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Effect of Temperature, Modified Atmosphere and Ethylene During Olive Storage on Quality and Bitterness Level of the Oil

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Abstract Mill olives (*Olea europaea* L. cv. 'Lechín'), harvested at the green mature stage of ripening, were stored for 72 h under six different storage conditions: in air, in a closed container, and in a closed container with 30 ppm ethylene either at 20 or at 40 °C. The use of 40 °C as the fruit storage temperature reduced oil bitterness, regardless of the atmosphere applied; however, it also induced a significant reduction in stability and pigment content of the oil extracted. At 20 °C, mill olives stored under air supplemented with 30 ppm ethylene engendered oils with middle bitterness intensity, whereas the oils obtained from fruit stored similarly, but without ethylene, or in an open container exhibited a strong intensity of this sensory attribute. Fruit respiration in the closed containers caused a CO_2 accumulation and an O_2 decrease in the storage atmosphere. This CO_2 concentration was increased by the previous ethylene addition, but O_2 presence did not suffer an additional reduction. The use of modified atmospheres in fruit storage induced off-flavor development in the oils extracted, producing a significant reduction in the overall grading of their sensory quality.

Keywords 3,4-DHPEA-EA · Firmness · Off-flavor · Oil quality · *Olea europaea* · Stability

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Introduction

Virgin olive oil (VOO) is by definition the oil obtained from the olive fruit through physical procedures and, as with any other fruit juice, its quality is directly related to the quality of the fruit from which it is extracted [1]. After the green mature stage of ripening, the increase in lipid content is scarce in olive fruit [2, 3]. Thus, it could be considered the most appropriate stage for fruit harvesting, because the olive cells contain enzymes that might cause alterations in the oils, such as lipases, lipoxygenases or hydroperoxide lyases, which can cause hydrolytic or oxidative alterations during fruit ripening and/or oil extraction [4]. As the olive matures, the possibility of these enzymes acting on the stored oil increases and, consequently, the quality of the oils tends to decline [5, 6]. Furthermore, when olive trees are harvested early, they produce a greater amount of fruit the following season. Nevertheless, the oils extracted from fruit at this stage have an excessive bitterness, which decreases with fruit ripening. For this reason, along with the fact that the oil yield obtained by physical extraction significantly increases with the level of maturity, harvesting in Spain is traditionally carried out when the fruit reaches a black skin color.

To develop a postharvest treatment, which when applied to green mature olives would cause acceleration in fruit ripening in order to control oil bitterness and improve oil yield, would be beneficial to the olive oil industry. In this sense, postharvest heat treatment applied to olive drupes before oil extraction can reduce the level of bitterness of the subsequently extracted oils without significantly affecting the physico-chemical parameters established for the evaluation of oil quality [7, 8]. However, an inadequate use of these treatments can cause an emulsion of the oil during the extraction process, producing a drastic loss in oil

yield, or can induce off-flavor development in the VOO extracted.

The fact that ethylene causes skin degreening in stored table olives is a well known fact [9]. Furthermore, this gas induces a softening of the fruit [10, 11]. However, the effect of the addition of ethylene to the olive storage atmosphere on the characteristics of the oil subsequently extracted has not yet been reported. To state whether ethylene may regulate the activity of the olive enzymes responsible for transferring bitter compounds to the oil during the extraction process would be valuable to the olive oil industry [12].

In the present work, the use of ethylene in closed containers during mill olive storage has been compared to heat treatment in the control of the bitterness intensity of the oils subsequently extracted.

Experimental Procedures

Olive fruit (*Olea europaea* L. cv. 'Lechín') was harvested during the 2005–2006 season in Osuna (Seville) at its green mature stage of ripening. Forty-two kg of healthy fruit were transported to the Instituto de la Grasa, where they were randomly divided into 21 groups of 2 kg each. Each group was put in a 3.5-L jar. The jars were then divided into seven treatment groups of three jars each. The first group was immediately processed to evaluate the initial values of the oil quality parameters. The jars from the second and third groups were left open at 20 and 40 °C, respectively; the jars from the fourth and fifth groups were hermetically closed and kept at 20 and 40 °C, respectively; and, finally, the jars from the sixth and seventh groups were hermetically closed, and gas ethylene was injected into each of them until reaching a concentration of 30 ppm in the internal atmosphere. Then they were also stored at 20 and 40 °C. The jars of these six different treatments were stored for a period of 72 h.

Prior to the distribution of the treatments, three batches of 100 healthy olives were taken from the original sample for the assessment of the initial values of skin color and fruit firmness, and, subsequently, the same number of fruit was randomly taken from each treatment after the storage period for monitoring the final values of these parameters. The color of these olives was determined on the equatorial zone, using a Minolta CR200 (Minolta Camera Co. Osaka, Japan) Chroma-Meter with a measuring area of 8 mm in diameter, diffuse illumination and a viewing angle of 0°. The CIE $L^*a^*b^*$ color notation system was applied to determine the parameters L^*, a^* and b^*; where L^* indicates lightness, a^* refers to the color axis from green to red and b^* from blue to yellow. By means of these parameters, a color index (CI) was calculated according to the formula: $CI = L^*(b^* - a^*)/100$. This equation has been used to monitor the changes in skin color during olive cold storage [13].

Fruit firmness was also evaluated on the equatorial zone of the same olives, using a Zwick 3300 hand densimeter (Zwick GmbH & Co. Ulm, Germany). The consistency of the fruit was measured without rupture applying the pressure of a 5-mm diameter disk [14].

After the storage period, gas samples (150 μL) were withdrawn from each jar's headspace using gas-tight syringes and analyzed by gas chromatography according to Pérez et al. [15]. CO_2 and O_2 contents were measured using a gas chromatograph Hewlett–Packard 5890 (Hewlett–Packard, Santa Clara, USA) equipped with a thermal conductivity detector, on a steel Carbosieve S-II (Supelco Inc. Bellefonte, USA) (3 m × 3 mm i.d.) column with helium as carrier gas. CO_2 was analyzed at 225 °C and O_2 at 33 °C. Ethylene was analyzed with the same apparatus using an activated alumina column (1 m × 3 mm i.d.) and a FID.

The olives from each jar were independently milled. A sample of 800 g paste was taken from each replicate and weighed in a metallic pitcher. The paste of each pitcher was homogenized using a spatula and the oil was extracted, using an Abencor analyzer (Comercial Abengoa S.A., Seville, Spain). This unit, consisting of three basic elements: a mill, a thermobeater, and a pulp centrifuge, simulates the industrial process of virgin olive oil production on a laboratory scale [16]. After centrifugation the oil was decanted into a graduated tube to measure the volume obtained in order to calculate the oil yield, which was expressed as a percentage of the fresh weight. Subsequently, the extracted oil was filtered and stored at −20 °C under N_2 atmosphere until analysis.

The titratable acidity, the peroxide index and the coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) were determined from the extracted oils according to the European Union standard methods (Annexes II and IX in European Community Regulation EEC/2568/91). For the panel test, the overall sensory quality of each oil sample was graded by a panel of eight trained (≥5 years experience) tasters according to a scale of nine points, "1" being the value for the poorest quality possible and "9" for the best. Bitterness intensity was determined by the same panel of tasters using a structured scale of five points: where "0" means the absence of bitterness; "1" simple perception of bitterness; "2" light presence of bitterness; "3" middle presence of bitterness; "4" strong intensity of bitterness; and "5" the highest possible intensity of bitterness. Oxidative stability was measured by the Rancimat method, which evaluates the time (h) of resistance to oxidation of 3 g oil samples exposed to a stream of dry air at a temperature of 100 °C [17]. 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 [18]. Phenolic fraction was isolated by solid phase extraction and analyzed by reversed phase HPLC using a diode array UV detector [19]. Quantification of the aldehydic form of the oleuropein aglycone (3,4-DHPEA-EA), the secoiridoid derivative most related to oil bitterness [20], was carried out at 280 nm using *p*-hydroxyphenylacetic acid as internal standard. The results were expressed in millimol per kilogram.

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 among the mean values, when ANOVA detected a significant ($P \leq 0.05$) effect due to treatment.

Results and Discussion

Modified Atmospheres

In the atmosphere of the hermetically closed jars, the concentrations of CO_2 and O_2 increased and decreased, respectively, due to fruit respiration, when they were heated to 20 as well as to 40 °C, especially when ethylene was initially added (Table 1). Since the concentrations of O_2 at 20 and at 40 °C were similar, it is possible to conclude that the presence of ethylene stimulated different enzymatic activities aside from respiration which were responsible for CO_2 liberation, because the increase in CO_2 found in the storage atmospheres supplemented with ethylene did not presuppose a proportional decrease in the final concentration of O_2. This fact could be explained by the development of fermentative activities witch could be stimulated by the presence of ethylene under a low O_2 and high CO_2 storage atmosphere. In Strawberry MA storage, the atmosphere can affect enzymes such as alcohol dehydrogenase, pyruvate decarboxylase or malate dehydrogenase inducing fruit fermentation [21].

At 20 as well as 40 °C, ethylene production was detected in the stored fruit, appreciable (0.1 ppm) in the ones left in open jars and considerable in the ones kept in the hermetically closed jars at 20 or 40 °C (3.0 and 2.0 ppm, respectively) (Table 1). In the containers to which 30 ppm of ethylene were added, this concentration remained unchanged at 20 °C and was reduced approximately by half when the storage temperature was 40 °C. This significant reduction demonstrates that the fruit exposed to this temperature metabolizes ethylene more efficiently. In any case, the presence of ethylene in a passive modified atmosphere induced a higher CO_2 production with lower oxygen consumption at both temperatures.

On the other hand, the addition of ethylene to the modified atmosphere accelerated the loss of the green skin color of the fruit, mainly at 40 °C, behaving in a similar way as it does in citrus degreening (Table 1). However it did not seem to exert any effect on fruit firmness (Table 1). In contrast, the use of 40 °C for 72 h induced a remarkable loss of texture in the olives, regardless of the storage atmosphere used.

Oil Bitterness

At 20 °C, the use of a passive MA induced a slight, non significant decrease in 3,4-DHPEA-EA content, that was translated into an equally small reduction in the presence of the bitter attribute in the oil (Table 2). Nevertheless, the addition of 30 ppm ethylene to this MA, induced a significant reduction in this attribute, as well as the presence of

Table 1 Concentrations of CO_2, O_2 and C_2H_4 found in the storage atmosphere of 'Lechín' olives maintained for 72 h at different temperatures and atmospheres and the effect of these storage conditions on skin color and firmness of the fruit

Temperature	Atmosphere	CO_2 (%)[a]	O_2 (%)[a]	C_2H_4 (ppm)[a]	Color index[b] (L(b − a)/100)	Firmness[b] (N/cm^2)
Initial value		0.0 ± 0.0c	20.9 ± 0.2a	0.0 ± 0.0d	31.0 ± 2.5a	45.3 ± 2.5a
20 °C	Air	0.0 ± 0.0c	20.9 ± 0.3a	0.1 ± 0.0d	30.1 ± 2.6a	44.9 ± 2.8a
	Modified	18.5 ± 1.2b	4.8 ± 0.8b	3.0 ± 0.3c	31.0 ± 3.0a	45.0 ± 2.3a
	Modified + ethylene[c]	32.0 ± 2.5a	4.7 ± 0.9b	31.9 ± 3.2a	24.8 ± 2.4b	44.9 ± 2.9a
40 °C	Air	0.0 ± 0.0c	21.0 ± 0.2a	0.1 ± 0.0d	29.5 ± 3.1a	40.6 ± 2.2b
	Modified	20.2 ± 1.7b	2.4 ± 0.7c	2.0 ± 0.4c	29.6 ± 3.2a	41.5 ± 2.7b
	Modified + ethylene[c]	29.8 ± 3.1a	5.9 ± 1.2b	12.9 ± 2.0b	7.3 ± 3.1c	39.4 ± 2.9b

In each column, values followed by the same lower-case letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

[a] Each point of this parameter is the mean value ± SD of three replicates

[b] Each point of this parameter is the mean value of 300 determinations

[c] 30 ppm

Table 2 Contents of 3,4-DHPEA-EA and bitterness level of the oils extracted from 'Lechín' olives previously stored at different temperatures for 72 h in different atmospheres

Temperature	Atmosphere	3,4-DHPEA-EA (mmol/kg)	Bitterness intensity[a]
Initial value		1.12 ± 0.05a	4.1 ± 0.3a
20 °C	Air	1.10 ± 0.04a	4.0 ± 0.4a
	Modified	0.95 ± 0.07a	3.8 ± 0.3a
	Modified + ethylene (30 ppm)	0.62 ± 0.14b	2.8 ± 0.4b
40 °C	Air	0.16 ± 0.02c	1.1 ± 0.1c
	Modified	0.15 ± 0.02c	1.0 ± 0.1c
	Modified + ethylene (30 ppm)	0.11 ± 0.01c	1.0 ± 0.0c

In each column, values followed by the same lower-case letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Each point is the mean value ± SD of three replicates

[a] Sensory evaluated in a structured scale, where 0 means no intensity and 5 means the highest intensity possible

3,4-DHPEA-EA in the oil extracted. Consequently, the increase in CO_2 and the decrease in O_2 in the storage atmosphere of the olive fruit had little influence on the control of bitterness in the oil later extracted. However, the addition of ethylene produced an acceptable level of this attribute. The increase in fruit storage temperature up to 40 °C induced a spectacular decrease in the bitterness level of the oils subsequently extracted, regardless of the atmosphere used and was therefore the most effective treatment, without requiring the aid of other more expensive systems.

Oil Quality

Neither the titratable acidity nor the ultraviolet absorbance of the oil extracted from the stored fruit underwent an appreciable alteration due to the treatments (Table 3). However, the addition of ethylene induced a significant increase in the peroxide value of the oils, when the olives were kept at 40 °C for 72 h (Table 3). Overall, the mean value exhibited by this treatment (8.15 mequiv O/kg) is considerably lower than the established limit for obtaining the best commercial quality category for virgin olive oils (20 mequiv O/kg). The use of passive MA for fruit storage induced the development of an unusual flavor in the oil extracted, which was accentuated with the increase in temperature and ethylene addition (Table 3). It resulted in a significant reduction in its sensory quality (panel test). In the past, Gutierrez et al. [22] detected a similar deterioration of this parameter in oils coming from olives that had been stored for a month at 5 °C under controlled atmospheres using 3% CO_2 and/or 5% O_2.

The use of MA induced a reduction in the photosynthetic pigment contents (chlorophylls and carotenes) of the oil (Table 4). Nevertheless, the addition of ethylene reduced this effect at 20 °C or induced an enormous increase in the concentrations of these compounds at 40 °C. Ethylene may induce an increase in fruit membrane permeability which has been associated with qualitative changes in its fatty acyl composition, resulting in increased saturation, as a first step in the acceleration of the ripening process [23, 24]. Thus, chloroplast membranes of ethylene treated olives may be modified accordingly, making these structures more sensitive to milling, to allow for a better pigment release and, in consequence, to obtain a higher presence of them in the extracted oils. Simultaneous

Table 3 Parameters of oil quality shown by oils extracted from 'Lechin' olives stored at different temperatures and atmospheres

Temperature	Atmosphere	Acidity (% oleic)	K_{232}	K_{270}	Peroxide value (mequiv O/kg)	Panel test[a]
Initial value		0.25 ± 0.03	1.70 ± 0.03	0.13 ± 0.03	6.02 ± 0.23b	7.7 ± 0.3a
20 °C	Air	0.22 ± 0.04	1.70 ± 0.03	0.12 ± 0.03	5.91 ± 0.31b	7.8 ± 0.3a
	Modified	0.20 ± 0.08	1.72 ± 0.03	0.13 ± 0.03	5.95 ± 0.36b	6.5 ± 0.2b
	Modified + ethylene (30 ppm)	0.25 ± 0.06	1.69 ± 0.03	0.13 ± 0.03	6.00 ± 0.41b	6.1 ± 0.2c
40 °C	Air	0.22 ± 0.05	1.64 ± 0.03	0.10 ± 0.03	6.22 ± 0.50b	7.8 ± 0.3a
	Modified	0.21 ± 0.07	1.63 ± 0.03	0.11 ± 0.03	6.10 ± 0.39b	5.8 ± 0.2c
	Modified + ethylene (30 ppm)	0.25 ± 0.03	1.60 ± 0.03	0.12 ± 0.03	8.15 ± 0.43a	5.2 ± 0.3d

Each point is the mean value ± SD of three replicates. In each column, values followed by the same lower-case letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Absence of lower-case letters in a column means no significant effect due to storage treatments on the tested variable ($P \leq 0.05$) according to ANOVA

[a] Panel test is evaluated according to a structured scale of nine points, "1" being the value for the poorest quality possible and "9" for the best

Table 4 Pigment contents and stability against oxidation of oils extracted from 'Lechin' olives stored at different temperatures and atmospheres

Temperature	Atmosphere	Carotenes[a] (mg/kg)	Chlorophylls[a] (mg/kg)	Stability[a] (h)
Initial value		34.5 ± 2.9b	42.5 ± 4.3b	59.8 ± 2.3a
20 °C	Air	35.1 ± 3.3b	42.6 ± 6.3b	58.9 ± 2.7a
	Modified	17.3 ± 2.6d	19.3 ± 3.3d	46.8 ± 3.3b
	Modified + ethylene (30 ppm)	26.0 ± 2.7c	30.1 ± 5.5c	38.7 ± 3.5c
40 °C	Air	24.9 ± 2.5c	19.9 ± 4.3d	24.8 ± 4.3c
	Modified	16.6 ± 3.3d	15.9 ± 5.6d	25.7 ± 4.1c
	Modified + ethylene (30 ppm)	117.9 ± 12.7a	244.5 ± 22.3a	26.3 ± 5.3c

In each column, values followed by the same lower-case letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

[a] Each point is the mean value ± SD of three replicates

storage at 40 °C could accelerate this process in the same way as chilling temperatures [25].

The use of MA at 20 °C in the olive storage caused a significant reduction in the oil stability against oxidation (Table 4). This effect was intensified by the addition of ethylene. However, the increase in the storage temperature to 40 °C was responsible for the highest deterioration of this parameter, independently of the atmosphere used. The effect observed in oil stability perfectly coincided with the reduction obtained in the levels of bitterness and 3,4-DHPEA-EA in the oils as a consequence of the storage conditions used, supporting the hypothesis that this secoiridoid derivative is strongly related to both parameters [22, 26].

In summary, heat treatment of olive fruit for 72 h at 40 °C in an open air atmosphere induced a significant reduction in oil bitterness, while maintaining the "extra" commercial category. Although this treatment also induced a significant reduction in pigment content and oil stability, this fact did not affect to the parameters legally established to measure olive oil quality. Most likely, storing the olive fruit in these moderately high temperatures impairs or slows down the development of the enzymatic activities responsible for the production of the compounds which cause bitterness in the oil. The use of MA atmospheres induced off-flavor development in the oil extracted, which was intensified by the ethylene addition. However, the use of ethylene requires more extensive study. It would be interesting to verify whether or not the ethylene treatments applied to the olives stored in an open air atmosphere would cause the development of negative sensory attributes in the oil subsequently extracted.

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