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Effect of Bruising on Respiration, Superficial Color, and Phenolic Changes in Fresh Manzanilla Olives (*Olea europaea pomiformis*): Development of Treatments To Mitigate Browning

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ABSTRACT: The aim of the work was to study the postharvest changes in Manzanilla olives and to find treatments to mitigate damages because of bruises. The phenolic content in bruised and unbruised fruits exposed to air always decreased, but the loss in phenols and the respiratory activity were greater in bruised olives; these changes were related to the appearance of brown spots. Immersion of the picked fruits in a cold (8 °C) acidic solution (pH 3), ascorbic acid solution (100 mM), or sodium metabisulfite solution (100 mM) significantly reduced the loss in phenols in olives and led to lighter brown bruised areas. This immersion did not affect the behavior of the fruits during the lye treatment and the subsequent fermentation. In the final product, no influence on the surface color of unbruised olives was observed and there was a significant color improvement in the bruised areas of damaged olives.

KEYWORDS: Bruise, harvest, Manzanilla cultivar, olive, phenol, respiration

INTRODUCTION

Traditionally, the harvesting of olives is performed by hand, using a technique known as "milking" the tree. The cost of this operation accounts for 50−70% of the final price of the fruits.[1,2] Currently, mechanical harvesting, using big machines that shake the olive tree or smaller machines that move the branches of the tree, is carried out only on cultivars with low sensitivity to bruising.[2,3]

The Manzanilla is the most common cultivar for processing Spanish-style green table olives but cannot be picked using mechanical harvesting. In fact, their fruits are very prone to the formation of brown spots because of the blows that the olives receive during the operation; these damages usually remain even after the complete fermentation process. As a result, the quality of the final product obtained from mechanically harvested olives is poor and unmarketable because of its unpleasant appearance.

The mechanism of the browning reaction in olives has been demonstrated using "*in vitro*" models.[4] First, there is an enzymatic release of hydroxytyrosol from oleuropein and hydroxytyrosol glucoside because of the action of the β-glucosidase enzymes present in the olive fruit.[5] Simultaneously, an additional hydroxytyrosol release can also be produced because the chemical hydrolysis of oleuropein.[6,7] In a second step, hydroxytyrosol and verbascoside are oxidized by polyphenoloxidase (PPO) from the fruits themselves. The whole process leads to browning. A chemical oxidation of hydroxytyrosol may also occur at the same time.[8] A scheme of the browning reaction mechanism has been presented by Segovia-Bravo et al.[4]

This mechanism of the browning reaction deduced from model solutions was in agreement with results obtained in bruised olives because the compound that decreased in the highest proportion during the postharvest period was oleuropein.[4]

A previous study showed that the maximum activity of PPO on Manzanilla olives was at pH 6.0. This activity was completely inhibited at pH below 3.0, regardless of the temperature; however, in alkaline conditions, the inhibition of the enzymatic reaction was observed at pH values above 9.0 and 11.0 for temperatures of 8 and 25 °C, respectively.[9]

The use of an acidic medium (pH 3) or an ascorbic acid solution (100 mM) prevented the oxidation of the phenolic compounds in model solutions even in the presence of aeration because no changes in their colors were observed.[10]

Ascorbic acid and sodium metabisulfite have shown a beneficial effect to mitigate the phenolic browning reaction in other products, such as artichokes,[11] mushrooms,[12] litchi fruits,[13] and pears.[14]

With respect to olive respiration, it has been demonstrated that its rate declined rapidly during the postharvest period.[15] However, the effect of the blows on the respiratory activity of fruits remains unknown.

The aim of this work was to study the effect that mechanical harvesting produced bruises have on the respiration and phenolic changes in olives as well as on the surface color of the bruised areas. Furthermore, treatments that could, potentially, prevent the oxidation of phenols and browning in the bruised areas of olives were also developed, particularly the effect of immersion in acidified (pH 3) and antioxidant (ascorbic acid and sodium metabisulfite) solutions.

MATERIALS AND METHODS

Fruits. The olives used in this study were from the Manzanilla cultivar (*Olea europaea pomiformis*). Fruits were harvested by hand in Coria del Rio, Sevilla, Spain, during the 2007 and 2008 seasons. Only fruits with the optimal green−yellow surface color (green maturation) were chosen for the experiment to work with homogeneous material.

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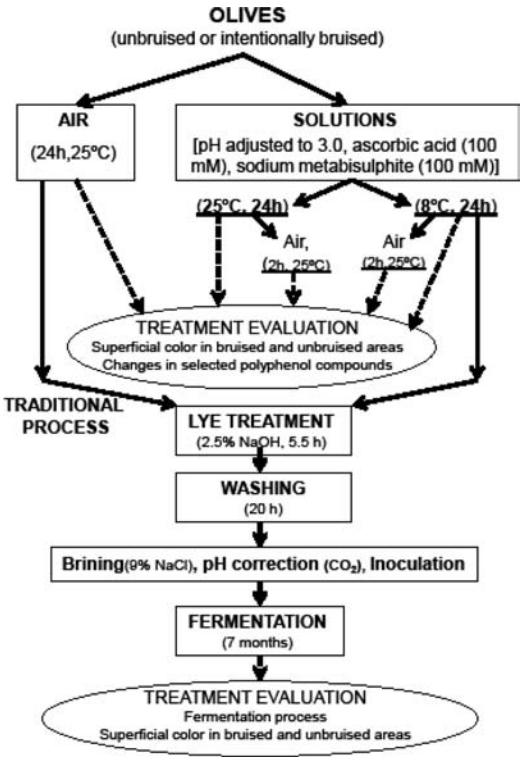

Figure 1. Scheme of different treatments applied to prevent browning in harvested olives.

The experiment was performed with fruits harvested in mid-September. The time elapsed from hand harvesting to the beginning of the experiments to prevent olive browning ranged from 1 to 2 h.

To reproduce the bruises caused by mechanical harvesting a pilot-plant scale device was applied.[9] It consisted of a sorting machine with a wooden block of 30 × 20 × 10 cm and a weight of 2.5 kg, which was maintained at a prefixed distance above a moving belt. The block was covered on its bottom surface with a piece of metallic mesh formed by 0.6 cm squares of 0.6 mm diameter wire, fixed to the surface, so that there was continuous contact between the surface of the wooden block and the mesh. The fruits were passed along the space left between the "floating" wooden block and the belt of the sorting machine. The distance between the sorting machine belt and the wooden block was regulated according to the olive size. The fruits were swept by the movement of the sorting machine belt. The procedure was checked to always produce homogenously distributed bruises on the olives and to cause similar damages to those observed in mechanical harvesting.

To simulate the conditions prevailing in farms during harvesting, bruised olives were kept in the air at room temperature (25 ± 1 °C) for 15 min before immersing them in the solutions.

Respiration Activity Measurement (Olives Exposed to Air). The experiences were carried out with a Micro-Oxymax O_2/CO_2 respirometer (Columbus Instruments, Columbus, OH), which measured the O_2 consumed and the CO_2 produced by the olives. A total of 10 bruised or not bruised olives were placed inside the glass jars (0.25 L) and kept at 8 or 25 °C in a thermostatic chamber (Selecta, Barcelona, Spain). Experiments were run in triplicate, and O_2 and CO_2 were monitored every 1.5 h. After each measurement, the air in the jars was replaced with fresh, dry air. Thus, the respiration of the olives was tested in what can be considered an open system.

Measurement of CO_2 Released by Olives Kept in Water. A total of 10 previously weighed olives were put in an A314 jar (Juvasa, Dos Hermanas, Spain). The jars were filled to the top with boiled, distilled water to eliminate any CO_2 in the solution and then conditioned to the desired temperature (8 or 25 °C). The jars were closed, avoiding the presence of any air bubbles in their interior, and the volume of water was recorded. The jars were kept at 8 or 25 °C, and after 24 h, the dissolved CO_2 in the water was analyzed.

Experiences To Prevent Olive Browning. A total of 2 kg of olives (unbruised or intentionally bruised) was placed in polyvinyl chloride (PVC) cylindrical vessels and kept for 24 h in contact with air at ambient temperature (25 °C) (traditional system);[1] this treatment was used as the control. The treatments to study the prevention of browning were carried out in vessels containing similar fruits but covered with the following cold (8 °C) or ambient temperature (25 °C) water solutions (1.5 L): acidic solution at pH 3 adjusted with HCl (2 N), ascorbic acid solution (100 mM), and sodium metabisulfite solution (100 mM). The containers were kept in the laboratory (25 °C) or in a cold room at 8 °C for 24 h. A scheme of the experiences is shown in Figure 1.

To simulate the industrial work conditions, a portion of the olives kept for 24 h in the above-mentioned solutions were exposed to air for 2 h, to mimic the air exposure during the handling previous to lye treatment and the possible changes in their phenolic composition studied in representative samples (about 200 g).

After the olives were maintained in cold solutions for 24 h or exposed to air for 24 h, they were put in a 2.5% (w/v) NaOH solution until the chemical reached $^2/_3$ of the distance from the skin to the pit (5.5 h).[1] Then, the olives were washed with tap water for 20 h and, finally, covered with a 9% (w/v) NaCl solution. After 48 h of brining, CO_2 was bubbled for 15 min through all treatments to control the pH to 5.5−6.0. After 1 day, the containers were inoculated with a starter culture of *Lactobacillus pentosus* IGLAC01 (previously isolated from table olives) and left for 7 months at ambient temperature (18−25 °C) to develop the characteristics of the fermentation process.[1]

All treatments were carried out in duplicate.

Analysis. The concentration of dissolved oxygen (mg of O_2/L) in the solutions was determined using a Jenway 9200 DO_2 meter (Barloworld Scientific, Ltd., Essex, U.K.).

The dissolved CO_2 in water was analyzed by the microdiffusion method adapted for olive brines.[16] A total of 10 mL of liquid was injected into a phosphate acid solution inside a jar closed with a "twist-off" cap, which contained a vial with NaOH. After incubation a 37 °C for 24 h, the alkaline solution was titrated. The results were expressed in milligrams of CO_2 per gram of olive in 24 h.

To know the concentration of different phenols in the olives, 15 fruits were put in a flask (A314) into which liquid nitrogen was added until the fruits were frozen. Liquid nitrogen was added once again, and the jar was closed to maintain an inert atmosphere. The flasks were put in a freezer at −30 °C (±4 °C) until analysis.

The phenolic extracts from the frozen fruits were obtained following the procedure described by Romero et al.[17] Phenolic compound extraction was achieved by a solution of methanol/water plus 100 ppm of sodium salt of diethyldithiocarbamic acid. A C_{18} cartridge was used to purify the phenolic extract. To quantify different phenols, syringic acid was added as an internal standard.

Phenolic compounds were determined by high-performance liquid chromatography (HPLC).[17] A Waters 2690 Alliance equipped with a Waters 996 photodiode array detector and controlled by Millenium32 software (Waters, Inc., Milford, MA) was employed using a Lichrospher 100 RP-18 column (250 × 4.6 mm, 5 μm particle size, Merck, Darmstard, Germany), and the following settings were used: elution solvent, solution of phosphoric acid (1.5 mL/L) at 1.0 mL/min flow rate, and a column oven set at 35 °C. Chromatograms were recorded at 280 nm. Retention times and UV spectra were used to identify the phenolic compounds by a comparison to commercial standards or pure compounds obtained by preparative HPLC, as shown by Romero et al.[17] Phenol determinations were performed in duplicate.

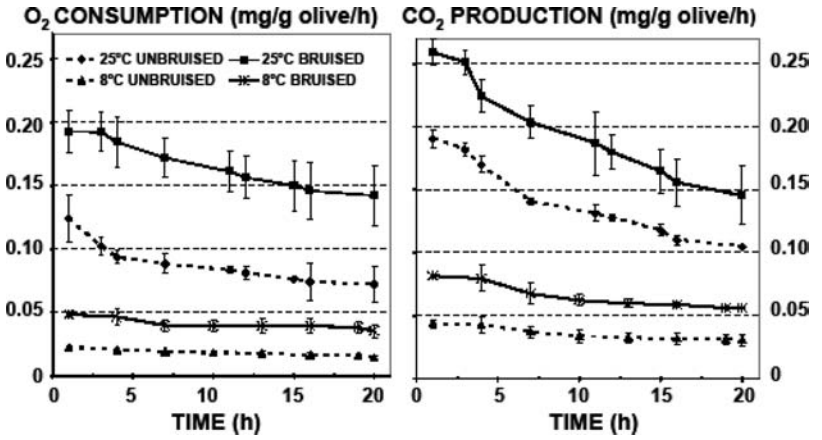

Figure 2. Effect of storage temperatures on the respiration rate of unbruised and intentionally bruised green olives from Manzanilla cv. The experiments started 1 h after harvesting, and the fruits were intentionally bruised immediately before starting the experiment. Each point is the average of three measurements. When error bars are not visible, determinations were within the range of the symbols on the graph.

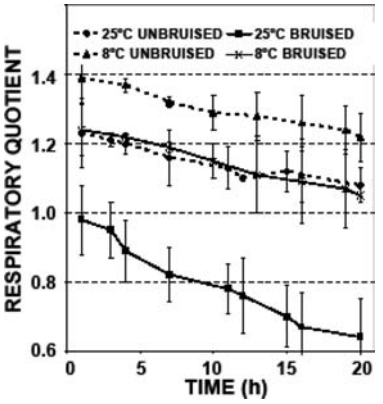

Figure 3. Effect of storage temperatures on the respiratory quotient of unbruised and intentionally bruised green olives from Manzanilla cv. The experiments started 1 h after harvesting, and the fruits were intentionally bruised immediately before starting the experiment. Each point is the average of three measurements. When error bars are not visible, determinations were within the range of the symbols on the graph.

The surface color of the fruits was measured using a BYK-Gadner model 9000 color view spectrophotometer (Silver Spring, MD). Interference by stray light was minimized by covering samples with a box, which had a matt black interior. Measurements were carried out using the illuminant "*C*" at 10°. Color was expressed in terms of the CIE L^* (whiteness or brightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness). From these values, chroma $[C = (a^{*2} + b^{*2})^{1/2}]$ and hue angle $[H = \tan^{-1}(b^*/a^*)]$ were also calculated. In the evaluation of *H*, the widely accepted international criterion of assigning the angle of 90° to the semi-axis + b^* (yellowness) was followed.

Also, in fermented fruits, the color was expressed as the color index (*Ci*), calculated according to Sánchez et al.[18]

$$Ci = \frac{-2R_{560} + R_{590} + 4R_{635}}{3}$$

where *R* stands for the reflectance at 560, 590, and 635 nm, respectively.

Data were the average of determinations in 10 olives, measuring the appropriate parameters on the bruised areas (bruised olives) or on any zone of unblemished fruits (unbruised olives).

Table 1. Production of CO_2 [mg (g of Olive)$^{-1}$ Day^{-1}] by Olives Maintained in Open Air and in Water at 25 and 8 °C[a]

	25 °C		8 °C	
	unbruised	bruised	unbruised	bruised
air	3.55 (0.04) b	4.73 (0.15) a	0.83 (0.04) d	1.54 (0.01) c
water	1.74 (0.12) b	2.16 (0.24) a	0.39 (0.01) d	0.45 (0.03) c

[a] Values were obtained with a respirometer. Standard deviation is given in parentheses. Strings values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

Statistical Analysis. Statistica software, version 6.0,[19] was used for data analysis. A comparison between treatments was carried out by Duncan's multiple range tests, and differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Respiration in Open Air during the Postharvest. The respiratory activity (RA) of olives in the open air, followed by monitoring the O_2 consumption and CO_2 production, depended upon time, temperature, and fruit type (Figure 2). RA always decreased with time and was lower at 8 °C than at 25 °C; in fact, the rate of O_2 consumption and CO_2 production was reduced to one-fourth (Figure 2), regardless of the type of fruit. For the same temperature, the RA was greater in bruised olives (higher O_2 consumption and CO_2 production) than in unbruised olives (Figure 2). This behavior has also been reported for European plums[20] and other vegetables.[21]

The respiratory quotient (RQ, CO_2 production/O_2 consumption, in moles) was superior to 1 at harvest, except for bruised fruits at 25 °C (Figure 3). It always declined, in parallel with RA, as the studied period became longer. Apparently, the initial value could be related to the CO_2 produced before picking and its subsequent release from the olive flesh during the first hours in closed vials.[22]

The RQ, for the same temperature, was lower in bruised olives than in unbruised olives. This behavior means that, for the same proportion of CO_2 released, the consumption of O_2 was higher. Part of this additional oxygen might have been used for

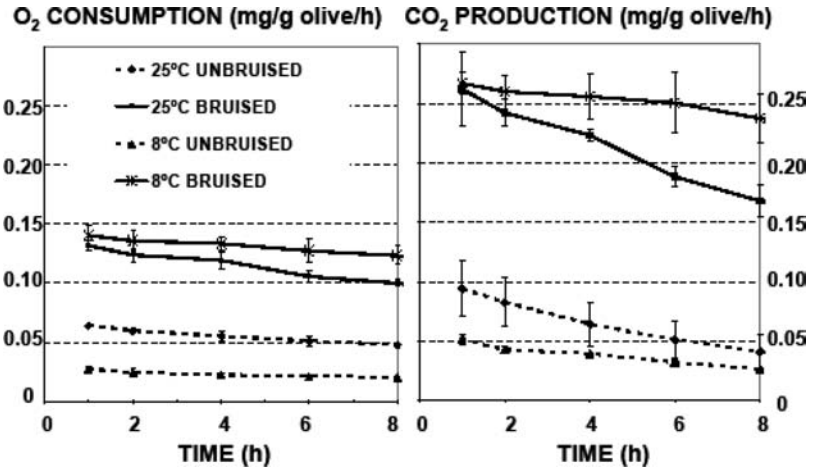

Figure 4. Effect of the previous storage of bruised and unbruised Manzanilla cv. fruits immersed for 24 h in water at 25 and 8 °C on the respiration of re-exposed to air olives. The experiments started 1 h after harvesting, and the fruits were intentionally bruised immediately before starting the experiment. Each point is the average of three measurements. When error bars are not visible, determinations were within the range of the symbols on the graph.

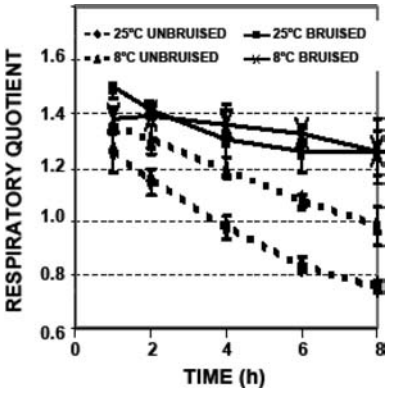

Figure 5. Effect of the previous storage of bruised and unbruised Manzanilla cv. fruits immersed for 24 h in water at 25 and 8 °C on the respiratory quotient of re-exposed to air fruits. The experiments started 1 h after harvesting, and the fruits were intentionally bruised immediately before starting the experiment. Each point is the average of three measurements. When error bars are not visible, determinations were within the range of the symbols on the graph.

producing the browning reactions (phenol oxidation), as demonstrated in "*in vitro*" experiments.[10]

In general, the RQs were higher at 8 °C than at 25 °C. In cold conditions, less oxygen was consumed because the low temperature reduces the metabolism of the fruits, the browning enzymatic reaction rate,[9,11] and CO_2 released from the fruits.[22]

CO_2 Released from Olives Held in Water. When the Manzanilla olives were immersed in water, the CO_2 released came from the gas accumulated in the fruits before being subjected to immersion in the liquid and from the current respiration of the submerged fruits, using the residual oxygen remaining in their interior atmosphere (Table 1). The values obtained at 25 °C were similar to those reported for Hojiblanca cv. unbruised fruits.[23] However, the proportion of CO_2 in the olives immersed in water was lower than the level observed when the olives were kept in air because of the absence of oxygen in the surrounding liquid.

In water, for the same temperature, a greater proportion (statically significant, $p < 0.05$) of CO_2 was released from bruised olives than from unbruised olives (Table 1), just as it occurred when the fruits were kept in the open air (Figure 2).

Respiration in Open Air of the Fruits Previously Kept in Water. When the liquid of the fruits kept in water for 24 h was removed and the olives were exposed again to open air at room temperature (8 h at 25 °C), the respiration process continued (Figure 4). In this case, the behavior observed was similar to that described in the olives kept in the open air during postharvesting (Figure 2); the bruised fruits showed a higher O_2 consumption and CO_2 release.

Initially, when the olives were re-exposed to air, RQ values were always similar (Figure 5). This must be due to the release of CO_2 trapped in the interior atmosphere of the olives, which could not be liberated while the fruits were immersed in water; however, once the olives were placed in the open air, the CO_2 was released more easily and there was always a decrease in RQ as time progressed (Figure 5).

In short, apparently, the respiratory process and gaseous exchanges in/from olives were reactivated when fruits previously kept in water were exposed to open air, even in the case of fruits subjected to low temperatures. As a result of this behavior, to prevent browning problems, the time of exposure of the olives to air, after removing the water solutions, should be as short as possible, even if they were kept in cold solutions.

Changes in Surface Color during the Postharvest. The damaged areas of intentionally bruised fruits, which followed the traditional process of exposure to open air, showed, 24 h after harvesting, the worst ($p < 0.05$) color parameter values (Table 2). This was due to the loss in luminance (L^*), the increase in a^* (which moved toward the red region), and the decrease in b^* values (which moved toward the less yellow region) with respect to values of freshly harvested fruit or unbruised olives after 24 h at air; in this case, the final chroma value and hue angle were the smallest in all treatments ($p < 0.05$). These changes were strongly related to the appearance of a brown tonality on the bruised surface of olives.

The immersion of the bruised fruits in acidic, ascorbic acid, or sodium metabisulfite solutions at ambient temperature (25 °C) or at 8 °C partially prevented browning in the bruised areas, which showed CIE L^*, a^*, and b^* values more similar to those observed for unbruised olives than to the bruised areas of olives

Table 2. Changes in the Surface Color (Expressed as CIE L^*, a^*, and b^* Parameters, Chroma, and Hue) of Unbruised Olives and in the Bruised Areas of Intentionally Bruised Fruits Exposed to Air (25 °C) or Immersed in Different Solutions (at 8 or 25 °C)[a]

	L^*	a^*	b^*	chroma	hue
unbruised					
initial	60.6 (0.6) a	−12.0 (0.4) a	40.9 (0.4) a	42.6 (0.5) a	106.1 (0.4) a
air (25 °C) (after 24 h)	56.3 (0.2) b	−9.59 (0.1) b	37.6 (1.1) bc	38.8 (1.0) b	104.3 (0.6) b
bruised (after 24 h)					
air (25 °C)	42.0 (1.1) e	−1.8 (0.5) f	22.9 (1.1) e	23.0 (1.2) e	94.4 (0.9) f
acidic solution (25 °C)	48.8 (0.2) d	−4.9 (0.3) e	35.8 (0.7) cd	35.8 (0.8) d	97.5 (0.3) e
acidic solution (8 °C)	49.9 (0.2) d	−5.9 (0.1) dc	37.7 (0.9) bc	38.2 (0.9) bc	98.8 (0.1) e
ascorbic acid (25 °C)	51.6 (0.3) c	−5.6 (0.5) de	35.8 (0.8) cd	36.2 (0.7) cd	98.9 (1.0) e
ascorbic acid (8 °C)	51.5 (0.6) c	−7.4 (0.5) c	38.3 (1.1) b	39.0 (1.0) b	100.9 (1.1) cd
metabisulfite (25 °C)	52.0 (0.2) c	−6.56 (0.8) cd	38.4 (1.1) b	38.9 (1.3) b	99.7 (0.8) de
metabisulfite (8 °C)	51.3 (0.2) cd	−8.8 (0.1) b	40.6 (0.1) a	41.5 (0.3) a	102.2 (1.1) c

[a] Measurements were made after 24 h of treatment, except at initial time (just picked fruits). Standard deviation is given in parentheses. Column values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

Table 3. Averages of Surface Color (Expressed as CIE a^* and b^* Parameters, Chroma, and Hue) of the Intentionally Bruised Area of Olives Immersed in Diverse Solutions According Treatment Temperature[a]

temperature (°C)	a^*	b^*	chroma	hue
25	−5.7 (0.3) b	36.5 (0.7) b	37.0 (1.0) b	98.8 (0.4) b
8	−7.3 (0.6) a	38.6 (0.6) a	39.6 (0.7) a	100.6 (0.7) a

[a] Measurements were made after 24 h of treatment. Standard deviation is given in parentheses. Column values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

Table 4. Averages (Regardless of the Temperature) of Surface Color (Expressed as CIE a^* and b^* Parameters, Chroma, and Hue) of the Intentionally Bruised Area of Olives Immersed in Diverse Solutions for 24 h[a]

type of solution	a^*	b^*	chroma	hue
acidic (pH 3)	−5.4 (0.3) c	36.6 (0.7) b	37.0 (0.7) b	98.3 (0.3) b
ascorbic acid	−6.5 (0.6) b	37.0 (0.8) b	37.6 (0.9) b	99.9 (0.7) a
metabisulfite	−7.6 (0.7) a	39.5 (0.7) a	41.0 (0.8) a	100.9 (0.8) a

[a] Standard deviation is given in parentheses. Column values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

Table 5. Averages of Luminance (L^*) of the Intentionally Bruised Area of Olives Immersed in Diverse Solutions for 24 h at 8 and 25 °C[a]

	temperature	
type of solution	25 °C	8 °C
acidic (pH 3)	48.8 (0.2) d	49.9 (0.2) cd
ascorbic acid	51.57 (0.3) ab	50.52 (0.6) bc
metabisulfite	51.28 (1.0) ab	52.0 (0.2) a

[a] Standard deviation is given in parentheses. Values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

that followed the traditional procedure (exposition to air) (Table 2). This behavior was in agreement with the good evaluation of the surface color (bruised areas) of submerged fruits.

The effects of treatments and temperatures were small and difficult to establish directly from the data in Table 2. The appropriate analysis of variance showed that, for CIE a^* and b^* parameters, chroma, and hue angle, the effects of the type of solution used and the temperature were significant ($p < 0.05$). However, for luminance (L^*), the effects of the type of solution and the interaction of the type of solution versus temperature were significant ($p < 0.05$).

Table 3 shows, according to the temperature at which the fruits were maintained, the averages of CIE a^* and b^* parameters, chroma, and hue angle. Better ($p < 0.05$) color evaluations (greener, yellowier, and greater values of chroma and hue angle) were always found in olives kept at 8 °C than in those maintained at ambient temperature (25 °C). Furthermore, in cold solutions, the values of these parameters (Table 3) were very similar to those initially obtained from freshly picked unbruised fruits (Table 2).

The statistical analysis of the data showed that the fruits immersed in a sodium metabisulfite solution retained the greenest tonality (lowest a^* value), the yellowest tonality (highest b^*), and subsequently, the highest chroma and hue angle (Table 4); on the contrary, the use of acidic solution led to the worst ($p < 0.05$) CIE a^* and b^* values. Immersion in ascorbic acid led to intermediate CIE a^* and b^* values.

These results can be related to the initial (before being added to olives) concentration of oxygen in the three solutions. The greatest concentration corresponded to the acidic solution (8.2 mg/L), followed by that with ascorbic acid (5.2 mg/L) because of its scavenger action.[24,25] However, the presence of a sodium metabisulfite solution produced the total disappearance of the initially dissolved oxygen.

According to these results, the use of an acidic solution should be the least effective in preventing the oxidation of the bruised areas, while the immersion in a metabisulfite solution would be the best, showing the use of ascorbic acid in an intermediate position.

In the case of luminance (L^*), the interpretation of the statistical analysis must consider the interaction between the type of solution versus temperature. Table 5 shows that, for each type of solution, the L^* value was statistically the same ($p < 0.05$)

Table 6. Concentrations of Phenols (mmol/kg) in the Olive Flesh of (i) Fresh Fruits (Initial), (ii) Unbruised Fruits after 24 h in Open Air (25 °C), (iii) Unbruised Fruits after 24 h of Immersion in an Acidic Solution at 8 °C, and (iv) Fruits from Treatment iii Re-exposed to Open Air at 25 °C for 2 h[a]

		after 24 h		
	fresh fruit	air (25 °C)	acidic solution (8 °C)	acidic solution (8 °C) (after +2 h on air, 25 °C)
hydroxytyrosol glucoside	36.22 (2.2) ab	28.81 (5.88) ab	37.46 (4.90) a	27.63 (0.60) b
hydroxytyrosol	14.72 (0.70) ab	14.21 (2.32) a	17.08 (0.96) a	13.63 (0.74) b
oleuropein	124.93 (6.66) a	71.23 (0.68) c	90.07 (2.85) b	78.47 (1.24) c
hydroxytyrosol compounds	175.87 (8.28) a	114.25 (3.20) c	144.61 (8.74) b	119.73 (2.31) c
verbascoside	0.11 (0.01) a	0.08 (0.01) b	0.10 (0.01) a	0.08 (0.01) b
tyrosol glucoside	1.14 (0.05) a	1.21 (0.20) a	0.97 (0.14) a	1.01 (0.05) a
tyrosol	2.85 (0.35) a	2.36 (0.09) a	2.73 (0.23) a	2.60 (0.76) a
total phenols	179.94 (8.08) a	119.98 (2.28) c	149.42 (8.36) b	123.42 (2.76) c

[a] Standard deviation is given in parentheses. Strings values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

at the two temperatures studied but the lowest values corresponded to olives immersed in the acidic solution, while the highest levels were related to fruits immersed in the sodium metabisulfite solution ($p < 0.05$).

In summary, the best system for the prevention of browning on the bruised areas of olives during the postharvest period should be immersion in a cold solution (8 °C) of sodium metabisulfite (100 mM). The use of an ascorbic acid solution (100 mM) or an acidic solution at pH 3 may also mitigate the appearance of brown spots but with lower efficiency.

Polyphenol Content Changes in Unbruised Olives during the Postharvest. The total phenol contents in unbruised fruits decreased during the postharvest period in open air at 25 °C, because of the loss of hydroxytyrosol compounds (sum of substances with a hydroxytyrosol nucleus); within these, oleuropein suffered the most remarkable decrease (Table 6). A decrease in the concentration of verbascoside was also observed ($p < 0.05$). Changes in the phenolic content were in agreement with the results obtained in the study of the browning reaction mechanism in olives.[4]

Similar reactions to those mentioned above can be produced during the time elapsed from harvesting to olive oil extraction and may be responsible for the deterioration of oil as the malaxation time increases.[26] In fact, the oxidative stability in the oils (Rancimat test) with large periods of malaxation was reduced because of the lower concentrations of phenols.[27]

Olives immersed in a cold acidic solution (8 °C) had lower losses in phenols than fruits maintained in the open air (25 °C). Apparently, in this case, the decrease in the total polyphenol content was primarily due to the loss in oleuropein as in olive extract solutions.[4]

When fruits kept in a cold acidic (pH 3) solution were exposed to air at room temperature, an additional loss of phenols occurred and their concentrations reached the same statistical values in only 2 h as olives exposed to air for 24 h.

In any case, there were no statistical differences ($p < 0.05$) in the concentrations of tyrosol glucoside and tyrosol during the postharvest period, regardless of the system used (Table 6). This agrees with the results obtained with model solutions, in which no changes in the concentrations of these phenols were observed[10] because of the fact that the tyrosol structure is less reactive than the hydroxytyrosol structure (catechol ring with two *ortho*-hydroxyl groups).

According to the above comments, when unbruised olives were maintained in the open air, a loss in total phenolic content was produced during the postharvest period. If the fruits were kept in an acidic cold solution, the direct contact with the air was prevented and the loss in phenols was reduced. However, if the fruits were exposed to the air after this immersion, a fast loss of phenols was produced again.

Polyphenol Content Changes in Bruised Olives during the Postharvest. Bruises produced an important loss in phenolic compounds in olives with respect to their initial values in the fresh fruits. In fact, the total initial concentration of 179.9 mmol/kg in fresh fruits (Table 6) was reduced to 128.8 mmol/kg (Table 7) just after the damage occurred. This oxidation of phenols is apparently responsible for the browning spots on the bruised areas of olives, as in stored artichoke[11] or olive model solutions, in which darkening correlated with the decrease of phenolic compound contents.[4,10]

When the bruised olives were kept in the air at 25 °C for 24 h, the loss in phenols continued to reach the lowest final concentration values (84.8 mmol/kg) (Table 7). This was due to the fact that bruised olives have pH values around 6.0, which is optimum for the PPO activity,[9] and in addition, the contact of the bruised areas of fruits with air (oxygen) allowed for completing the browning reaction with the formation of dark compounds,[4] the subsequent shift of the reaction balance toward the formation of end products, and the decrease of phenols in olives.

The loss in total phenols in the bruised olives was reduced in a significant proportion ($p < 0.05$), in comparison to fruits just maintained in the open air at 25 °C, when the fruits were kept in cold solutions (8 °C), where they reached 117.5 mmol/kg (acidic, pH 3), 132.6 mmol/kg (ascorbic acid), and 112.8 mmol/kg (sodium metabisulfite) (Table 7). Ascorbic acid solutions had a significantly ($p < 0.05$) higher effect on mitigating phenol oxidation than sodium metabisulfite and acidic solutions. The above-described protective effect of acidic solutions for preventing the oxidation of phenols in unbruised fruits for at least 24 h (Table 6) was also noticed in bruised olives and for sodium metabisulfite and ascorbic acid solutions as well (Table 7).

The protective effects continued when fruits kept in the cold liquids for 24 h were re-exposed to air. In fact, after 2 h at ambient temperature (25 °C), the total final concentrations of phenols in such olives (between 113.1 and 127.2 mmol/kg) were

Table 7. Concentration of Phenols in the Olive Flesh (mmol/kg) of Intentionally Bruised Fruits (i) Immediately after Bruising, (ii) after 24 h in Open Air at 25 °C, (iii) after 24 h of Immersion in Different Solutions at 8 °C, or (iv) Treatment iii Plus 2 h of Exposition to Open Air at 25 °C[a]

		after 24 h of bruising						
			solutions at 8 °C					
	immediately after bruising	air (25 °C)	acidic (pH 3)	acidic (pH 3) (+2 h air, at 25 °C)	ascorbic acid	ascorbic acid (+2 h air, at 25 °C)	metabisulfite	metabisulfite (+2 h air, at 25 °C)
hydroxytyrosol glucoside	26.7 (2.2) a	25.7 (2.1) a	20.4 (0.6) c	20.8 (0.9) c	20.2 (0.3) c	25.3 (0.5) a	18.0 (0.2) d	22.1 (0.2) b
hydroxytyrosol	13.1 (2.5) ab	12.7 (2.0) ab	11.9 (0.1) ab	12.0 (0.6) ab	11.8 (0.4) ab	11.0 (0.6) b	13.8 (0.1) a	14.2 (0.3) a
oleuropein	85.4 (3.3) bc	43.3 (3.3) e	81.9 (3.4) bc	76.9 (0.5) cd	96.9 (2.5) a	87.15 (2.05) b	77.4 (1.7) cd	75.4 (2.3) d
hydroxytyrosol compunds	125.2 (2.2) ab	81.7 (3.3) d	114.2 (3.9) c	109.7 (1.9) c	128.9 (1.8) a	123.5 (1.0) b	109.2 (1.7) c	111.8 (2.1) c
verbascoside	0.11 (0.03) a	0.09 (0.04) a	0.10 (0.01) a	0.10 (0.01) a	0.10 (0.02) a	0.08 (0.01) a	0.10 (0.01) a	0.1 (0.01) a
tyrosol glucoside	1.05 (0.18) a	0.95 (0.16) a	1.09 (0.36) a	1.35 (0.06) a	1.36 (0.01) a	1.35 (0.43) a	1.04 (0.15) a	1.07 (0.05) a
tyrosol	2.38 (0.58) a	2.02 (0.60) a	2.08 (0.21) a	1.88 (0.76) a	2.27 (0.14) a	2.31 (0.47) a	2.46 (0.79) a	2.29 (0.26) a
total phenols	128.8 (1.9) b	84.8 (2.6) d	117.5 (3.3) c	113.1 (2.8) c	132.6 (2.0) a	127.2 (0.1) b	112.8 (1.1) c	115.2 (1.7) c

[a] The fruits were from the same batch as the experiments with unbruised olives (Table 6). Standard deviation is given in parentheses. Strings values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

Table 8. Color of Bruised and Unbruised Surface Areas of Olives (Expressed as CIE L^*, a^*, and b^* Parameters, Chroma, and Hue) and Color Index (Ci) after 7 Months in Brine, According to the Treatment Received during Postharvesting (24 h of Exposition to Open Air at 25 °C and Immersion in Different Solutions at 8 °C)[a]

previous treatment	color index (Ci)	L^*	a^*	b^*	chroma	hue
unbruised						
air (25 °C)	28.8 (0.4) a	54.7 (0.9) a	3.0 (0.1) a	40.5 (1.0) ab	40.6 (1.0) ab	85.8 (0.3) a
acidic solution (pH 3, 8 °C)	28.9 (1.3) a	54.5 (0.8) a	4.1 (0.1) b	40.6 (0.9) ab	40.8 (0.9) ab	84.3 (0.1) b
ascorbic acid solution (8 °C)	30.1 (0.5) a	55.8 (0.3) a	3.9 (0.2) b	40.1 (1.4) b	40.3 (1.4) b	84.4 (0.1) b
metabisulfite solution (8 °C)	29.0 (1.3) a	55.0 (0.2) a	3.3 (0.3) a	42.3 (0.7) a	42.5 (0.7) a	85.6 (0.3) a
bruised						
air (25 °C)	21.9 (0.5) d	46.9 (0.7) c	7.4 (0.3) e	30.3 (0.9) d	31.2 (0.9) d	76.3 (0.4) f
acidic solution (pH 3, 8 °C)	25.6 (0.6) b	49.4 (0.1) b	5.8 (0.4) d	35.0 (1.1) c	35.5 (1.1) c	80.6 (0.6) e
ascorbic acid solution (8 °C)	24.9 (1.1) b	50.0 (1.3) b	5.6 (0.1) d	36.7 (0.7) c	37.1 (0.6) c	81.4 (0.2) d
metabisulfite solution (8 °C)	23.7 (0.5) c	48.8 (0.6) b	4.8 (0.1) c	36.8 (0.7) c	37.1 (0.7) c	82.6 (0.1) d

[a] Standard deviation is given in parentheses. Column values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple range test.

statistically higher ($p < 0.05$) than in olives maintained in the open air (25 °C) for 24 h (84.4 mmol/kg).

Furthermore, when acidic or metabisulfite solutions were used, the concentration of total phenols in their respective olives, after 2 h in the open air, were statically the same ($p < 0.05$) as at the end of the period in the liquid. The protective effect of ascorbic solutions was very marked and preserved total phenols better than any other solution not only after the 24 h of immersion (in fact, showing the highest content, 132.6 mmol/kg) but also after the additional 2 h of exposure to open air (127.2 mmol/kg), despite the significant ($p < 0.05$) decrease in the last period. In this case, the olives showed the highest concentration.

The protective effect of the immersion in these cold solutions on phenol oxidation also prevented the appearance of browning in bruised areas. This effect could be observed visually and objectively because the values of the CIE L^*, a^*, and b^* parameters (data not shown) were statistically equal ($p < 0.05$) to those found just after removing the solutions (acidic pH 3, ascorbic acid, and sodium metabisulfite) (Table 2).

Effect of Treatments To Prevent Browning on Elaboration Processing and Color of Fermented Olives. No differences in debittering (lye treatment) were observed among olives maintained in any of the tested solutions at 8 °C for 24 h or in those that followed the traditional postharvest process in the open air at 25 °C. In addition, changes in physicochemical characteristics (pH, free acidity, etc.) in the brines of all treatments during fermentation were similar (data not shown). Furthermore, after 7 months in brine, their final physicochemical values were statiscally the same ($p < 0.05$).

At the end of elaboration process, no statistically significant differences ($p < 0.05$) in the color index (Ci) of unbruised areas were observed between olives maintained in the tested solutions with respect to those that followed the traditional procedure (fruits maintained in air at ambient temperature, 25 °C) (Table 8). In addition, only small changes in the CIE a^* and b^* parameters in treated olives were noticed with respect to those obtained following the traditional process, while the values of luminance (L^*) were statistically the same ($p < 0.05$).

When the color evaluation was made on the intentionally bruised areas of fruits, the worst ($p < 0.05$) color index (Ci) and luminance value (L^*) corresponded to those olives that followed the traditional process (21.9 and 46.9, respectively) (Table 8). This was due to the browning process as shown by changes in the other CIE parameters; the values of a^* increased and moved toward a redder region, whereas the b^* values decreased and moved toward a less yellow region. Such modifications also led to the lowest chroma (31.2) and hue angle values (76.3°), which match with a brown shade. These changes could also be detected visually as a brown tonality on the bruised surface of the olives.

The same values were found ($p < 0.05$) for CIE L^* and b^* and chroma in the bruised areas of fermented fruits from olives immersed in solutions at 8 °C during postharvesting, regardless of the type of solution (Table 8). However, there were differences in the CIE a^* parameter; the lowest statistically significant ($p < 0.05$) value (lowest red tonality) was obtained when sodium metabisulfite solution was used (Table 8). This is consistent with the results obtained at the end of the postharvest period (Tables 2−4) because the use of this solution was, in this case, more effective for browning mitigation.

As a result, the immersion of the olives after harvesting in acidic solution (pH 3), ascorbic acid solution (100 mM), or sodium metabisulfite solution (100 mM) at 8 °C was effective to mitigate browning on the bruised areas during postharvesting and led to final products in which the damaged areas were barely distinguishable from the unbruised zones. The best results were obtained when sodium metabisulfite solution (100 mM) was used.

In summary, the phenol content in olives decreased during the postharvest exposure of fruits to open air at 25 °C, mainly because of the loss in oleuropein. The losses were more pronounced in bruised fruits than in unbruised fruits. This correlated with the appearance of brown stains in the bruised areas.

There was greater respiratory activity in bruised olives than in unbruised olives for the same temperature. However, a lower respiration quotient in the bruised olives was observed. This would indicate that more O_2 was consumed for the same CO_2 produced; part of this additional oxygen might have been used in the browning reactions (phenol oxidation), as demonstrated in "*in vitro*" experiments.[10]

The immersion of olives in cold (8 °C) acidic (adjusted at pH 3 with HCl), ascorbic acid (100 mM), or sodium metabisulfite (100 mM) solutions significantly reduced the loss in polyphenols in bruised and unbruised fruits. When fruits immersed in these solutions for 24 h were re-exposed to air, the respiratory activity continued and the oxidation of phenols was reactivated. However, the maintaining of olives in an acidic, ascorbic acid, or sodium metabisulfite medium at 8 °C not only prevented phenol oxidation during the immersion but also when the fruits were re-exposed to air for a period of up to 2 h. The film of liquid that adhered to the olive surface could eventually have been responsible for the prevention of phenol oxidation. Consequently, the browning reactions on bruised areas were also reduced.

In conclusion, the results suggest that the immersion of fruits after harvesting in an acidic medium at pH 3 or in solutions containing ascorbic acid (100 mM) or sodium metabisulfite (100 mM) may prevent the bruised area (because of manual or mechanical harvesting) and browning during the postharvest olive handling. These treatments do not affect the behavior of olives in the subsequent lye and fermentation processes as green olives. The superficial color of unbruised olives was similar to those that followed the traditional process; however, there was a significant improvement in the bruised areas in olives subjected to antioxidant solutions after harvesting but not in the bruised olives, which followed the traditional process.

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REFERENCES

(1) Sánchez, A. H.; García, P.; Rejano, L. Elaboration of table olives. *Grasas Aceites* **2006**, *57*, 86–94.

(2) Ferguson, L. Trends in olive harvesting. *Grasas Aceites* **2006**, *57*, 9–15.

(3) Kouraba, K.; Gil-Ribes, J.; Blanco-Roban, J. L.; de Jaime-Revuelta, M. A.; Barranco-Navero, D. Suitability of olive varieties for mechanical harvester shaking. *Olivae* **2004**, *101*, 39–43.

(4) Segovia-Bravo, K. A.; Jaren-Galan, M.; García-García, P.; Garrido-Fernández, A. Browning reactions in olives: Mechanism and polyphenols involved. *Food Chem.* **2009**, *114*, 1380–1385.

(5) Fernández-Bolaños, J.; Rodríguez, R.; Guillén, R.; Jiménez, A.; Heredia, A. Activity of cell wall-associated enzymes in ripening olive fruits. *Physiol. Plant.* **1995**, *93*, 651–658.

(6) Brenes, M.; García, P.; Durán, M. C.; Garrido, A. Concentration of phenolic compounds change in storage brines of ripe olives. *J. Food Sci.* **1993**, *58*, 347–350.

(7) Brenes, M.; Rejano, L.; García, P.; Sánchez, A. H.; Garrido, A. Biochemical changes in phenolic compounds during Spanish style green olive processing. *J. Agric. Food Chem.* **1995**, *43*, 2702–2706.

(8) Brenes, M.; García, P.; Garrido, A. Phenolic compounds related to the black color formed during the elaboration of ripe olive. *J. Agric. Food Chem.* **1992**, *40*, 1192–1196.

(9) Segovia-Bravo, K. A.; Jaren-Galan, M.; García-García, P.; Garrido-Fernández, A. Characterization of polyphenol oxidase from the Manzanilla cultivar (*Olea europaea pomiformis*) and prevention of browning reactions in bruised olive fruits. *J. Agric. Food Chem.* **2007**, *55*, 6515–6520.

(10) Segovia-Bravo, K. A.; Jaren-Galan, M.; García-García, P.; Garrido-Fernández, A. Treatment to inhibit the browning reaction in model solutions of olive fruit extracts. *Food Chem.* **2010**, *123*, 741–746.

(11) Lattanzio, V.; Linsata, V.; Palmieri., S.; Van Sumere, C. The benefical effect of citric and ascorbic acid on the phenolic browning reaction in stored artichoke (*Cynara scolmymus* L.) heads. *Food Chem.* **1989**, *33*, 93–106.

(12) Golan-Goldhirsh, A.; Whitaker, J. R. Effect of ascorbic acid, sodium bisulfite, and thiol compounds on mushroom polyphenol oxidase. *J. Agric. Food Chem.* **1984**, *32*, 1003–1008.

(13) Jiang, Y.; Fu, J.; Zauberman, G.; Fuchs, Y. Purification of polyphenol oxidase and the browning control of litchi fruit by glutathione and citric acid. *J. Sci. Food Agric.* **1999**, *79*, 950–954.

(14) Arias, E.; González, J.; Oria, R.; López-Buesa, P. Ascorbic acid and 4-hexylresorcinol effects on pear PPO and PPO catalyzed browning reaction. *J Food Sci.* **2007**, *72*, 422–429.

(15) García, P.; Brenes, M.; Romero, C.; Garrido, A. Respiration and physicochemical changes of harvested olive fruits. *J. Hortic. Sci.* **1995**, *70*, 925–933.

(16) García, P.; Durán, M. C.; Garrido, A. Modificaciones del proceso de fermentación de aceitunas negras al natural en salmuera para evitar alteraciones. *Grasas Aceites* **1982**, *33*, 9–17.

(17) Romero, C.; García, P.; Brenes, M.; Garrido, A. Hydroxytyrosol 4-β-D-glucoside, an important phenolic compound in olive fruits and derived products. *J. Agric. Food Chem.* **2002**, *50*, 3835–3839.

(18) Sánchez, A. H.; Rejano, L.; Montaño, A. Determinación del color en las aceitunas verdes aderezadas de la variedad Manzanilla. *Grasas Aceites* **1985**, *36*, 258–261.

(19) StatSoft. *Statistica for Windows (Computer Program Manual)*; StatSoft: Tulsa, OK, 2001.

(20) Lippert, F.; Blanke, M. M. Effect of mechanical harvest and timing of 1-MCP application on respiration and fruit quality of European plums *Prunus domestica* L. *Postharvest Biol. Technol.* **2004**, *34*, 305–311.

(21) Fonseca, S. C.; Oliveira, F. A. R.; Brecht, J. K. Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: A review. *J. Food Eng.* **2002**, *52*, 99–119.

(22) Kadder, A. A. Respiration gas exchange of vegetables. In *Postharvest Physiology of Vegetables*; Weichman, J., Ed.; Marcel Decker: New York, 1987; pp 25−43.

(23) Romero, C.; Brenes, M.; García, P.; Garrido, A. Respiration of olives stored in sterile water. *J. Hortic. Sci.* **1996**, *71*, 739–745.

(24) Zerdin, K.; Rooney, M. L.; Vermue, J. The vitamin C content of orange juice packed in an oxygen scavenger material. *Food Chem.* **2003**, *82*, 387–395.

(25) Robertson, G. L.; Samaniego, C. M. L. Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. *J. Food Sci.* **1986**, *51*, 184–187.

(26) García, J. M.; Yousfi, K. The postharvest of mill olives. *Grasas Aceites* **2006**, *57*, 16–24.

(27) García, J. M.; Gutiérrez, F.; Castellano, J. M.; Perdiguero, S.; Morilla, A.; Albi, M. A. Influence of storage and temperature on fruit ripening and olive oil quality. *J. Agric. Food Chem.* **1996**, *44*, 264–267.