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Browning reactions in olives: Mechanism and polyphenols involved

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ABSTRACT

The mechanism of the browning reaction in olives has been disclosed by following the polyphenol changes in healthy and bruised fruits and using "*in vitro*" models. It was later validated in independent experiments with active and denatured enzymatic extracts, treated or not with ascorbic acid to prevent oxidation. The proposed mechanism would consist of two steps. First, there is an enzymatic release of hydroxytyrosol, due to the action of the fruits' β-glucosidases and esterases on oleuropein and hydroxytyrosol glucoside; additional hydroxytyrosol can also be produced (in a markedly lower proportion) by the chemical hydrolysis of oleuropein. In a second phase, hydroxytyrosol and verbascoside are oxidized by the fruits' polyphenoloxidase (mainly) and by a chemical reaction, which occurs to a limited extent due to the olive flesh pH, ≈5.0.

1. Introduction

Olives of the Manzanilla cultivar are very prone to the formation of brown spots due to the blows that the fruits receive during harvesting (Kouraba, Gil-Ribes, Blanco-Roban, de Jaime-Revuelta, & Barranco-Navero, 2004). Injuries to the skin of the olive remain, even after the fermentation process.

The phenol content in olives changes with maturation (Ryan, Robards, & Lavee, 1999). When the fruits of the Manzanilla cultivar are harvested with the proper green-yellow tonality for green Spanish-style processing, the main phenolic compounds in the flesh are oleuropein (a heterosidic ester of elenolic acid and hydroxytyrosol), hydroxytyrosol 4-β-D-glucoside (4-β-D-glucosyl-3-hydroxyphenylethanol), hydroxytyrosol (3,4-dihydroxyphenylethanol) and other minor compounds, such as tyrosol, tyrosol glucoside, rutin and verbascoside (Romero, Brenes, García, & Garrido, 2002; Ucella, 2001).

Both esterase and β-glucosidase are naturally present in the olive flesh (Briante et al., 2002; Fernández-Bolaños, Rodríguez, Guillén, Jiménez, & Heredia, 1995). Esterases hydrolyze (Fig. 1) the ester bonds of oleuropein (1), producing hydroxytyrosol (2) and glucosyl derivate (3) (Capozzi, Piperno, & Uccella, 2000). B-Glucosidases break the liaison of glucose with the aglycon (4) which, in turn, may also react with esterases, releasing hydroxytyrosol (2) and elenolic acid (5) (Briante, Patumi, Febbraio, & Nucci, 2004; Mazzei, Giorno, Mazzuza, Spadafora, & Drioli, 2006). Both pathways produce hydroxytyrosol, glucose and elenolic acid as final compounds. Furthermore, oleuropein may suffer acidic or alkaline chemical hydrolysis (Brenes & de Castro, 1998; Brenes, García, Durán, & Garrido, 1993; Gikas, Papadopoulos, & Tsarbopoulos, 2006). In any case, hydroxytyrosol is always a final product from oleuropein hydrolysis.

The browning reaction, which results from mechanical injury during post-harvest storage or processing of fruits and vegetables, is a widespread phenomenon. Polyphenol oxidase (PPO) is the main enzyme that causes browning. PPO catalyzes the oxidation of *o*-dihydroxyphenols to *o*-quinones. The quinones then condense to form dark pigments (Martinez & Whitaker, 1995).

In olives of the Manzanilla cv., the PPO enzyme was recently characterized, using 4-methylcatechol as phenolic substrate (Segovia-Bravo, Jaren-Galan, García-García, & Garrido-Fernández, 2007.

The purpose of this work was to reveal the mechanisms of the browning reaction produced on the surface of the fresh Manzanilla olive cultivar due to bruises caused during hand or mechanical harvesting. The role played by the different phenols in the browning reaction and the involvement of the enzymes present in the olive flesh have also been studied.

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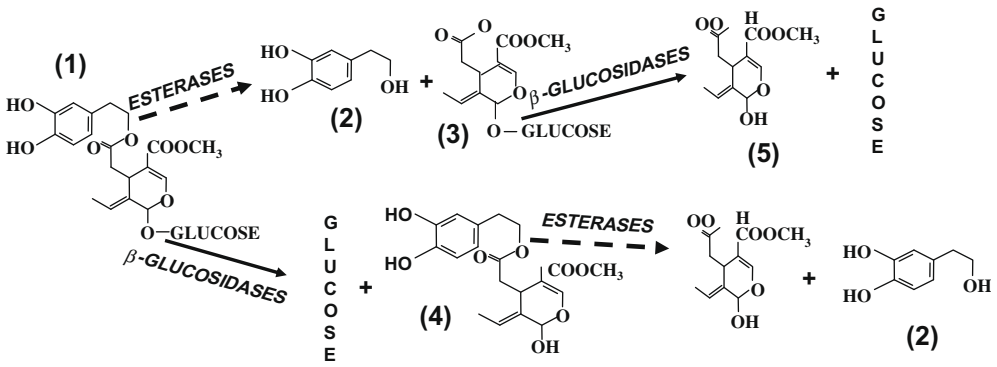

Fig. 1. Scheme of enzymatic degradation of oleuropein and products formed according to the reaction type: (1) oleuropein, (2) hydroxytyrosol, (3) glucosyl derivate, (4) aglycone, and (5) elenolic acid.

2. Materials and methods

2.1. Fruits

The olives were from the Manzanilla cultivar (*Olea europaea pomiformis*). Fruits were harvested in mid-September, by hand, from an orchard located in Coria del Rio, Sevilla (Spain) during the 2006 and 2007 seasons. Only fruits with the optimal green-yellow surface colour (green maturation) (Sánchez, García, & Rejano, 2006) were chosen for the experiment, in order to work with homogeneous material. The time elapsed from harvesting to the beginning of the trials ranged from 1 to 2 h.

To reproduce the bruises caused by mechanical harvesting, a pilot plant scale device was developed (Segovia-Bravo et al., 2007). It consisted of a sorting machine with a wooden block of $30 \times 20 \times 10$ cm and a weight of 2.5 kg which was maintained at a fixed 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, so that there was continuous contact between the surface of the wooden block and the mesh. The distance between the sorting machine belt and the wooden block was regulated according to the olive size. The fruits were passed along the space left between the "floating" wooden block and the belt. The procedure was checked to produce always homogeneously distributed bruises on the olives and to cause similar injuries to those observed in mechanical harvesting.

To study the changes in phenols in the olive flesh during the post-harvest period, fruits (bruised and unbruised) were left uncovered at room temperature (25 °C) for 24 h.

2.2. Extraction and purification of crude enzymes

A protein precipitate (acetone powder) of the fruit was prepared from 50 g of triturated unbruised pitted olives. The fruit paste was extracted with 100 ml of acetone containing 2.5 g of polyethylene glycol at −30 °C and the residue was re-extracted three times with 100 ml of acetone at −30 °C and, finally, the pellet obtained was dried and weighed; 0.5 g of this acetone powder were re-suspended in 25 ml of a 50 mM phosphate buffer, pH 6.2 (extraction buffer), containing 1 M KCl. The suspension was stirred at 4 °C for 30 min, and then centrifuged at 20,000 *g* for 20 min at 4 °C. The pellet was discarded and the supernatant divided into two aliquots, one was used as the active enzymatic extract (Hornero-Méndez, Gallardo-Guerrero, Jarén-Galán, & Minguéz-Mosquera, 2002; Sciancalepore & Longone, 1984), and the other was used to obtain a denatured enzymatic extract by boiling this supernatant aliquot for 20 min. The enzymatic extracts were stored at 4 °C and 2 h; before their use, they were stored in room temperature (25 °C).

The designation of the polyphenoloxidase enzyme has changed several times, the latest being E.C. 1.14.18.1 according to Sciancalepore and de Stefano (1995).

2.3. Extraction and purification of phenolic extract

The phenolic extract of olives was obtained by following the procedure of Romero et al. (2002). The method consisted of extracting the phenolic compounds with a solution of methanol/water plus 100 mg/l of the sodium salt of diethyldithiocarbamic acid. A C_{18} cartridge was used to purify the phenolic extract. To quantify the phenols, syringic acid was added as internal standard. The phenolic extracts were frozen at −30 °C and 2 h before their use they were brought to room temperature (25 °C).

2.4. Reaction buffer

The reaction buffer was prepared from a mixture of phosphate and 0.2 M borate each, adjusted with NaOH to pH 5.0 (Segovia-Bravo et al., 2007) and stored at room temperature (25 °C). A variant of this reaction buffer solution was prepared, adding ascorbic acid, 2 h before its use, at a final concentration of 100 mM.

2.5. Measurement of the enzymatic activity

During the enzymatic reaction, diverse coloured compounds are formed. The overall enzymatic activity can be evaluated by measuring the increase in absorbance during the reaction time at a previously established wavelength, usually 410 nm.

The enzymatic reaction took place in the spectrophotometric cuvette at room temperature (25 °C) after adding 0.25 ml of phenolic extract solution and 2.5 ml of reaction buffer. The reaction was initiated by adding 0.25 ml of crude enzyme extract solution. Absorbance at 410 nm was recorded with 0.5 min interval for 10 min in a thermostatted spectrophotometric cuvette placed in a Cary1E Spectrophotometer (Varian, Mulgrave, Vi, Australia).

The reaction time was established after confirming that the measurement of the reaction fulfilled the conditions for an enzymatic reaction, namely linearity over the time that the experiment lasted, appropriate increase in absorbance and a measurable total absorbance increment at the end of the reaction. All the experiments were performed in duplicate.

2.6. Monitoring of the reaction between olive phenolic compounds and olive enzyme extracts

During the enzymatic reaction, which leads to the coloured compound formation, oxygen and the phenolic compounds, initially present in the reaction medium, are consumed. To reveal

their evolution, 2.5 ml of enzymatic extract were added to 25 ml of reaction buffer; then the reaction was started by adding 2.5 ml of phenolic extract. The reaction conditions, concentrations of enzyme and phenolics and temperature were similar to those used for measuring the enzymatic activity. All the experiments were performed in duplicate.

Oxygen uptake in the solution was recorded periodically during the 10 min of reaction using a Jenway 9200 DO_2 meter (Barloworld Scientific Ltd, Essex, UK).

Changes in phenols during the reaction were monitored by periodically transferring them to an Eppendorf tube, with 0.1 ml of phosphoric acid and 1 ml of the reacting solution. Phenolic compounds were determined by HPLC (Romero et al., 2002). Reacting and phenolic extract solutions of olives were filtered through a 0.45 μm nylon filter. The system consisted of a Waters 2690 Alliance (which included a pump, column, heater, and auto sampler modules), equipped with a Waters 996 Photodiode Array Detector and controlled by Millenium32 software (Waters Inc., Milford, MA, USA). A 25 cm × 46 mm i.d. 5 μm Lichrospher 100 RP-18 (Merck, Darmstard, Germany) column, phosphoric acid solution (1.5 ml/l) at 1 ml/min flow rate and a temperature of 35 °C were used. Chromatograms were recorded at 280 nm. Phenol determination was performed in duplicate.

3. Results and discussion

3.1. Phenol changes in the olive flesh

Changes in the concentration of polyphenols in olives during the following 24 h after harvesting have been studied. They differed according to the chemical structure and condition of the fruits. Tyrosol glucoside and tyrosol levels in the flesh of bruised and unbruised fruits did not undergo significant changes during this period of time (Table 1). However, oleuropein and verbascoside decreased and reached their lowest concentrations in the bruised fruits. There was also a drop in hydroxytyrosol glucoside concentration in bruised and unbruised olives (Table 1), which indicated a parallel breaking of the sugar–hydroxytyrosol union regardless of the condition of the olive's health.

Some of these compounds can be grouped. Hydroxytyrosol, hydroxytyrosol glucoside and oleuropein are phenols that have a hydroxytyrosol nucleus in their structure, whose sum is usually defined as "hydroxytyrosol compounds". The final concentration of "hydroxytyrosol compounds" was significantly lower in the damaged than in the healthy fruits (Table 1). Their decrease could be related to an eventual enzymatic activity, which was more intense in stressed fruits because of the leakage of enzymes from the damaged cells (Fernández-Bolaños et al., 1995). Due to the structure of the "hydroxytyrosol compounds", all of them can be used as substrates by PPO enzymes and, consequently, are involved in the browning reaction observed on the skin of the bruised olives (Segovia-Bravo et al., 2007).

As a result, it appears that the browning reaction might be related to the loss of "hydroxytyrosol compounds" and, mainly, to oleuropein, which showed the greatest concentration reduction in fruits during the 24 h, post-harvesting period.

Table 1
Changes in the concentration of phenols in bruised and unbruised fruits during a 24 h period after harvesting. Olives were left to stand at ambient temperature (25 °C).

	Initial	After 24 h	
		Unbruised	Bruised
Hydroxytyrosol glucoside	23.8 (1.06)[a]a[b]	17.3 (0.74)b	17.7 (1.56)b
Oleuropein	115 (3.74)a	84.2 (0.83)b	44.0 (0.10)c
Hydroxytyrosol	22.1 (0.12)b	14.7 (1.27)c	27.6 (0.81)a
Hydroxytyrosol compounds	161 (3.74)a	116 (0.30)b	89.3 (1.37)c
Verbascoside	0.12 (0.01)a	0.11 (0.01)a	0.08 (0.01)b
Tyrosol glucoside	1.49 (0.20)a	1.33 (0.10)a	1.21 (0.18)a
Tyrosol	2.84 (0.41)a	2.23 (0.10)a	2.47 (0.10)a
Total phenols	165 (7.37)a	120 (0.98)b	93.1 (1.43)c

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

3.2. "In vitro" study of the browning mechanism

Despite evidence relating the modifications of phenols in olive fruits to browning, it is difficult to isolate this effect from many other biological reactions that can occur in them simultaneously. So, to study the browning reaction mechanism it was necessary to reproduce the phenomenon with "*in vitro*" models. To achieve this, olive phenol extracts were put into contact with crude enzymatic olive extracts in a solution buffered at the same pH as the olive flesh (5.0). The ratio between enzymes and the concentration of phenols in the reaction medium was 10-fold higher than that usually found in the fruits. Following the changes in the contents of the different phenols, it was possible to discriminate the compounds involved in the reaction, the products formed and their effects on colour (also analysed).

The sum of tyrosol and tyrosol glucoside concentrations remained constant (Fig. 2C) during the reaction time. A certain hydrolysis of tyrosol glucoside (Fig. 2A), with liberation of tyrosol (Fig. 2B) could have occurred but, as the global balance of these compounds was unchanged, it may be deduced that, overall, tyrosol did not undergo any modification and therefore can hardly be related to any reaction.

The concentrations of verbascoside (Fig. 2A), oleuropein (Fig. 2B) and hydroxytyrosol glucoside (Fig. 2B) decreased during the reaction time. The greatest decrease was observed in oleuropein whose concentration was reduced, in approximately one minute, to a level below 40% of its initial content. At the same time, hydroxytyrosol (Fig. 2B) showed an increase, followed by a slow decreasing evolution.

The initial increment in the levels of hydroxytyrosol may be due to the release of hydroxytyrosol from oleuropein and hydroxytyrosol glucoside by a chemical and/or enzymatic reaction. The enzymatic reaction may have been caused by the actions of esterase and β-glucosidase, which produce hydroxytyrosol from oleuropein according to the scheme included in Fig. 1 (Briante et al., 2002, 2004; Capozzi et al., 2000; Mazzei et al., 2006). The presence of these enzymes in green fruits harvested in mid-September has been demonstrated; furthermore, their activities were similar and their combination showed a maximum at this time (Briante et al., 2002). The chemical reaction can be produced by an acidic hydrolysis due to the relatively low acidic pH (≈5.0) in the medium (Brenes, Rejano, García, Sánchez, & Garrido, 1995; Brenes et al., 1993; Gikas et al., 2006).

Declining concentrations of hydroxytyrosol glucoside and tyrosol glucoside can only be due to the action of the β-glucosidases (Capozzi et al., 2000).

Globally, the sum of all phenolic compounds (Fig. 2C) decreased with time while a progressive coloration of the reaction medium was noticed. As previously described in the literature (Martinez & Whitaker, 1995), browning can be related to the consumption of phenols by PPO enzymes which may use them as substrates for the formation of dark polymers. However, not all phenols followed the same trend. As mentioned above, tyrosol compounds did not change. By contrast, "hydroxytyrosol compounds", which constituted the greatest proportion of phenols, decreased progressively during the reaction time; furthermore, the evolution observed for the sum of the concentrations of all phenolic

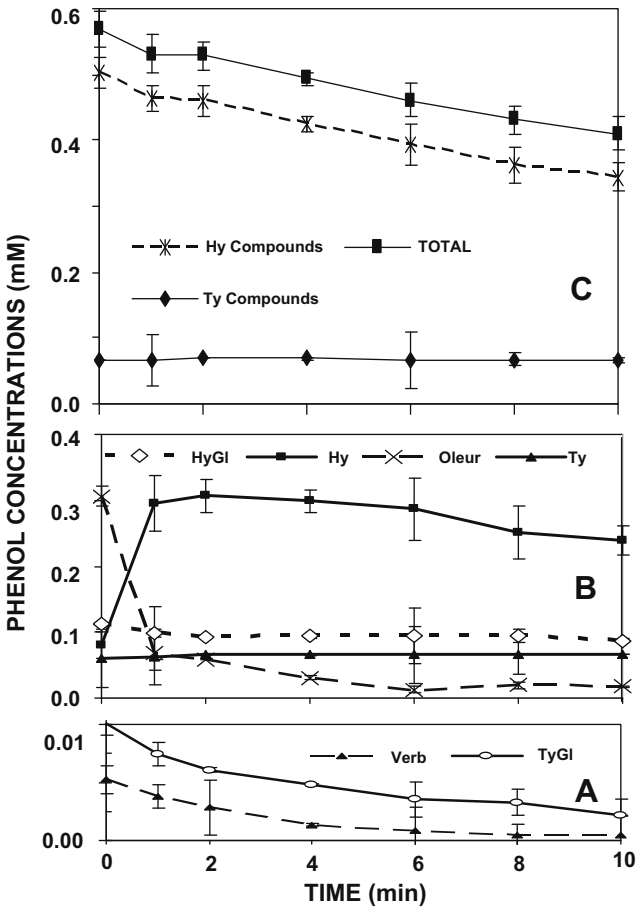

Fig. 2. Changes in the concentration of different phenols during the study of olive browning reaction in a model system. Verbascoside (Verb), tyrosol glucoside (TyGl), tyrosol (Ty), hydroxytyrosol glucoside ((HyGl), hydoxytyrosol (Hy) and oleuropein (Oleur). Tyrosol compounds (Ty compounds) are the sum of Ty + TyGl concentrations; hydroxytyrosol compounds (Hy compounds) are the sum of HyGl + Hy + Oleur concentrations. Values are the means of duplicate experiments. When error bars are not visible, determinations were within the range of the symbols on the graph.

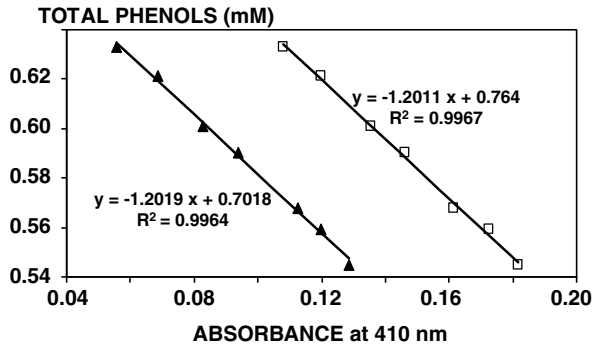

Fig. 3. Relationship between the loss in total phenol concentration and the colour increase in the solution.

compounds was similar to that of the "hydrotyrosol compounds" due to their predominance.

The "hydroxytyrosol compounds" and verbascoside concentration decrease could indicate that they were simultaneously consumed in another reaction, presumably that involving PPOs, as described in the literature (Martinez & Whitaker, 1995).

In parallel to the consumption of "hydroxytyrosol compounds" and verbascoside, the solution became progressively brown (increase in absorbance) and the browning reaction was fairly well correlated with the decrease in the concentration of these compounds (Fig. 3). In two successive experiments, the correlation coefficient (R^2) was >0.996 and the slopes were statistically the same ($p < 0.05$). The different intercept values were due to the diverse initial colorations of the buffered phenolic solutions.

3.3. Validation of the browning reaction mechanism hypothesis with "in vitro" models

The above-observed trend in the polyphenol concentration in the reaction medium (total and specific compounds) and the development of colour in the solutions may indicate that a possible mechanism of the browning reaction in Manzanilla olives would imply the release of hydroxytyrosol from hydroxytyrosol glucoside and oleuropein by β-glycosidase and/or esterase, although, simultaneously, a chemical acidic hydrolysis of oleuropein is possible. So hydroxytyrosol and verbascoside may be oxidized by PPO enzymes, reactions that could lead to the formation of brown polymers. A purely chemical oxidation of hydroxytyrosol has been related to the formation of the characteristic brown-black colour in ripe olives (Brenes, García, & Garrido, 1992).

The validation of the above-outlined hypothesis of the reaction mechanism was accomplished by means of independent experiments in which crude and denatured enzymatic extracts, treated or not with ascorbic acid (100 mM) to prevent the browning reaction, were used (Martinez & Whitaker, 1995; McEvily & Iyengar, 1992; Segovia-Bravo et al., 2007).

When the crude enzymatic extract was put into contact with the phenolic extract, there was a rapid decrease in the oleuropein content (Fig. 4). However, the reaction rate was slower when the denatured enzymatic extract was applied. This would confirm that oleuropein can suffer two types of reactions, enzymatic which takes place at a high rate (Briante et al., 2002, 2004; Capozzi et al., 2000; Mazzei et al., 2006), and a chemical hydrolysis, which produces a slow decline in the concentration of this phenol (Brenes et al., 1993, 1995; Gikas et al., 2006). When using the crude extract, both reactions occur simultaneously so that changes are faster.

The transformation of oleuropein, by enzymatic reaction and chemical hydrolysis, involves an increase in the hydroxytyrosol concentration (Fig. 4). In the first case, β-glycosidase and esterase break the glycoside and ester bonds of oleuropein and release hydroxytyrosol (Briante et al., 2002; Mazzei et al., 2006), which experience a rapid increase. However, in the case of the addition of the denatured enzymatic extract, only the chemical reaction took place, and the rate of hydroxytyrosol formation was markedly slower.

In addition, the further evolution of hydroxytyrosol concentration produced a different behaviour in the reactions with both crude and denatured enzymatic extracts, depending on the absence or addition of ascorbic acid (Fig. 4). The concentrations were lower in the absence of the antioxidant, regardless of the type of extract (Fig. 4). Apparently, under these conditions, there was a gradual oxidation of hydroxytyrosol, a process that was coupled with verbascoside oxidation (data no shown), that led to an overall rapid decrease in the total phenolic concentration (Fig. 4).

When a denatured enzymatic extract was used in the presence of ascorbic acid (100 mM), the total phenolic concentration remained stable over time (Fig. 4). At the same time, there was neither an increase in the coloration of the solution (Fig. 5) nor oxygen consumption (Fig. 6). However, if the denatured enzymatic extract was used without ascorbic acid, there was a slow decrease in the total and "hydroxytyrosol compound" phenols (Fig. 4), slight darkening (Fig. 5) and fairly low oxygen consumption (Fig. 6). This behaviour could be explained by the chemical oxidation of

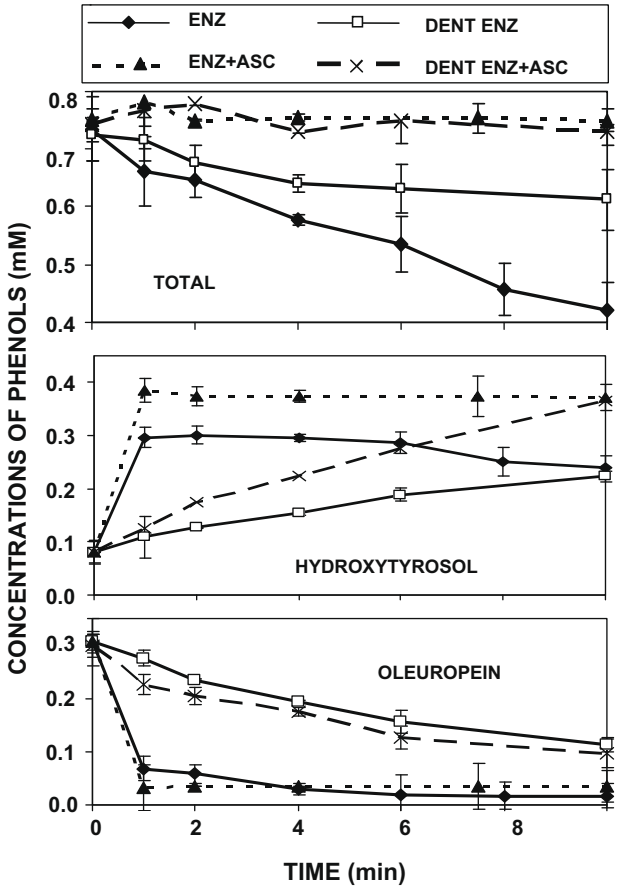

Fig. 4. Evolution of oleuropein, hydroxytyrosol and total phenol concentrations in the experiments with denatured (DENT ENZ) or crude enzymatic (ENZ) extracts treated with ascorbic acid (ASC). Values are the means of duplicate experiments. When error bars are not visible, determinations were within the range of the symbols on the graph.

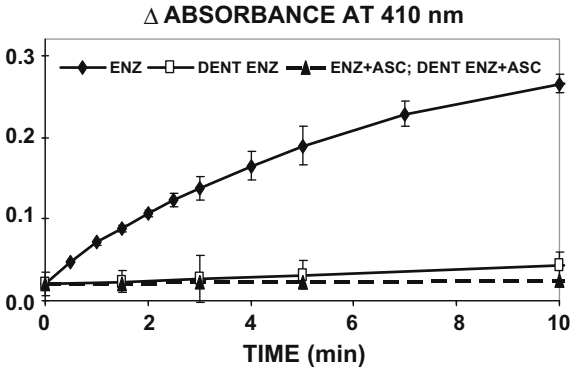

Fig. 5. Changes in the colour of the solutions corresponding to the experiments with denatured (DENT ENZ) or crude (ENZ) enzymatic extracts treated with ascorbic acid (ASC). Values are the means of duplicate experiments. When error bars are not visible, determinations were within the range of the symbols on the graph.

hydroxytyrosol (Brenes et al., 1992) because, in this case, there was no enzymatic activity. So a pure chemical oxidation of phenols, manly hydroxytyrosol and verbascoside, may also contribute to the browning reaction (because, in these extracts, the enzyme was denatured and non-enzymatic oxidation was expected) although it should be relatively limited.

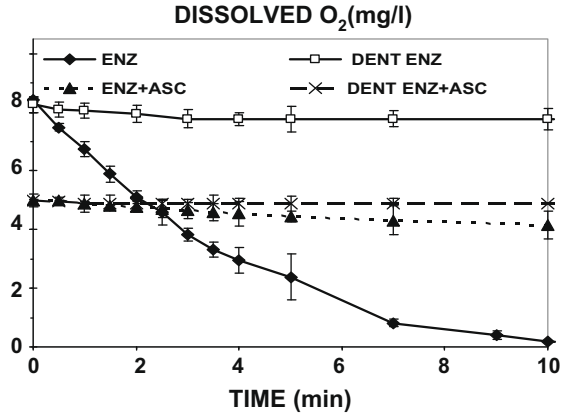

Fig. 6. Changes in the concentration of dissolved oxygen in the solution corresponding to the experiments with denatured or crude enzymatic extracts treated with ascorbic acid. Points are the means of duplicate experiments. When error bars are not visible, determinations were within the range of the symbols on the graph.

In the presence of crude enzymatic extract, the oxidation of hydroxytyrosol and verbascoside, by the action of the PPO enzymes (Martinez & Whitaker, 1995; McEvily & Iyengar, 1992; Segovia-Bravo et al., 2007), was faster (Fig. 4, hydroxytyrosol and total) and led to a progressive darkening of solutions (Fig. 5) and oxygen consumption (Fig. 6). By contrast, the same extract, in the presence of ascorbic acid, led to an increase in the concentration of hydroxytyrosol and total phenols which reached its maximum in one minute and then remained constant. This means that the addition of ascorbic aid prevented the oxidation of the hydroxytyrosol and a change in the total concentration of phenols over time. As result, the solution did not darken (Fig. 5) and the oxygen consumption was minimal (Fig. 6).

3.4. Hypothesis of the browning mechanism in bruised olive fruits

Bearing in mind the results of our experiments, a mechanism for bruised olive fruit darkening after hand or mechanical harvesting can be developed. First, there is an enzymatic release of hydroxytyrosol from oleuropein and hydroxytyrosol glucoside, due to the action of the β-glycosidase and esterase present in the olive fruit. Simultaneously, an additional hydroxytyrosol release can also be produced because of the chemical hydrolysis of oleuropein, although it is slower and more limited than the enzymatic reaction. In a second phase, hydroxytyrosol (initially present plus that released in the enzymatic and chemical reactions), together with verbascoside, is oxidized by the PPOs from the fruits themselves. A chemical oxidation of hydroxytyrosol may also occur simultaneously but its contribution to deterioration, due to the pH value of the olive flesh (around 5 units), is limited when compared to the effect caused by the enzymatic action (Brenes et al., 1992; García, Brenes, Vattan, & Garrido, 1992). In any case, the whole process leads to browning in the damage areas of fruits.

The presence of ascorbic acid did not prevent the reactions that produce the release of hydroxytyrosol from the oleuropein and hydroxytyrosol glucoside and its effect was solely related to the further prevention of the oxidation of hydroxytyrosol and verbascoside.

This hypothesis of the browning reaction mechanism is in agreement with the results in Table 1, because oleuropein is the compound that decreased most when the olives were bruised, and with the results from the "in vitro" experiments, where the decrease in the sum of the concentrations of compounds that contain hydroxytyrosol in the molecule is mainly responsible for the de-

crease in total phenols in olives. In both experimentals, there was also a slight decrease in the concentration of verbascoside.

In fresh healthy olives, the changes in the concentration of phenols were similar to those observed in bruised fruits, but the sum of "hydroxytyrosol compounds" and the total concentration of phenols was higher ($p < 0.05$). This may indicate that the enzymatic process was always active in olive fruits during post-harvest storage but its activity increased under stress (as was observed in bruised fruits) (Fernández-Bolaños et al., 1995).

These reactions can eventually be produced during the time elapsed from harvesting to olive oil extraction and could be responsible for the deterioration of oil as the maceration time increases (García & Yousfi, 2006). In fact, the oxidative induction time in the obtained oils was reduced due to the lower concentrations of phenols (García et al., 1996). This phenomenon can be markedly increased when milling the olives. Strong reactions of hydrolysis and oxidation can be produced during the malaxation of olive pastes in the extraction of olive oil because the phenolic concentration in virgin olive oil progressively decreases as the olive paste time of exposure to air is longer (Clodoveo, Delcuratolo, Gomes, & Colelli, 2007; Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2003a, 2003b).

These findings on the browning reaction of olives, due to hand or mechanical harvesting bruising, can be an essential tool for future studies related to the design of treatments to prevent browning on the surface of bruised fruits from both hand and mechanical harvesting.

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