Inhibition of Polyphenol Oxidases and Peroxidase Activities in Green Table Olives by some Anti-browning Agents

Maryam MOHSENABADI [1]
Mahmood GHASEMNEZHAD [1]
Abuzar HASHEMPOUR [2] (✉)
Reza HASAN SAJEDI [3]

Summary

Almost, all table olive cultivars are susceptible to the formation of brown spots due to mechanical damage during harvesting and processing. Therefore, application of some anti-browning agents might be an effective strategy to minimize unfavourable effects of enzymatic browning in green table olives. The aim of this study was to assess the effect of ascorbic acid (AA), citric acid (CA), oxalic acid (OA), 4-hexylresorcinol (HR) and sodium hexametaphosphate (NaHMP) on reducing enzymatic browning of four green table olive cultivars ('Mari', 'Shengeh', 'Manzanilla' and 'Zard') fruit. The results showed that 'Mari' and 'Shengeh' potentially had the highest browning index. AA could reduce peroxidase (POD) activity just in 'Mari', but NaHMP could beneficially suppress its activity in both cvs. 'Manzanilla' and 'Mari'. In general, the monophenolase activity of polyphenol oxidase (PPO) was significantly higher than its diphenolase activity in all studied cultivars. The highest inhibitory effect on monophenolase activity was found in 'Manzanilla' fruit by CA and OA, while HR and NaHMP could suppress monophenolase activity of 'Mari' fruits. Diphenolase activity of PPO with pyrocatechol and dopamine HCl substrates was also dependent on olive cultivars. The minimum diphenolase activity of PPO for both substrates was found in 'Zard' fruit with the lowest browning index. The inhibitory effect of anti-browning agents on reducing diphenolase activity of PPO with dopamine hydrocholoride (DPOA) substrates was cultivar-dependend. Total phenolic content of treated fruits was higher than control. Overall, this result confirms that the potency of anti-browning agents on suppressing POD and PPOs enzymes that are involved in fruits browning was completely cultivar-dependend.

Key words

anti-browning agents, enzymatic browning, peroxidase, polyphenol oxidase, table olive

[1] Department of horticulture science, Faculty of Agriculture Sciences, University of Guilan, Rasht, Iran
[2] Citrus and Subtropical Fruits Research Center, Iranian Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Ramsar, Iran
✉ e-mail: a.hashempour@areeo.ac.ir
[3] Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Received: February 21, 2017 · Accepted: July 29, 2017

Introduction

Almost, all table olive cultivars are susceptible to the formation of brown spots due to mechanical damage during harvesting (Kouraba et al., 2004). Injuries to the skin of the olive remain, even after the fermentation process. As a result, the final product obtained is of poor quality or, in fact, unmarketable, because of its unpleasant appearance (Segovia-Bravo et al., 2010).

Browning reaction that results from mechanical injury during harvesting or processing of fruits and vegetables is a widespread phenomenon. In this process, oxidative reactions occurred and phenols oxidize to quinones. The main oxidative reactions are enzymatic browning. These oxidation reactions progress in the presence of oxygen and are catalyzed primarily by two oxidoreductases enzymes: polyphenoloxidase (PPO, EC 1.10.3.1) and peroxidase (POD, EC 1.11.1.7). PPO catalyzes two reactions; the first, a hydroxylation of monophenols to diphenols, which is relatively slow and results in colourless products. The second, the oxidation of o-dihydroxyphenols to o-quinones, which is rapid and gives coloured products (Queiroz et al., 2008). The quinones then condense to form dark pigments (Martinez and Whitaker, 1995). The substrates involved in these reactions are located in the vacuoles, while enzymes are in the cytoplasm; the reactions can take place only if they are mixed and in the presence of oxygen (Sommer et al., 1994; Murata et al., 1997). So, all mechanical injuries during harvesting or processing of fruits lead to the starting of browning reactions that result in the final product of poor quality (Toivonen and Brummell, 2008).

Several chemical agents are used to control enzymatic browning. In the past, sulfites have been the most widespread. However, their use has been restricted, because of consumer concerns over their harmful effects. Hence, several anti-browning chemicals such as ascorbic acid (AA) or 4-hexylresorcinol (HR) have shown to be good alternatives to sulfites as anti-browning agents (Pizzocaro et al., 1993; Monsalve-Gonzalez et al., 1995). Previous studies show that AA is the effective agent at preventing enzymatic browning of fruits tissue (Özoglu and Bayiindirli, 2002; Rojas-Grau et al., 2006). Using other anti-browning agents such as the HR in minimal processing of apple fruits was also suggested (Dong et al., 2000; Son et al., 2001). Inhibitory effects of oxalic acid (OA) on browning have been also proven (Son et al., 2000; Son et al., 2001). Citric acid (CA) was introduced as an effective inhibitor of production of unwanted brown pigment in minimally processed fruits and vegetables (Ahvenainen, 1996).

Yoruk and Marshall (2003) reported PPO enzyme activity and browning reaction was reduced to 80-85 percent in litchi fruits, when treated with citric acid (CA). Also, Pilizota and Sapers (2004) reported that using of sodium hexametaphosphate (NaHMP) in 'Granny Smith' apple fruits were successful in anti-browning control, but the results in 'Fuji' apples were contrary.

The PPO enzyme was characterized in olives using 4-methylcatechol as phenolic substrate (Segovia-Bravo et al., 2007). Goupy et al. (1991) described PPO activity related to oleuropein concentration. The browning reaction might be related to the loss of phenolic compounds and, mainly, to oleuropein in olive fruits during the 24 h post-harvesting period (Segovia-Bravo et al., 2009). Segovia-Bravo et al. (2010) found that low pH or ascorbic acid solutions may be useful to prevent browning in the bruised areas of hand- or mechanically-harvested 'Manzanilla' table olives. However, all the previous studies deal only with one or two anti-enzymatic browning agents and deal only with one olive cultivar. In this study, the effect of AA, CA, OA, HR and NaHMP on suppressing enzymatic browning of four olive cultivars was investigated.

Materials and methods

Fruits

Fruits of four olive (*Olea europaea* L.) cultivars (cvs. 'Mari', 'Shengeh', 'Manzanilla' and 'Zard') were used in this study. Fruits were harvested with maturity index of one (yellowish green skin color) from Roudbar olive Research Center in Guilan Province, Iran, during mid-September. Uniform fruits with no visual damage at the exocarp were chosen for the experiment (Sanchez et al., 2006).

The fruit were divided into two groups, the first group selected for evaluation of browning potential and the second ones selected for evaluation of anti-browning agents effects on monophenolase and diphenolase activity of PPO and POD activity. To mimic mechanical damage, a pilot plant scale device was developed (Segovia-Bravo et al., 2007). It has 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 fixed distance above a moving belt. The procedure was checked to produce homogeneously distributed bruises on the olives. Bruised fruits from each cultivar dipped immediately in AA (5.7 mM), CA (2.6 mM), OA (60 mM), HR (0.5 mM) and NaHMP (1.6 mM) solutions for 30 min. Distilled water was used as control treatment. After treatment, the fruits were left at room temperature for two hours. For biochemical assay, fruit samples was frozen in liquid nitrogen and kept at -80°C until further biochemical analysis.

Measurement of browning index

Evaluation of browning scale was subjectively based on a numeral scoring index. The severity of fruit skin browning was assessed visually on the surface of olive fruits. The browning severity was divided into 5 classes: 0 as no browning, 1 as slight browning (less than 25% of fruit surface brown), 2 as mild browning (more than 25% and less than 50% of fruit surface brown), 3 as severe browning (more than 50% and less than 75% of fruit surface brown), and 4 as very severe browning (more than 75% of fruit surface brown). Browning index was expressed according to Wang et al. (2006) as:

$$\text{Browning Index} = \sum [(\text{Browning level}) \times (\text{Number of fruits at each browning level})] / (5 \times \text{Total number of fruits in the treatment}).$$

Extraction enzymes

Tissues of green olive drupe that was bruised and thereafter treated with anti-browning agents were used in the experiments (Segovia-Bravo et al., 2010). Fifty grams of flesh sample was homogenized once with 100 mL of cold acetone (–20°C) and 2.5 g of polyethyleneglycol in order to remove the lipo-soluble fraction. The residue was re-extracted three times with 100 mL of cold acetone (–20° C) and the homogenate obtained was separated by filtration, finally dried and weighed. Half of a gram of this acetone powder was re-suspended in 25 mL of 50 mM phosphate buffer, pH 6.2 (extraction buffer), containing 1 M KCl. The pellet was stirred at 4°C for 30 min and then centrifuged at 15,000 g for 20 min at 4°C. The pellet was discarded and the supernatant was used as the active enzymatic extract (Segovia-Bravo et al., 2010).

Enzyme assay

POD assay

POD activity was assayed by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm (Chance and Maehly, 1955). The reaction mixture contained 100 µL of crude enzyme extract, 500 µL of 5 mM H_2O_2, 500 µL of 28 Mm guaiacol, and 1900 µL of 50 mM potassium phosphate buffer (pH 7.0). POD activity of the extract was expressed as activity µmol g^{-1} FW min^{-1}.

Monophenolase and diphenolase assay of PPO

Monophenolase and diphenolase activity of PPO were assessed with different substrates according to the method of Vidhan et al. (2010) with some modification.

Monophenolase activity of PPO was determined spectrophotometrically by adding 300 µL of enzyme extract to 300 µL pyrogallol 0.02 M and 2400 µL phosphate buffer solution (0.1 M, pH 7.2). Absorbance was read at 420 nm every 10 s up to 3 min according to Narpinder et al. (1999). The activity was expressed as µmol 100 g^{-1} FW min^{-1}.

Diphenolase activity of PPO was determined using two different substrates: pyrcatechol and dopamine hydrochloride (dopamine HCl). Briefly, for the first substrate, 300 µL of enzyme extract was added to 300 µL pyrcatechol 0.5 M and 2400 µL phosphate buffer solution (0.1 M, pH 7.2). Absorbance was read at 420 nm according to Vidhan et al. (2010) and the activity was expressed as µmol 100 g^{-1} FW min^{-1}. All determinations were performed in triplicate. With the second substrate, 10 µL of enzyme extract was added to 500 µL dopamine hydrocholoride solution 60 mM with MBTH 5 mM, 2% (v/v), phosphoric acid 5% and 490 µL phosphate buffer solution (0.1 M, pH 7.2). Absorbance for dopamine hydrocholoride substrate was read at 475 nm every 10 s up to 3 min according to Juan et al. (1995). The activity was expressed as µmol g^{-1} FW min^{-1}.

Total phenolics content

Total phenolics content was analyzed spectrophotometrically using the modified Folin–Ciocalteu colorimetric method as described by Ghasemnezhad et al. (2011) with some modifications. One gram of fruit flesh was extracted with 10 mL methanol; l50µL of the methanolic extract were mixed with 350 µL of distilled water in a test tube, and then 2.5 mL of 10% Folin–Ciocalteu reagent was added and allowed to stand for 6 min. Then, 2 mL of 7.5% sodium carbonate solution was added. Each sample was allowed to stand for 90 min at room temperature in darkness and the absorbance was measured at 760 nm using an UV/Vis spectrophotometer model PG Instrument +80, (Leicester, UK). Results were expressed as mg gallic acid (mg GAE) g^{-1} FW.

Statistical analyses

The experiment was performed in a randomized complete design in factorial arrangement. The values are mean values ± S.E of three replicates. Statistical analysis was carried out using SAS software (Version 9.1, SAS Instituted, Cary, NC, USA). Analysis of variance between treatment means was carried out using LSD test at $p \leq 0.05$.

Results and discussion

Browning index

Browning index was used to determine the browning intensity of olive fruit tissues after artificial mechanical damage. The results showed that browning index of fruit tissues was significantly different in studied olive cultivars (Fig. 1). 'Mari' olives showed the highest browning index, while the lowest index was found in 'Zard' olives in comparison with other cultivars.

Previous study confirmed that the browning potential of various fruits and sometimes of different cultivars of the same species have been shown to be directly related to the phenol content, the PPO and POD activity or the combination of these factors (Sciancalepore and Longone, 1984). The mechanical injuries during harvesting or processing of fruits lead to the starting of browning reactions, which lead the final product of poor quality (Toivonen and Brummell, 2008). So, to avoid browning reaction several methods can be proposed such as selection and cultivation of mechanical harvesting damage tolerant cultivars, as well as application of inhibitor agents to inhibit POD and specially PPO activity. This study demonstrated that 'Zard' can be considered as the most mechanical harvesting damage tolerant cultivar; in contrast 'Mari' is the most mechanical damage sensitive cultivar (Fig. 1).

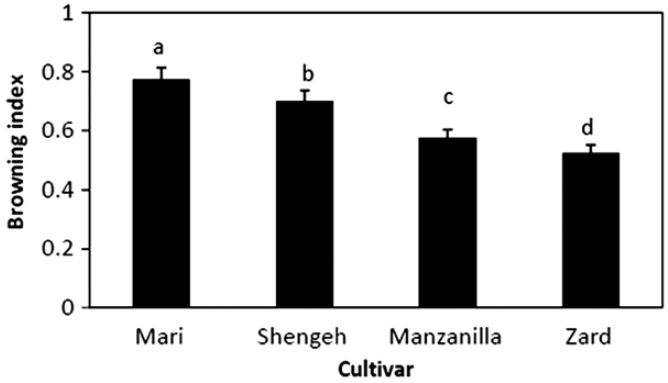

Figure 1. Browning index of fruit tissue in cvs. 'Mari', 'Shengeh', 'Manzanilla' and 'Zard' (Note: means in each column with the same letter are not significantly different according to the LSD test at $p \leq 0.05$ level. Vertical bars represent SE based on three replicates).

Enzyme activity

POD activity

POD activity of four olive cultivars tissues that were treated with anti-enzymatic browning agents are presented in Fig 2. The results showed a significant difference of POD activity in the enzymatic extracts of fruits tissues after treatment with anti-enzymatic browning agents. The highest POD activity was assayed in 'Manzanilla' olive fruit treated with AA (4.38 µmol g^{-1} FW min^{-1}), while the lowest POD activity (0.79 µmol g^{-1} FW min^{-1}) was assayed in 'Mari' extract treated with NaHMP. Among the anti-browning agents, NaHMP decreased POD activity in 'Manzanilla' and 'Mari' fruit compared with other treatments; while HR and AA treatments even increased POD activity compared with control treatments (Fig. 2). HR not only did not decrease POD activity compared with control, but also increased significantly the enzyme activity in 'Shengeh', 'Mari' and 'Manzanilla' fruit.

Browning reactions of fruit tissues progress in the presence of oxygen and are catalyzed primarily by PPO and POD enzymes (Lamikanra and Watson, 2001). Previous studies showed that AA could reduce the POD enzyme activity in fresh cuts of apple (Li et

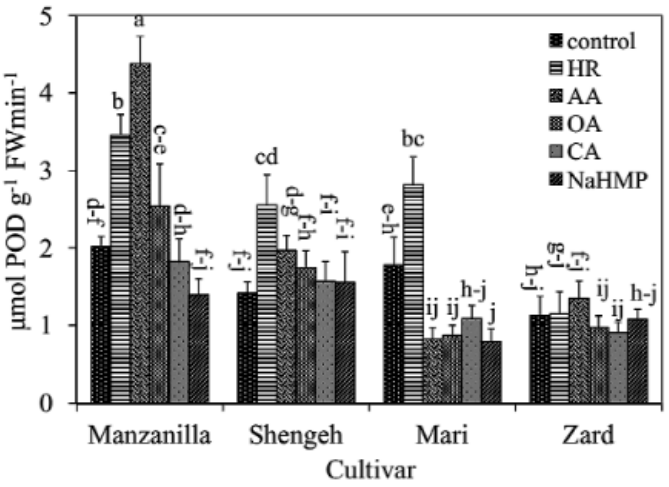

Figure 2. POD activity in fruit tissues treated with distilled water (control), 4-hexaresorcinol (HR), ascorbic acid (AA), oxalic acid (OA), citric acid (CA) and sodium hexametaphosphate (NaHMP) in cvs. 'Mari', 'Shengeh', 'Manzanilla' and 'Zard' (Note: means in each column with the same letter are not significantly different according to the LSD test at $p \leq 0.05$ level. Vertical bars represent SE based on three replicates).

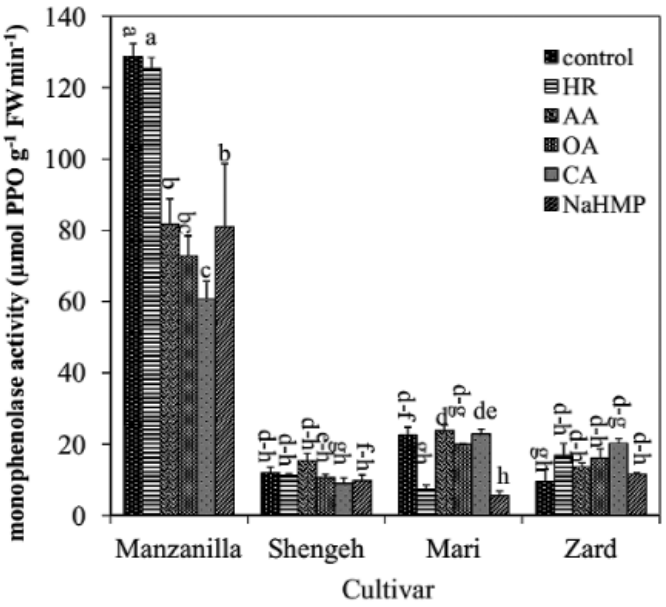

Figure 3. The monophenolase activity of PPO by pyrogallol substrate in fruit tissues treated with distilled water (control), 4-hexaresorcinol (HR), ascorbic acid (AA), oxalic acid (OA), citric acid (CA) and sodium hexametaphosphate (NaHMP) in fruit of cvs. 'Mari', 'Shengeh', 'Manzanilla' and 'Zard' (Note: means in each column with the same letter are not significantly different according to the LSD test at $p \leq 0.05$ level. Vertical bars represent SE based on three replicates).

al. 2008), litchi (Sun et al. 2010) and peach (Zhu et al., 2009) compared with control. In this study, the potency of AA on suppressing POD activity was completely dependent on cultivar. Its application reduced POD activity only in 'Mari' cultivar, but did not have effect on other cultivars. Preventing effect of NaHMP on POD activity was observed in some olive cultivars such as cvs. 'Manzanilla' and 'Mari'. NaHMP is a natural polyphosphate and can be considered as anti-browning agent (Dziezak, 1990). In agreement with our finding, Pilizota and Sapers (2004) showed that NaHMP was successful in browning reduce in 'Granny Smith' apples, but not successful in the 'Fuji' apples.

Monophenolase and diphenolase activity of PPO

Pyrogallol as a monophenolic substrate, and pyrochatechol and DPOA as diphenolic substrates were assayed in fruit tissue of different olive fruit cultivars.

Monophenolase activity of PPO

The monophenolase activity of PPO by pyrogallol substrate in olive fruits tissues that were treated with anti-enzymatic browning agents are presented in Fig 3. When pyrogallol was used as a substrate, there was a significant difference in monophenolase activity among the enzymatic extracts of different olive cultivars after treatment with anti-enzymatic browning agents. In general, the results showed that the monophenolase activity of PPO was significantly higher than their diphenolase activity. As the results showed, the minimum monophenolase activity was assayed in 'Zard' with the lowest fruit browning index (Fig. 3). The results also showed that application of anti-enzymatic browning could decrease monophenolase activity in cvs. 'Manzanilla' and 'Mari' compared with untreated fruit (control). The highest inhibitory effect on monophenolase was found in 'Manzanilla' cultivars when dipped in CA and OA (53% and 43%, respectively) compared to control. While, in 'Mari' olives, NaHMP and HR were the most effective treatments (75% and 67%, respectively) compared with control treatment (Fig. 3).

The monophenolase activity of PPO is generally considered as the first step in enzymatic browning, as the enzyme catalyses the hydroxylation of monophenols to o-diphenols (Sánchez-Ferrer et al., 1995). Using other anti-browning agents such as the HR in minimal processing of apple fruits were also suggested (Dong et al., 2000; Son et al., 2001). In addition, inhibitory effects of OA on browning have been also proven (Son et al., 2000; Son et al., 2001). The OA inhibitory effect on PPO enzyme activity of cv. Red Delicious apple were compared to malonic, glutaric and succinic acids and it was found that OA had the greatest inhibitory impact on the enzyme activity (Yoruk and Marshall, 2003). On other hand, CA compound was introduced as an effective inhibitor agent of the enzymatic browning in minimally processed fruits and vegetables (Ahvenainen, 1996). Yoruk and Marshall (2003) reported that PPO enzyme activity and browning reaction was reduced to 80-85% in litchi fruits when treated with CA. Citric acid treatment inhibits PPO due to its chelating action (Jiang et al., 1999). Pilizota and Sapers (2004) also reported that an acidic browning inhibitor formulation containing NaHMP was successful in suppressing shelf life limiting core tissue browning in fresh-cut apples.

Diphenolase activity of PPO

The diphenolase activity of PPO enzyme was assayed using two substrates; pyrocatechol and dopamine HCl (Figs. 4, 5). There was a significant difference among different olive cultivars that were treated with anti-enzymatic browning agents for diphenolase activity of PPO with pyrocatechol substrate (Fig. 4). The minimum diphenolase activity of PPO using the pyrocatechol substrate was assayed in cv. 'Zard' (0.02 µmol 100 g^{-1} FW min^{-1}) with the lowest

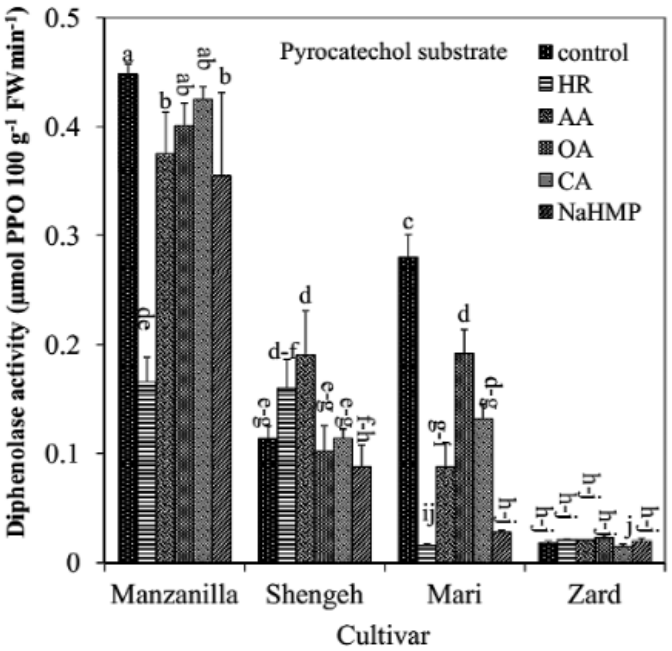

Figure 4. The diphenolase activity of PPO using the pyrocatechol substrate in fruit tissues treated with distilled water (control), 4-hexaresorcinol (HR), ascorbic acid (AA), oxalic acid (OA), citric acid (CA) and sodium hexametaphosphate (NaHMP) in cvs. 'Mari', 'Shengeh', 'Manzanilla' and 'Zard' (Note: means in each column with the same letter are not significantly different according to the LSD test at $p \leq 0.05$ level. Vertical bars represent SE based on three replicates).

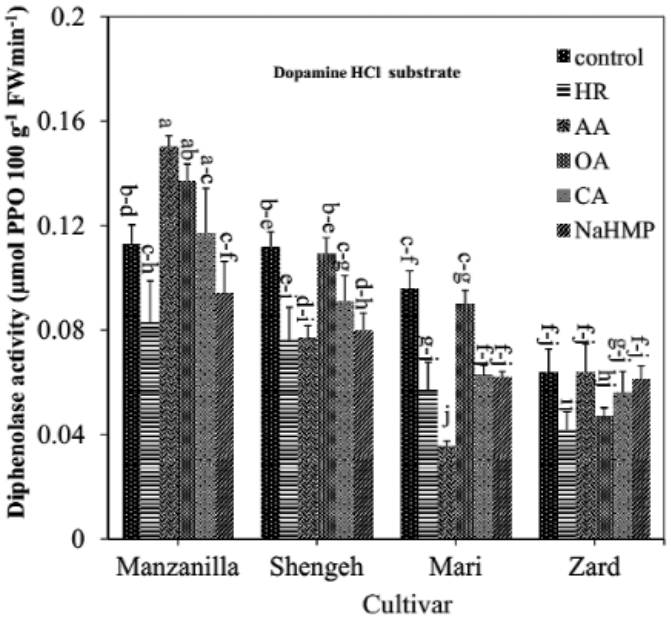

Figure 5. The diphenolase activity of PPO using the dopamine HCl substrate in fruit tissues treated with distilled water (control), 4-hexaresorcinol (HR), ascorbic acid (AA), oxalic acid (OA), citric acid (CA) and sodium hexametaphosphate (NaHMP) in cvs. 'Mari', 'Shengeh', 'Manzanilla' and 'Zard' (Note: means in each column with the same letter are not significantly different according to the LSD test at $p \leq 0.05$ level. Vertical bars represent SE based on three replicates).

browning index, while cvs. 'Manzanilla', 'Mari' and 'Shengeh' (0.45, 0.28 and 0.11 µmol 100 g^{-1} FW min^{-1}, respectively) showed higher diphenolase activity (Fig. 4).

The result also showed that inhibitory effect of browning agents on diphenolase activity of PPO with pyrocatechol substrate was completely dependent on cultivars (Fig. 4). HR showed the highest inhibitory effect on diphenolase activity of PPO using the pyrocatechol substrate in fruits of cvs. 'Mari' and 'Manzailla' (94% and 63%, respectively) compared to untreated fruits, while NaHMP and OA were more effective in 'Shengeh' cultivar (22% and 11%, respectively) than control (Fig.4). In contrast, in cv. 'Zard', none of agents could inhibit enzyme activity compared with control.

There was a significant difference among different olive cultivars treated with anti-enzymatic browning agents for diphenolase activity of PPO with dopamine HCl substrate (Fig. 5). Cvs. 'Manzanilla' and 'Zard' showed the highest and the lowest diphenolase activity of PPO (0.11 and 0.06 µmol 100 g^{-1} FW Min^{-1}, respectively) using the dopamine HCl substrate in untreated fruits. The potency of anti-browning agents on reducing diphenolase activity of PPO with DPOA substrate was completely dependent to olive cultivars. For example, NaHMP in cv. 'Manzanilla', AA in cv. 'Shengeh', HR in cv. 'Mari' and OA in cv. 'Zard' had high inhibitory effect on diphenolase activity of PPO compared to untreated fruit (Fig. 5).

Our result is in accordance with Jang and Moon (2011) and Özen et al. (2004) who used two substrates to measure the enzyme activity in different apple genotypes and demonstrated that diphenolase activity of PPO was higher than monophenolase activity. Furthermore, previous studies also showed differences in the diphenolase activity of apple genotypes using pyrocatechol and dopamine HCl substrates (Özen et al., 2004). In olive, inhibitory effects of other anti-browning agents on PPOs was also dependent on cultivars. AA is a widely used natural inhibitor of PPO. In this study, the highest inhibitory effect on diphenolase activity of PPO in 'Mari' cultivar was found by AA. Previous study showed that AA inhibits browning reactions by reducing the o-quinones back to the phenolic substrates that are generated by the action of the PPO enzymes (Robert et al., 2003). However, AA is consumed during the process and provides only temporary protection against discoloration unless very high concentrations are used (Gill et al., 1998). In this study, HR showed the highest inhibitory effect on monophenolase and diphenolase activity of PPO in the most of studied olive cultivars. HR is generally recognized as safe and inhibits browning by generating an inactive complex with the enzyme. Kinetic studies demonstrated that HR inhibits PPO activity either by a competitive type (Jiménez and García-Carmona, 1999) or a slow-binding inhibition mechanism (Jiménez and García-Carmona, 1997) depending on the substrate. In mango purees, the main inhibition effect was observed with HR treatment (Guerrero-Beltránetet al., 2005) and in apple juice HR had long term inhibitory effect (Iyidogan and Bayiindirli, 2004). On other hand, CA also inhibits PPO due to its chelating action (Jiang et al., 1999). Segovia-Bravo et al. (2010) reported that low pH or ascorbic acid

solutions may be useful to prevent browning in the bruised areas of hand- or mechanically-harvested cv. 'Manzanilla' table olives. In addition, inhibitory effects of OA on browning have been also proven (Son et al., 2000; Son et al., 2001). The OA inhibitory effect on PPO enzyme activity of cv. Red Delicious apple was compared to malonic, glutaric and succinic acids and it was found that OA had the greatest inhibitory impact on the enzyme activity (Yoruk and Marshall, 2003)., The NaHMP formulation was also used successfully with 'Granny Smith' apples, while it showed inconsistent results with 'Fuji' (Pilizota and Sapers, 2004).

Total phenolic content

In the current study, the total phenolic content was significantly lower in untreated fruits (control) than in treated fruits, except for 'Mari' (Fig. 6). Olive treated with HR in cvs. 'Manzanilla' and 'Shengeh' showed the highest total phenolic content (16.56 and 14.62 mg galic acid/g FW) compared to other treatments. While, cvs. 'Mari' and 'Zard' treated with CA and OA had the highest total phenolic content (14.11 and 13.44 mg galic acid/g FW for CA treatment; 13.73 and 13.38 mg galic acid/g FW for OA treatment, respectively) compared with other treatments.

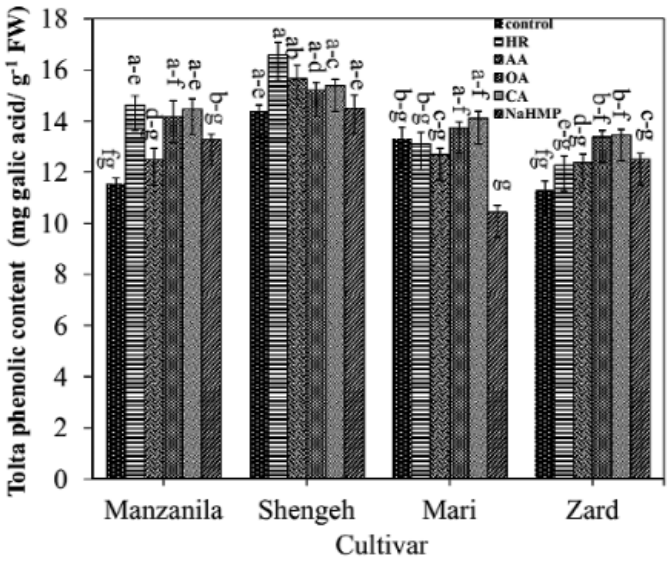

Figure 6. The total phenolic content in fruit tissues treated with distilled water (control), 4-hexaresorcinol (HR), ascorbic acid (AA), oxalic acid (OA), citric acid (CA) and sodium hexametaphosphate (NaHMP) in cvs. 'Mari', 'Shengeh', 'Manzanilla' and 'Zard' (Note: means in each column with the same letter are not significantly different according to the LSD test at $p \leq 0.05$ level. Vertical bars represent SE based on three replicates).

Browning can be related to the consumption of phenols by PPO enzymes which may use them as substrates for the formation of dark polymers (Martinez and Whitaker 1995). In the current study, the total phenolic content was significantly lower in untreated fruits (Control) than treated fruits (Fig. 6). Phenolic compounds decrease could be related to an eventual enzymatic activity, which was more intense in untreated fruits (control) than treated fruits because of more activity of enzymes related to browning in the damaged cells (Fig. 6). Phenolic compounds 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). So, the chemical agent treatments that were used to control enzymatic browning can inhibit the phenolic content decrease. In our study, the fruits tissue treated with HR in cvs. 'Manzanilla' and 'Shengeh' showed the highest total phenolic content compared with other treatments. HR is generally recognized as safe and inhibits browning by generating an inactive complex with the enzyme. Kinetic studies demonstrated that HR inhibits PPO activity either by a competitive type (Jiménez and García-Carmona, 1999) or by a slow-binding inhibition mechanism (Jiménez and García-Carmona, 1997) depending on the substrate. In apple juice HR had long term inhibitory effect (Iyidogan and Bayiindirli, 2004). However, fruits tissue of cvs. 'Mari' and 'Zard' treated with CA and OA had the highest total phenolic content compared to other treatments. Citric acid (CA) inhibits PPO due to its chelating action (Jiang et al., 1999). Citric acid has proved to be effective in delaying of browning reactions (Lattanzio et al., 1989). In addition, inhibitory effects of OA on browning have been also proven (Son et al., 2001; Yoruk and Marshall, 2003).

Conclusions

The results of current study showed that olive fruits of cv. 'Zard' had the lowest browning index. Inhibitory effects of each anti-enzymatic browning agent on activity of PPOs and POD were completely dependent on cultivar. HR and NaHMP treatments suppressed diphenolase activity of PPO with pyrocatechol substrate at 'Mari' and 'Manzanilla' fruits. The fruits tissue treated with HR in 'Manzanilla' and 'Shengeh' showed the highest total phenolic content compared to other treatments. Overall, monophenolase activity of PPO was higher than diphenolase activity in all studied cultivars and the highest monophenolase and diphenolase activity was found in 'Manzanilla'. Cv. 'Zard' that has the lowest POD and PPOs showed also the lowest tissue browning. Overall, HR agent was the most effective agent on suppressing of PPOs activity and browning in olive fruits.

References

Ahvenaien R. (1996). New approaches in improving the shelf life of minimally processed fruit and vegetables. Trends Food Sci. Technol 7: 367-380.

Chance B., Maehly S. K. (1955). Assay of catalase and peroxidase. Methods Enzym 2: 764-775.

Dong X., Wrolstad R. E., Sugar D. (2000). Extending shelf life of fresh-cut pears. J Food Sci 65: 181–186.

Dziezak J. D. (1990). Phosphates improve many foods. Food Technol 44: 80-92.

Ghasemnezhad M., Sherafati M., Payvast A. (2011). Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. J Functional Foods 3: 44-49.

Gil M. I., Gorny J. R., Kader A. A. (1998). Responses of 'Fuji' apple slices to ascorbic acid treatments and low-oxygen atmospheres. Hort Sci 33: 305-309.

Goupy P., Fleuriet A., Amiot M. J., Macheix J. J. (1991). Enzymatic browning, oleuropeincontent, and diphenol oxidase activity in olive cultivars (*Olea europaea* L.). J Agric and Food Chem 39: 92–95.

Guerrero-Beltrán J. A., Swanson B. G., Barbosa-Cánovas G. V. (2005). Inhibition of polyphenoloxidase in mango puree with 4-hexylresorcinol, cysteine and ascorbic acid. LWT – Food Sci and Tecnhol 38: 625–630.

Iyidogan N. F., Bayiindirli A. (2004). Effect of L-Cysteine, kojic acid and 4-hexylresorcinol combination on inhibition of enzymatic browning in Amasya apple juice. J. Food Eng 62: 299–304.

Jang J. H., Moon K. (2011). Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. J. Food Chem 124: 444-449.

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

Jiménez M., Garcáa-Carmona F. (1997). 4-Substituted resorcinols (sulfite alternatives) as slowbinding inhibitors of tyrosinasechatecholase activity. J. Agric. Food Chem 45: 2061–2065.

Jiménez M., Garcáa-Carmona F. (1999). Oxidation of the flavonolquercetin by polyphenol oxidase. J. Agric. Food Chem 47: 56–60.

Juan C. E., Mercedes M., Ramon V., Jose T., Francisco G. C. (1995). A continuous spectrophotometric method for determining the monophenolase and diphenolase activities of apple polyphenol oxidase. Anal biochem 231: 237–246.

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

Lamikanra O., Watson M. A. (2001). Effects of ascorbic acid on peroxidase and polyphenol oxidase activities in fresh-cut cantaloupe melon. J. food sci 66: 1283-1286.

Lattanzio V., Palmieri V. L. S., Christiaanand F., Van S. (1989). The beneficial effect of citric and ascorbic acid on the phenolic browning reaction in stored artichoke (*Cynarascolymus*L.) heads. Food Chem 33:93-106.

Li Z., Eun J. S., Jun H. L. (2008). Effect of ascorbic acid and citric acids on CIE colour values of fresh cut apple cubes. J. Food Technol. 6: 20- 24.

Martinez M. V, Whitaker J. R. (1995). The biochemistry and control of enzymatic browning. J. Food Technol 6: 195-200.

Monsalve-Gonzalez A., Barbosa-Ca´novas G. V., Cavalieri R. P., McEvily A. J., Iyengar R. (1995). Inhibition of enzymatic browning in apple products by 4-hexylresorcinol. J. Food Technol 49: 110-118.

Murata M., Tsurutani M., Hagiwara S., Homma S. (1997). Subcellular location of polyphenol oxidase in apples. Biosci. Biotechnol., Biochem 61: 1495–1499.

Narpinder S., Randhir S., Kulwinder K., Harmit S. (1999). Studies of the physico-chemical properties and polyphenoloxidase activity in seeds from hybrid sunflower (*Helianthus annuus*) varieties grown in India. Food Chem 66: 241–247.

Özen A., Colak A., Dincer B., Güner S. (2004). A diphenolase from persimmon fruits (*Diospyros kaki* L. Ebenaceae). J. Food Chem 85: 431–437.

Özoglu H., Bayiindirli A. (2002). Inhibition of enzymatic browning in cloudy apple juice with selected antibrowning agents. Food Contorl 13: 213–221.

Pilizota V., Sapers G. (2004). Novel browning inhibitor formulation for fresh-cut apples. J. Food Sci 69: 140–143.

Pizzocaro F., Torreggiani D., Gilardi G. (1993). Inhibition of apple polyphenoloxidase (PPO) by ascorbic acid, citric acid and sodium chloride. J. Food Process. Preserv. 17: 21-30.

Queiroz C., Lopes M. L. M., Fialho E., Valente-Mesquita V. L. (2008). Polyphenol oxidase: Characteristics and mechanisms of browning control. Food Rev Int 24: 361-375.

Robert C. S. F., Martın O. B. (2003). New advances in extending the shelf life of fresh-cut fruits: a review. Trends Food Sci. Technol 14: 341-353.

Rojas-Grau M. A., Sobrino-Lopez A., Tapia M. S., Martın-Belloso O. (2006). Browning inhibition in fresh-cut 'Fuji' apple slices by natural antibrowning agents. J. Food Sci 71: 59–65.

Sanchez A. H., Garcia P., Rejano L. (2006). Elaboration of table olives. Grasas Aceites 57: 86–94.

Sanchez-Ferrer A., Rodriguez-Lopez J. N., Garcia-Canovas F., Garcia-Carmona F. (1995). Tyrosinase: A comprehensive review of its mechanism. Biochim. Biophys. Acta 1247: 1–11.

Sciancalepore V., Longone V. (1984). Polyphenol oxidase activity and browning in green olives. J. Agric. Food Chem 32: 320–321.

Segovia-Bravo K. A., Jaren-Galan M., Garcia-Garcia P., Garrido-Fernandez A. (2007). Characterization of polyphenol oxidase from the 'Manzanilla' cultivar (*Olea europaea* pomiformis) and prevention of browning reactions in bruised olive fruits. J. Agric. Food Chem 48: 2732-2735.

Segovia-Bravo K. A., Jaren-Galan M., Garcia-Garcia P., Garrido-Fernandez A. (2010). Treatment to inhibit the browning reactions in model solutions of olive fruit extracts. Food Chem 123: 741–746.

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

Sommer A., Ne'eman E., Steffens J., Mayer A., Harel E. (1994). Import, targeting and processing of a plant polyphenol oxidase. Plant Physiol 105: 1301–1331.

Son S. M., Moon K. D., Lee C. Y. (2000). Kinetic study of oxalic acid inhibition on enzymatic browning. J. Agric. Food Chem 48: 2071–2074.

Son S. M., Moon K. D., Lee C. Y. (2001). Inhibitory effects of various antibrowning agents on apple slices. Food Chem 73: 30-33.

Sun D., Guobin L., Jianghui X., Xintao L., Yiwei M. (2010). Improved preservation effects of litchi fruit by combining chitosan coating with ascorbic acid treatment during postharvest storage. Afr. J. Biotechnol 22: 3272-3279.

Toivonen P. M. A., Brummell D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biol. Technol 48: 1-14.

Vidhan J., Ara D., John R. P. (2010). Anthocyanins and polyphenol oxidase from dried arils of pomegranate (*Punica granatum* L.). Food Chem 118: 11–16.

Wang L., Chen S., Kong W., Li S., Archbold D. D. (2006). Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. Postharvest Biol. Technol 41:224-251.

Yoruk R., Marshall M. R. (2003). A survey on the potential mode of inhibition for oxalic acid on polyphenol oxidase. J. Food Sci 68:2479–85

Zhu L. Q., Zhou J., Zhu S., Guo H., Hui L. (2009). Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. Food Chem. 114: 174-179.

acs82_71