Antifungal Effects of Savory Essential Oil, Gum Arabic, and Hot Water in Mexican Lime Fruits

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Abstract. *Penicillium digitatum* is one of the most important causes of postharvest decay of Mexican lime fruit. The first stage of this study dealt with examining the effect of savory (*Satureja hortensis*) essential oil on *P. digitatum* mycelial growth in vitro. Savory essential oil (SEO) applied at concentrations of 0, 200, 400, 600, 800, 1000, and 1200 $\mu\text{L}\cdot\text{L}^{-1}$ to potato dextrose agar (PDA) medium. The results revealed that the application of SEO at concentrations of 1000 and 1200 $\mu\text{L}\cdot\text{L}^{-1}$ completely prevented the growth of *P. digitatum*. Gas chromatography–mass spectrometry results indicated that the dominant components of SEO were carvacrol (55.67%) and γ -terpinene (31.98%). In the second phase of the experiment, in vivo assays were conducted to evaluate the efficiency of SEO (800 and 1000 $\mu\text{L}\cdot\text{L}^{-1}$), hot water (40 and 50 °C), and gum arabic coating (2.5% and 5%) in restricting the fungi activity on Mexican lime fruit. The Mexican lime fruit were immersed in the aqueous solutions of SEO and gum arabic or in the hot water for 5 minutes, and then stored at 8 °C for 30 days. Savory essential oil at the concentration of 800 $\mu\text{L}\cdot\text{L}^{-1}$ proved to be the most effective treatment in conserving bioactive compounds of the fruits such as total phenols. This treatment also optimally maintained antioxidant activity and suppressed the activity of polyphenol oxidase (PPO) in the fruit peel. Moreover, hot water at 40 °C caused the least physicochemical changes and the highest appearance quality during storage.

Fruit decay caused by microorganisms may start before, during, or after harvest, especially in tropical regions where high temperature and relative humidity (RH) provide a suitable condition for the development of fungi on fruits. To preserve quality and increase the shelf life of horticultural crops, postharvest treatments are usually needed. Green mold caused by *P. digitatum* is one of the most damaging postharvest diseases of citrus fruits (Smilanick et al., 2006). Chemical fungicides are widely used to control green mold. However, concerns about chemical pesticides residues and human health problems resulted in the public demand for fresh produce without pesticides (Liu et al., 2016). Furthermore, there is a universal desire to explore new alternatives to expand the shelf life of fruit, in which such negative side

effects could be minimal for consumers and the environment (Bautista-Baños et al., 2006).

Essential oils extracted from medicinal plants have been shown to contain different amounts of phenolic compounds with antimicrobial effects (Al-Zoreky, 2009). Savory (*S. hortensis* L.) is an annual medicinal plant with antifungal (Sabzghabae et al., 2012), antibacterial (Karami-Osboo et al., 2010), and antioxidative properties (Fathi et al., 2011). It has been shown that SEO inhibits the growth of bacteria (Gram positive and negative) and *Penicillium* sp. in fruits of apple and pepper, extends storage life, increases moisture retention, and reduces wrinkles and wilt on the surface of fruits and vegetables (Kraśniewska et al., 2014).

Another method to maintain the quality of fruit and vegetables is the use of edible coatings. These coatings are thin layers of materials that reduce the exchange of moisture, oxygen and dissolved substances in food, and postpone their deterioration. Gum arabic is obtained from acacia branches especially *Acacia senegal*. A combination of gum arabic (10%) and cinnamon essential oil (4%) was effective for controlling papaya

and banana anthracnose rot and maintained the quality of the fruits (Maqbool et al., 2011). Coverage of tomato and mango fruit with gum arabic at a concentration of 10% reduced respiration and ethylene production and maintained antioxidant capacity, phenolics, and carotenoid content (Ali et al., 2010).

Prestorage immersion of fruits in hot water is considered as another effective and easy method for preventing decay and chilling damage during storage (Ansari and Feridoon, 2007; Porat et al., 2000). ‘Valencia’ oranges dipped in hot water (45 °C for 42 s) had a lower rate of moisture loss and remained firmer in cold storage (Williams et al., 1994).

The present study was conducted to determine the best concentration of SEO for controlling green mold in vitro and on Mexican lime fruit. Moreover, the effectiveness of SEO on maintaining the appearance and internal quality of Mexican limes was compared with gum arabic edible coating, and hot water treatments.

Material and Methods

Extraction and analysis of SEO. To extract SEO, the aerial parts of *S. hortensis* were used at the full-flowering stage. After harvest, they were air-dried at room temperature (less than 25 °C) in a shady location for 10 d. The plant sample was hydro-distilled for 3 h using an all-glass Clevenger-type apparatus to extract the SEO. The SEO was separated from the aqueous layer by decantation and dried over anhydrous sodium sulfate. It was light yellow in color with a yield of 2.33% (v/w, dry weight basis). The EO samples were stored in sealed vials at a low temperature (4 °C) until analyzed by gas chromatography (GC) and GC–mass spectrometry (GC–MS) and future experiments.

After the injection of SEOs to a gas chromatograph (7890A; Agilent Technologies, Wilmington, DE), the most appropriate thermal planning for the column was determined. Then the essential oil was diluted by dichloromethane and injected to GC–MS, and mass spectra and chromatograms were obtained. GC–MS (Model C5975; Agilent Technologies) column was a HP-5MS of 30 m length, 0.32 mm diameter, and thickness of the resident phase layer was 0.25 μm . The temperature of column container was adjusted at 280 °C. The carrier gas was nitrogen at 1 $\text{mL}\cdot\text{min}^{-1}$ import pressure. Ionization energy was equal to 70 eV. The detector temperature was adjusted at 280 °C and the carrier gas was helium with 1 $\text{mL}\cdot\text{min}^{-1}$ import pressure (Ramezani et al., 2016). The composition of the SEO was identified quantitatively and qualitatively using the retention time, retention index, the whole mass, and comparison with standard compounds (Adams, 2001), and using the information existing in the SATURN software. To calculate the index of inhibition, 9–23 carbons normal hydrocarbons were used in the heat-planning conditions.

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In vitro experiments. *Penicillium digitatum* was isolated from infected Mexican limes (*Citrus aurantiifolia*), single spore-purified, and propagated on PDA (extract of 300 g boiled potato, 20 g dextrose, 15 g agar). To measure direct effects of EO on growth and sporulation of *P. digitatum*, different concentrations (0, 200, 400, 600, 800, 1000, and 1200 $\mu\text{L}\cdot\text{L}^{-1}$) of SEO were used in the culture medium. Tween 80 was used as an emulsifier at a concentration of 0.5 $\text{mL}\cdot\text{L}^{-1}$ of medium. PDA blocks (5 mm diameter) containing *P. digitatum* single spores were separated with a cork borer and placed in the center of culture media, 24 h after preparation. Culture media were incubated at 25 °C for 8 d. The diameter of the colonies was measured every 24 h and the mycelial growth inhibition (MGI) was calculated according to the following formula:

$$\text{MGI}(\%) = [(d_c - d_t)/d_c] \times 100$$

where d_c = mean diameter of the control colonies and d_t = mean diameter of colonies treated with different concentrations of SEO.

In vivo experiments. A factorial experiment in a completely randomized design was used with seven treatments including control, SEO (800 and 1000 $\mu\text{L}\cdot\text{L}^{-1}$, based on *in vitro* results), hot water (40 and 50 °C), gum arabic (2.5% and 5%), using three replications and 15 fruit per replication. Fruit were handpicked from a commercial orchard in Dehrood region of Fars Province, Iran (lat. 28°81'3"N and long. 52°55'0"E). After transport to the laboratory, the fruit were washed and disinfected with 10% commercial Clorox and 70% ethanol. The air-dried fruit were inoculated with 10 μL of spore suspension at a concentration of 1×10^5 spores/mL through superficial wounds (2×2 mm) (Mahmoodabadi et al., 2005). The infected fruit were treated with the aforementioned treatments and packed in perforated polyethylene bags and stored at 8 °C and 85% RH. The following measurements were made at 15 and 30 d of storage.

The appearance of fungus colonies on the surface of each fruit was considered as a decayed fruit (Sharples et al., 1979). Decay was estimated based on the following equations:

$$\text{decay}(\%) = \frac{\text{decayed fruit}}{\text{total fruit}} \times 100$$

$$\text{decay rate} = \text{Arc sin } \sqrt{\text{decay}(\%)}$$

Weight loss was measured during storage by weighing the fruit at the initial stage (W_1) and at the different sampling dates (W_2), and calculated using the following equation (Habibi and Ramezani, 2017):

$$\text{weightloss}(\%) = \frac{W_1 - W_2}{W_1} \times 100$$

Ascorbic acid content was measured spectrophotometrically and expressed as fresh weight basis (Habibi and Ramezani, 2017). Briefly, 100 μL of fruit juice was mixed with 10 mL metaphosphoric acid

(1%). One milliliter of the solution was mixed with 9 mL dichloroindophenol, and shaken vigorously for a few seconds. The solution absorbance was measured at 515 nm. Juice total soluble solids (TSS) and pH were measured with a handheld refractometer (ATAGO, Japan) and by a pH meter (JENWAY 351, England), respectively. Titratable acidity (TA) was measured by titrating fruit juice against 0.2 N NaOH (Hassani et al., 2010).

The concentration of carotenoids and chlorophyll in fruit peel were measured spectrophotometrically and expressed as fresh weight basis. Briefly, 1 g of peel tissue was mixed with acetone and filtered. The absorbance was read at 646, 663, and 470 nm for the measurement of chlorophyll a and b and carotenoids, respectively (Lichtenthaler, 1987).

Antioxidant activity of each pulp extract was determined by the diphenylpicrylhydrazyl (DPPH) free radical scavenging assay

(Moon and Terao, 1998). Briefly, 100 μL of extract mixed with 1 mL DPPH (0.1 mM) and 1 mL Tris-HCl (pH = 7.5) buffer, and maintained for 30 min at room temperature. The absorbance was measured in a microplate spectrophotometer (Epoch Biotech, Germany) at 517 nm. Antioxidant activity was calculated using the following formula:

Antioxidant activity (%)

$$= \left[1 - \frac{A_{\text{Sample}}(517\text{nm})}{A_{\text{Control}}(517\text{nm})} \right] \times 100$$

For measuring the activity of PPO, 0.2 g of fresh peel tissue was mixed with 0.02 M potassium phosphate buffer, pH 8.6 at 4 °C. Then, the extract was cold centrifuged at 11,269 g_n for 15 min, and the supernatant was used to measure the activity of PPO. The reaction mixture contained 100 μL of enzyme extract, 500 μL of 5 mM hydrogen peroxide, and 500 μL of 0.02 mM methyl catechol in

Table 1. The composition of savory essential oil.

Compound	Retention time	Retention index	(%)
α -Thujene	6.29	924	1.16
α -Pinene	6.49	932	0.64
Camphene	6.82	946	0.06
Hepten-1-ol	7.19	958	0.05
Sabinene	7.36	969	0.02
β -Pinene	7.52	974	0.22
Myrcene	7.77	988	1.15
Phellandrene	8.22	1,002	0.24
α -Terpinene	8.61	1,014	3.75
ρ -Cymene	8.69	1,020	2.20
Sylvestrene	8.96	1,025	0.37
β -E-Ocimene	9.46	1,044	0.07
γ -Terpinene	10.04	1,054	31.98
Terpinolene	10.886	1,086	0.05
<i>trans</i> - α -Sabinene hydrate	11.01	1,098	0.07
Isoborneol	13.42	1,155	0.07
Terpinen-4-ol	13.92	1,174	0.20
α -Terpineol	14.45	1,186	0.10
Carvacrol, methyl ether	16.41	1,241	0.09
Thymol	18.17	1,289	0.80
Carvacrol	18.89	1,298	55.67
Thymol acetate	20.47	1,349	0.03
Carvacrol acetate	21.19	1,370	0.08
Caryophyllene	23.95	1,417	0.36
Aromadendrene	24.71	1,439	0.08
α -Humulene	25.19	1,454	0.02
Bicyclogermacrene	26.79	1,500	0.18
Bisabolene	27.27	1,505	0.21
Spathulenol	29.43	1,577	0.045

Table 2. The effect of savory essential oil on growth inhibition (%) of *Penicillium digitatum* mycelia during 8 d incubation at 25 °C.

Treatment	Incubation period (days)			
	2	4	6	8
Control	0.00 g ^z	0.00 g	0.00 g	0.00 g
Tween	5.33 e-g	3.93 e-g	2.10 fg	1.49 fg
EO 200 $\mu\text{L}\cdot\text{L}^{-1}$	14.10 e	12.26 ef	9.09 e-g	7.20 e-g
EO 400 $\mu\text{L}\cdot\text{L}^{-1}$	62.06 d	60.89 d	59.50 d	57.10 d
EO 600 $\mu\text{L}\cdot\text{L}^{-1}$	86.99 bc	85.05 c	83.00 c	82.62 c
EO 800 $\mu\text{L}\cdot\text{L}^{-1}$	97.18 ab	97.27 ab	96.53 ab	91.28 a-c
EO 1000 $\mu\text{L}\cdot\text{L}^{-1}$	100.00 a	100.00 a	100.00 a	100.00 a
EO 1200 $\mu\text{L}\cdot\text{L}^{-1}$	100.00 a	100.00 a	100.00 a	100.00 a

^zMeans followed by similar letters are not significantly different according to Duncan's multiple range test ($P = 0.05$).

EO = essential oil.

1900 μL of potassium phosphate buffer, at a pH of 6.1. The absorbance of the reaction mixture was determined at 410 nm in a Microplate Reader (Epoch Biotech). The results were expressed as fresh weight basis (Ghanati et al., 2002).

Fruit firmness was measured using a texture analyzer (CT3; Brookfield Engineering Laboratories Inc., Middleboro, MA). The instrument gave the 10-mm deformation after application of a compression load at a rate of $1\text{ mm}\cdot\text{S}^{-1}$ at the equatorial region of the fruit (Navarro-Tarazaga et al., 2008).

Folin–Ciocalteu reagent was used to measure the total phenolic content of pulp tissue and the results were expressed as fresh weight basis. Seven hundred microliter of extract mixed with 900 μL of 2% sodium carbonate and maintained at room temperature. Then, 180 μL of 50% Folin was added, and the samples were maintained for 30 min at room temperature. The mixture absorbance was read at 750 nm. The concentration of the total phenolic content was expressed as g GAE/kg (Meyers et al., 2006).

Fruit color parameters (L , a^* , and b^*) were measured by a Minolta colorimeter (CR400, Japan).

Experimental design and statistical analysis. The treatments were arranged as a complete randomized design with three replications. Physicochemical data were analyzed using two-factor analysis of variance (treatments and storage time) procedures. Mean comparisons were performed using the Duncan's multiple range test ($P = 0.05$). All analyses were performed by the SAS software (SAS Institute Inc., Cary, NC) version 9.1 for Windows.

Results and Discussion

SEO analysis. The essential oil of savory plants was 1.44% of the dry weight. GC–MS revealed 29 components in the SEO (Table 1). The most important constituents were carvacrol (55.67%), terpinene (31.98%), α -terpinene (3.75%), ρ -cymene (2.20%), α -thujene (1.16%), and myrcene (1.15%).

In vitro growth inhibition of *P. digitatum*. Savory essential oil at concentrations of 1000 and 1200 $\mu\text{L}\cdot\text{L}^{-1}$ completely prevented the growth of *P. digitatum* colonies (Table 2). Savory essential oil's antimicrobial activity on *Candida albicans* has also been reported (Moradian et al., 2013). It has been reported that carvacrol causes cell death by reducing plasma membrane integrity and inducing leakage of intracellular ATP and potassium ions (Tahmasbpour et al., 2015).

In vivo experiment. As shown in Fig. 1, control fruit had the highest decay rate in the first sampling date (0.82%). Fruit treated with 40- and 50 °C-hot water showed no decay during storage. The inhibitory effects of heat treatment on citrus fruit decay have been reported by others (Cohen et al., 1990; Droby et al., 1993). Wound healing following heat treatment prevents growth and development of fungi due to the synthesis of lignin-like

Fig. 1. Effect of savory essential oil at concentrations of 800 $\mu\text{L}\cdot\text{L}^{-1}$ (EO800) and 1000 $\mu\text{L}\cdot\text{L}^{-1}$ (EO1000), hot water at 40 (HW40) and 50 °C (HW50), and gum arabic at 2.5 (GA2.5) and 5% (GA5) on decay rate (A) and weight loss (B) of Mexican lime fruit inoculated with *Penicillium digitatum* after 15 and 30 d of storage at 8 °C. Vertical bars represent $\pm\text{SE}$ of means, where values are larger than the symbol.

Fig. 2. Effect of savory essential oil at a concentration of 800 $\mu\text{L}\cdot\text{L}^{-1}$ (EO800) and 1000 $\mu\text{L}\cdot\text{L}^{-1}$ (EO1000), hot water at 40 (HW40) and 50 °C (HW50), and gum arabic at 2.5 (GA2.5) and 5% (GA5) on firmness (N) of Mexican lime fruit inoculated by *Penicillium digitatum* after 15 and 30 d of storage at 8 °C. Vertical bars represent $\pm\text{SE}$ of means, where values are larger than the symbol.

Fig. 3. Effect of savory essential oil at concentrations of 800 $\mu\text{L}\cdot\text{L}^{-1}$ (EO800) and 1000 $\mu\text{L}\cdot\text{L}^{-1}$ (EO1000), hot water at 40 (HW40) and 50 °C (HW50), and gum arabic at 2.5 (GA2.5) and 5% (GA5) on fruit peel lightness (L^*) of Mexican lime fruit inoculated by *Penicillium digitatum* after 15 and 30 d of storage at 8 °C. Vertical bars represent $\pm\text{SE}$ of means, where values are larger than the symbol.

substances (Ben-Yehoshua and Porat, 2005). Moreover, increase in free phenolic compounds and phenylalanine ammonia lyase (PAL) activity also limits fruit decay after heat treatment (Droby et al., 1993). The synthesis of pseudo-lignin materials in the damaged tissue of fruit exocarp reduces the fungal growth (Brown, 1990). In our study, the application of SEO at 800 or 1000 $\mu\text{L}\cdot\text{L}^{-1}$ reduced the fruit decay up to 15 d of storage. High carvacrol content of SEO may damage the fungus plasma membrane and reduce fungal contamination (Tahmasbpour et al., 2015).

At the end of the storage period, the lowest weight loss was found in the fruit treated with 50 °C-hot water (4.56%) (Fig. 1B). Fruit weight loss is the result of transpiration and respiration (Li et al., 2012). Moreover, wounding increases the rate of transpiration and respiration. Heat treatment suppresses the rate of water loss by stimulating lignin synthesis in the wounds and cracks (Lurie et al., 1995). SEO and edible coatings probably reduces the rate of water and weight loss during storage by stabilizing cell structure and providing a physical barrier (Khorram et al., 2017).

Fruit firmness decreased until the end of the storage period. The highest firmness was found in the fruit treated with 2.5% gum arabic after 15 d of storage (Fig. 2), and it was 3.2 N more than the nontreated fruit after 30 d of storage. Application of gum arabic has been shown to reduce the activity of cell wall-degrading enzymes during ripening (Ali et al., 2011). It has been reported that heat treatment decreases the activity of cell wall-degrading enzymes by inducing heat shock proteins and maintaining cell turgor pressure (Khaliq et al., 2015).

Fruit treated with 40 °C hot water showed maximum lightness (L^*) after 15 d of storage (Fig. 3). It was 10.18% higher than the control fruit after 30 d of storage, and was significantly more than other treated fruit at the end of the storage period. The control fruit had the lowest L^* value (67.15), which was significantly different compared with the other treatments. It has been shown that proper heat treatment prevents pigments oxidation and maintains the brightness of fresh-cut 'Spring Belle', 'Flavor Crest', and 'Fayette' peaches (Koukounaras et al., 2008). Early studies have indicated that heat treatment prevents fruit color changes during storage by changing the activity of some enzymes (i.e., inhibition of chlorophyllase activity), and delaying the formation of certain proteins (Brodli, 1989).

At the end of the storage period, the highest fruit color intensity (chromaticity) was found in the fruit treated with 800 $\mu\text{L}\cdot\text{L}^{-1}$ of SEO, which was 13.06% higher than that in the control fruit. However, there was no significant difference between 800 $\mu\text{L}\cdot\text{L}^{-1}$ SEO and 40 °C hot water treatments at both sampling times. After 30 d of storage, the control fruit had the lowest chromaticity (48.54, Fig. 4). The effects of SEO on improving color intensity during

Fig. 4. Effect of savory essential oil at concentrations of 800 $\mu\text{L}\cdot\text{L}^{-1}$ (EO800) and 1000 $\mu\text{L}\cdot\text{L}^{-1}$ (EO1000), hot water at 40 (HW40) and 50 °C (HW50), and gum arabic at 2.5 (GA2.5) and 5% (GA5) on fruit peel chromaticity of Mexican lime fruit inoculated by *Penicillium digitatum* after 15 and 30 d of storage at 8 °C. Vertical bars represent $\pm\text{SE}$ of means, where values are larger than the symbol.

Fig. 5. Effect of savory essential oil at concentrations of 800 $\mu\text{L}\cdot\text{L}^{-1}$ (EO800) and 1000 $\mu\text{L}\cdot\text{L}^{-1}$ (EO1000), hot water at 40 (HW40) and 50 °C (HW50), and gum arabic at 2.5 (GA2.5) and 5% (GA5) on ascorbic acid content ($\text{g}\cdot\text{kg}^{-1}$) (A), total soluble solids/titratable acidity (TSS/TA) (B) of Mexican lime fruit inoculated by *Penicillium digitatum* after 15 and 30 d of storage at 8 °C. Vertical bars represent $\pm\text{SE}$ of means, where values are larger than the symbol.

Fig. 6. Effect of savory essential oil at concentrations of 800 $\mu\text{L}\cdot\text{L}^{-1}$ (EO800) and 1000 $\mu\text{L}\cdot\text{L}^{-1}$ (EO1000), hot water at 40 (HW40) and 50 °C (HW50), and gum arabic at 2.5 (GA2.5) and 5% (GA5) on chlorophyll content ($\text{g}\cdot\text{kg}^{-1}$) of Mexican lime fruit peel inoculated by *Penicillium digitatum* after 15 and 30 d of storage at 8 °C. Vertical bars represent $\pm\text{SE}$ of means, where values are larger than the symbol.

storage could be due to its strong antioxidant property and the prevention of the fruit skin pigments' oxidation (Chen et al., 2014).

The treated fruit had more ascorbic acid content during storage compared with the nontreated control fruit (Fig. 5A). The highest content of ascorbic acid was found in the fruit treated with 40 °C hot water at day 15 of storage, which was 8.2 times greater than the control. The ascorbic acid content of the fruit showed a reducing trend during storage. However, the rate of decrease in ascorbic acid content was lower in the treated fruit compared with the nontreated control fruit. Heat treatments have been reported to overcome oxidative stress enzymatically and deactivate ascorbic acid oxidase (Chuah et al., 2008). The ascorbic acid content was significantly higher in SEO-treated fruit than control. However, the effect of SEO was less than the hot water treatments. Carvacrol, the dominant component detected in SEO (55.67%), is a phenolic compound which can be involved in prevention of ascorbic acid oxidation (Mathooko, 2003).

TSS/TA decreased in all treatments compared with the control (Fig. 5B). The lowest TSS/TA was found in the fruit treated with 40 °C hot water at day 15 of storage, and its ratio was 92.8% lower than that in the nontreated control fruit after 30 d of storage. The total acidity, as an important factor in maintaining fruit quality, directly depends on the concentration of organic acids in fruits (Jiang et al., 2013). Hot water treatment maintains the organic acids generated in the process of respiration (Chen and Paull, 2001). Fruit respiration rate can be reduced by hot water treatments and this may have reduced the use of organic acids in the respiratory enzyme reaction (Rabiei et al., 2011).

The highest chlorophyll content was found in the peel of the fruit treated with 40 °C hot water after 15 d of storage (Fig. 6), in which its amount was 5.2 times higher than in the nontreated control fruit. The lowest total chlorophyll content was measured in gum arabic (2.5%) treated fruit. It has been reported that heat treatment suppresses degrading enzymes, such as chlorophyllase, peroxidase, and oxidase, and reduces the degradation of chlorophyll and chlorophyll precursors (Kaewsuksaeng et al., 2011).

The highest total phenolic content (0.48 g·kg⁻¹) was found in the fruit treated with 800 µL·L⁻¹ of SEO (Fig. 7A), where phenolic content was 2.53 times higher than that in the nontreated control fruit after 30 d of storage. The lowest amount of total phenols was found in the nontreated control fruit (0.19 g·kg⁻¹) at the end of the storage period.

Generally, the antioxidant activity declined until the end of the storage period. The highest antioxidant activity (49.75%) was found in the fruit treated with 800 µL·L⁻¹ of SEO after 15 d of storage (Fig. 7B), where the antioxidant levels were 3.83 times higher than the control treatment. The lowest antioxidant activity (12.97%) was found in the nontreated control fruit.

The least PPO activity was found in the fruit treated with 800 µL·L⁻¹ of SEO after 15 d of storage; 9.50 times less than in the nontreated control fruit at 30 d of storage. The highest PPO activity was observed in the nontreated control fruit, and was significantly different from other treatments (Fig. 8). In both sampling times, the highest PPO activity was observed in control fruit.

SEO antioxidant activity was reported to be mostly related to the ability to increase phenolics (Chen et al., 2014). It has been found that SEO increases the activity of PAL, which can absorb and neutralize free radicals

(Pennycooke et al., 2005), thereby reduces the phenol degrading enzymes activity (Gardner et al., 2000). Overall, PPO activity increased during storage. Essential oils are phenolic compounds that react with the enzyme active site, thereby inhibiting the enzymes such as PPO (Alikhani-Koupaei, 2014). The ameliorative effect of heat treatment on PPO has been reported in pineapple fruit (Torres et al., 2010).

Conclusions

Hot water treatments for 5 min prevented decay development on the Mexican lime

Fig. 7. Effect of savory essential oil at concentrations of 800 µL·L⁻¹ (EO800) and 1000 µL·L⁻¹ (EO1000), hot water at 40 (HW40) and 50 °C (HW50), and gum arabic at 2.5 (GA2.5) and 5% (GA5) on total phenolic content (g·kg⁻¹) (A), antioxidant activity (%) (B) of Mexican lime fruit inoculated by *Penicillium digitatum* after 15 and 30 d of storage at 8 °C. Vertical bars represent ±SE of means, where values are larger than the symbol.

Fig. 8. Effect of savory essential oil at concentrations of 800 µL·L⁻¹ (EO800) and 1000 µL·L⁻¹ (EO1000), hot water at 40 (HW40) and 50 °C (HW50), and gum arabic at 2.5 (GA2.5) and 5% (GA5) on polyphenol oxidase enzyme activity (U·min⁻¹·g⁻¹) of Mexican lime fruit inoculated by *Penicillium digitatum* after 15 and 30 d of storage at 8 °C. Vertical bars represent ±SE of means, where values are larger than the symbol.

fruit. Hot water treatment at 40 °C caused the least negative physicochemical changes and maintained the quality of Mexican lime fruit during storage. SEO at 800 $\mu\text{L}\cdot\text{L}^{-1}$ was the best treatment to preserve bioactive compounds such as phenolic contents, increased antioxidant activity in fruit pulp, and restricted PPO activity in the fruit peel. Overall, both the hot water at 40 °C and SEO at 800 $\mu\text{L}\cdot\text{L}^{-1}$ are useful treatments for reducing the Mexican lime decay and preserving fruit quality during storage.

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