stored at ambient conditions than in those from fruit stored at 5 °C. The phenol compounds of the VOO are mainly formed from the hydrolysis of glycosylated phenolic compounds, such as oleuropein, verbascoside and/or ligstroside (Ryan *et al*. 1999) and it may occur in the fruit cells or during the process of oil extraction (Kalua *et al*., 2006). Therefore, their presence depends on the interaction of many factors: genetic (variety), environmental (cultivation, harvest and post-harvest conditions), physiological (fruit age and health) and the conditions of processing (type of milling, malaxation time and temperature, amount of water used in centrifugation) (Servili *et al.,* 2004; Clodoveo, 2012). This should be the cause of the disparity of results found in the scientific literature.

4. CONCLUSIONS

'Arbequina' olives from hedgerow cultivation, mechanically harvested, showed a rapid deterioration of their physiology during the waiting time before processing for VOO extraction. The reduction in storage temperature delayed the increase of parameters used to evaluate the progress of this physiological deterioration. Thus, the progress of the incidence of post-harvest decay, weight loss, skin de-greening, softening, respiration rate and ethylene production in the fruits were delayed with an effectiveness inversely proportional to the storage temperature used. Subsequently, the extracted VOO showed quality according to the degradation level of the original fruit. Storage at 2 °C preserved the fruit physiology to maintain the best level of VOO quality for a period of 12 days.

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