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Full Length Research Paper

Apple and quince peroxidase activity in response to essential oils application

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Enzymatic browning arises by peroxidase in fruits. However, essential oils are recognized as natural antioxidant agents. So in this study, the effect of thyme, coriander and rosemary essential oils were evaluated on the reduction of peroxidase activity in apples (*Malus domestica* Mill. cv Golden delicious), (*M. domestica* Mill. cv Red delicious) and quince (*Cydonia oblonga* Mill. cv. Esfahan). In this study, 50, 75, 100 or 200 µl/100 ml concentrations of pure essential oil were applied *in vivo* and *in vitro*. The results revealed the control samples of golden delicious, red delicious and quince had 185, 142.5 and 300 (units/min/g) peroxidase activity, respectively. *In vitro* 75 µl/100 ml of thyme and *in vivo* 50 µl/100 ml of thyme and 200 µl/100 ml of rosemary essential oils exerted the highest antioxidant ability (reductions in peroxidase activity by 92%) in the golden delicious. *In vitro* experiments were focused on the coriander essential oil treatments offering potential antioxidant benefits in the red delicious. Peroxidase activity was affected by *in vivo* 50 µl/100 ml concentrations of pure rosemary essential oil (reductions in peroxidase activity by 90%) when the enzyme was obtained from quince.

Key words: Peroxidase, essential oil, antioxidant, apple, quince.

INTRODUCTION

Enzymatic browning in fruits and vegetables tissues can cause undesirable quality changes (Nicoli et al., 1991; Khan and Robinson, 1993; Nicoli et al., 1994; Valderrama and Clemente, 2004). This reaction catalyzed by mostly peroxidase (EC 1.11.1.7) activity (Ponce et al., 2004; Alikhani et al., 2009). While peroxidase widely occurs, its main limitations are enzymatic stability and low production yield (Conesa et al., 2002; Eisenmenger and Corcuera, 2009). Therefore, inactivation of the peroxidase is considered necessary to minimize the possibility of deterioration and is important in the preservation of fruits and vegetables (Nicoli et al., 1991; Ponce et al., 2004; Valderrama and Clemente, 2004). Some reports revealed the peroxidase activity in apple (Leja et al., 2003; Wang et al., 1991). Leja et al. (2003) investigated the peroxidase involved in oxidative reactions of apple. Soluble peroxidase and peroxidase isoenzymes were investigated from the peel and pulp of apples cultivars Gala and Fuji by Valderrama and Clemente (2004). The inhibitory effects of chlorine dioxide on browning were studied in Golden Delicious apples (Fu et al., 2007). Inhibition of enzymatic browning in fruits is generally achieved using physical or chemical treatments such as heating (Nicoli et al., 1994; Hemeda and Klein, 1990; Valderrama and Clemente, 2004). Anti-browning agents, such as ascorbic acid treatment was evaluated for the elevation of shelf-life and decrease of browning of apple (Baldwin et al., 1996; Lee et al., 2003; Son et al., 2001). In general, it is accepted that peroxidase, unlike other enzymes which are inactivated by heat, remains active (Valderrama and Clemente, 2004). The commercial heat treatments used for processing fruit and vegetables is not very effective for irreversible inactivation of the peroxidase (Khan and Robinson, 1993), as well as increasing of soluble peroxidase activity was observed in apples kept in air and CA (Leja et al., 2003). Combination of an edible coating material and ascorbic acid is applied for extending the shelf life of quince (Yurdugul, 2005). Ascorbic acid is an antioxidant that sequesters harmful free radicals, which inhibit browning of fruit; hence, recently the use of natural compounds to control post-harvest browning is restricted. A number of studies demonstrated that antioxidant properties of essential oils were obtained from a

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reduction of enzymatic browning and development of the shelf life of fruit and vegetable with conservation of its quality (Lanciotti et al., 2004; Ponce et al., 2004; Alikhani et al., 2009). Because peroxidase requires oxygen for its catalytic activity, exposure to air via tissue injury will cause browning to occur (Ball, 1997). Essential oils eliminate the co-substrate oxygen and by this way could prevent enzymatic browning. Ponce et al. (2004) evaluated the effectiveness of natural essential oils to reduce peroxidase activity of leafy vegetable. Natural antioxidant phenolics of tea leaves on the activities of apple peroxidase were studied (Omiadze et al., 2004). Alikhani et al. (2009) stated that thyme essential oil using a dose of 250 or 400 ppm minimizes the peroxidase activity and enzymatic browning in the pear. Antioxidant activities of edible coatings enriched with natural plant extracts such as rosemary were studied *in vitro* and *in vivo* by Ponce et al. (2008).

The possible application of essential oils as natural antioxidant agents may be an alternative to chemical compounds and heat treatment preservation in fruits. Therefore, the purpose of this experiment was to use essential oils to reduce peroxidase activity in apple (Golden delicious and Red delicious) and quince.

MATERIALS AND METHODS

Raw materials

The fruits were bought at the local markets during experimentation. Apples (*Malus domestica* Mill. cv (Golden delicious), (*M. domestica* Mill. cv Red delicious) and Quince (*Cydonia oblonga* Mill. cv. Esfahan) were used as raw materials for peroxidase.

Antioxidant application

Two types of antioxidant application were used: (1) Extract of fresh raw fruits with no treatment supplied (*in vitro* samples), afterwards 0.03 ml of antioxidants solution were added to the extract for the determination of peroxidase activity. (2) Fresh raw fruits immersed in antioxidant solution (essential oils, ascorbic acid or water) and was dried by exposure to room temperature (*in vivo* samples) and then prepared for peroxidase assay (Ponce et al., 2008).

Antioxidant source

The essential oils utilized for antioxidant source were thyme (*Thymus vulgaris*), coriander (*Coriandrum sativum*) and rosemary (*Rosmarinus officinalis*). Essential oils were obtained with hydro-distillation by Clevenger. Pure concentration, 200, 100, 75, and 50 µl/100 ml of essential oils used were obtained from MIC that was taken as the concentration of essential oils that inhibit the native microflora. For supply of solutions, essential oils were solved in ethanol 96% and Tween 80, then it diluted with distilled water (Ponce et al., 2003; Moreira et al., 2005).

Extracts preparing (Source of enzyme)

10 g of each fruits were chopped and then 30 ml of water added during homogenisation. The slurry was centrifuged (SIGMA-3K30) at 10000 g for 15 min at 4°C. The supernatant, which contained peroxidase activity, was used as the enzyme source for the experiment (Ponce et al., 2004).

Substrate preparing

The substrate mixture contained 10 ml of 1% guaiacol, 10 ml of 0.3% hydrogen peroxide and 100 ml of 0.05 M sodium phosphate (pH 6.5) buffer. The reaction cuvette contained 2.87 ml substrate mixture, 0.1 ml crude extract, and 0.03 ml treatment solution (essential oils, ascorbic acid and water) in a total volume of 3 ml (Ponce et al., 2004).

Determination of enzyme activity

Peroxidase activity was determined at 25°C with a spectrophotometer (PD-303UV) at 470 nm using guaiacol as the substrate and H_2O_2 as the hydrogen donor. One unit of activity is defined as a change in absorbance of 0.001 min^{-1} (Ponce et al., 2004).

Essential oils antioxidant activity

In order to compare essential oils antioxidant activity, the deionized water and synthetic ascorbic acid were utilized as a control sample and chemical control, respectively. For each enzyme source instead of essential oils, 0.03 ml deionized water or ascorbic acid (0.017 g/100 L) were added to reaction cuvette (Ponce et al., 2004).

Statistical analysis

For data collection, the experiment was established in factorial trait using complete randomized design with 3 factors including (antioxidant application (*in vitro* and *in vivo)*, antioxidant source (thyme, coriander, rosemary, ascorbic acid and water) and concentration. Concentration supplemented in 7 levels (pure, 50, 75, 100 and 200 µl/100 ml for essential oils, 0.017 g/100 L for ascorbic acid and water). All experiments were defined in 4 replications. The data of tests were normalized by LOG. The ANOVA was performed for analysis and the means were done by least significant difference (LSD) test (P<0.01). Group comparison was used for analysis of these comparisons: C_1- Comparison applying thyme, coriander and rosemary essential oils with control. C_2- Comparison between applying thyme with coriander. C_4- Comparison between applying thyme with rosemary. C_3- Comparison between applying coriander with rosemary (Soltani, 2007). Data were analyzed by SAS software (2001).

RESULTS

In vivo and *in vitro* peroxidase activity

Based on ANOVA (Table 1) and group comparison (Table 3), the results revealed that there was a significant difference (P<0.01) in the essential oils effects on peroxidase activity in the apples and quince. As shown in Table 1, the trilateral interaction of experimental factors (antioxidant application × antioxidants source × antioxidant concentration) is significant (P<0.01).

It was found that quince has higher peroxidase activity than apple cultivars (Table 2). Control of quince, golden

Table 1. ANOVA of antioxidant treatment relative whit peroxidase activity of golden delicious, red delicious and quince.

S.O.V	d.f	Sum of square		
		Golden delicious	Red delicious	Quince
Application (a)	1	0.49*	0.03^{ns}	0.07^{ns}
Antioxidant (b)	2	0.93**	0.08^{ns}	0.29**
Concentration (c)	4	1.39**	0.54**	0.74**
a×b	2	0.007^{ns}	0.23**	0.75*
a×c	4	1.67**	0.35**	0.13^{ns}
b×c	8	4.45**	0.21^{ns}	0.40*
a×b×c	8	2.47**	0.73**	0.70**
error	99	7.57	1.61	2.05
CV %		17.74	7.96	7.88

** (P<0.01), * (P<0.05), ^{ns} (P>0.05).

delicious and red delicious had 300, 185 and 142.5 (units/min/gr) peroxidase activities, respectively. On basis of results, there were significant difference between *in vitro* and *in vivo* application of essential oils in the red delicious and quince. The activity of the peroxidase obtained from red delicious was not significantly affected by thyme and rosemary. Yet its activity was strongly influenced by coriander when it was exerted *in vitro* (Table 2 and Figure 2). In this study, *in vivo* application of rosemary essential oil had high ability to minimize the peroxidase activity in the quince (Table 2 and Figure 3). There was no significant difference (P>0.05) between *in vitro* and *in vivo* application of essential oils in the golden delicious (Table 2 and Figure 1). As shown in group comparison (Table 3), there was a significant difference between the application of coriander and rosemary as reduction of peroxidase activity in the quince (Table 3).

In vitro antioxidant activity of essential oil

From the results (Table 3), the antioxidant activity of thyme, coriander and rosemary essential oils was significantly different in golden delicious, red delicious and quince (P<0.01). As shown in Table 2, the highest percentage of antioxidant activity (92%) was obtained under *in vitro* 75 µl/100 ml of thyme in the golden delicious (P<0.01). *In vivo* and *in vitro* experiments were focused on the pure concentration of thyme, coriander and rosemary essential oils treatments offering potential antioxidant benefits in the red delicious, which had no significant difference with the application of ascorbic acid (Table 2). In red delicious, *in vitro* concentrations of coriander caused 79% antioxidant activity (Table 2).

In vivo antioxidant activity of essential oil

As shown in Table 2, the highest percentage of antioxidant activity (92%) was obtained under *in vivo* 50 µl/100 ml of thyme and 200 µl/100 ml of rosemary in the golden delicious (P<0.01). Also, the highest percentage antioxidant activity (90%) was attained under 50 µl/100 ml and pure concentrations of rosemary essential oil in the quince when it was applied *in vivo* (Table 2). Antioxidant ability observed via *in vivo* and *in vitro* application of ascorbic acid in the golden delicious, red delicious and quince was 84, 79 and 85%, respectively (Table 2).

DISCUSSION

In vivo and *in vitro* peroxidase activity

The results revealed that peroxidase content of golden delicious (185 units/min/gr) is higher than red delicious (142.5 units/min/g). Valderrama and Clemente (2004) reported that a higher enzymatic activity for soluble and ionically bound peroxidase fractions was extracted from apple cultivars Gala and Fuji. They reported that the peroxidase activity in the enzymatic extracts of pulp of the apple cultivars Gala and Fuji was 960 and 8470 units, respectively. Leja et al. (2003) observed in apples of Sampion and Jonagold cultivars, the activity of peroxidase was 8.20 and 2.10 (mg AA 100g $^{-1}$ f.w.s^{-1}), respectively.

In this study, the peroxidase was not completely inactivated at the tested antioxidants. The residual activety of the peroxidase is at least 15 unit/min/g, (a maximum of 92% reduction of peroxidase) indicating a minimum inactivation of peroxidase in the golden delicious.

In vitro and *in vivo* antioxidant activity of essential oil

In this study, pure concentration of thyme, coriander and rosemary essential oils had grand antioxidant ability in

Table 2. Effect of antioxidant agents (thyme, coriander, rosemary and ascorbic acid) on peroxidase content (Units/min/g) and percentage of peroxidase activity reduction (Numbers in bracket) of golden delicious, red delicious and quince.

Activity	Antioxidant	Concentration (µl/100 ml)	Golden delicious	Red delicious	Quince
In vitro	Thyme	50	30 (84) ^{c}	60 (58) ^{bc}	60 (80) ^{def}
		75	15 (92) ^{d}	75 (47) ^{b}	105 (65) ^{bc}
		100	90 (52) ^{ab}	30 (79) ^{d}	75 (75) ^{cde}
		200	45 (76) ^{bc}	45 (68) ^{cd}	120 (60) ^{b}
		pure	105 (43) ^{ab}	30 (79) ^{d}	45 (85) ^{ef}
	Coriander	50	105 (43) ^{ab}	30 (79) ^{d}	60 (80) ^{def}
		75	30 (84) ^{c}	30 (79) ^{d}	60 (80) ^{def}
		100	90 (51) ^{ab}	30 (79) ^{d}	75 (75) ^{cde}
		200	60 (68) ^{bc}	30 (79) ^{d}	60 (80) ^{def}
		pure	30 (84) ^{c}	30 (79) ^{d}	60 (80) ^{def}
	Rosemary	50	30 (84) ^{c}	45 (68) ^{cd}	90 (70) ^{bcd}
		75	30 (84) ^{c}	30 (79) ^{d}	105 (65) ^{bc}
		100	45 (76) ^{bc}	60 (58) ^{bc}	90 (70) ^{bcd}
		200	30 (84) ^{c}	60 (58) ^{bc}	97.5 (67) ^{bc}
		pure	45 (76) ^{bc}	30 (79) ^{d}	45 (85) ^{ef}
	Ascorbic acid	0.017 (g/100 L)	30 (84) ^{c}	30 (79) ^{d}	45 (85) ^{ef}
In vivo	Thyme	50	15 (92) ^{d}	30 (79) ^{d}	90 (70) ^{bcd}
		75	30 (84) ^{c}	30 (79) ^{d}	105 (65) ^{bc}
		100	30 (84) ^{c}	75 (47) ^{b}	75 (75) ^{cde}
		200	60 (68) ^{bc}	60 (58) ^{bc}	60 (80) ^{def}
		pure	45 (76) ^{bc}	30 (79) ^{d}	90 (70) ^{bcd}
	Coriander	50	45 (76) ^{bc}	45 (68) ^{cd}	120 (60) ^{b}
		75	45 (76) ^{bc}	60 (58) ^{bc}	60 (80) ^{def}
		100	45 (76) ^{bc}	75 (47) ^{b}	90 (70) ^{bcd}
		200	30 (84) ^{c}	30 (79) ^{d}	90 (70) ^{bcd}
		pure	30 (84) ^{c}	30 (79) ^{d}	45 (85) ^{ef}
	Rosemary	50	45 (76) ^{bc}	45 (68) ^{cd}	30 (90) ^{f}
		75	30 (84) ^{c}	45 (68) ^{cd}	75 (75) ^{cde}
		100	30 (84) ^{c}	60 (58) ^{bc}	45 (85) ^{ef}
		200	15 (92) ^{d}	45 (68) ^{cd}	45 (85) ^{ef}
		pure	30 (84) ^{c}	30 (79) ^{d}	30 (90) ^{f}
	Ascorbic acid	0.017 (g/100 L)	30 (84) ^{c}	30 (79) ^{d}	45 (85) ^{ef}
	Control	0	185 (0) ^{a}	142.5 (0) ^{a}	300 (0) ^{a}
	LSD		30.3	24.57	33.51

Means followed by the same letter are not significantly different at 1% by LSD. Numbers in bracket: percentage of peroxidase activity reduction.

the red delicious. Our results showed the 92% antioxidant activity in the golden delicious when 50 µl/100 ml of thyme and 200 µl/100 ml of rosemary were applied *in vivo*, and 75 µl/100 ml of thyme was used *in vitro*. Omiadze et al. (2004) reported apple peroxidase was inhibited by lower concentrations of the antioxidative tea polyphenols. The *in vivo* experiments were focused on the pure and 50 µl/100 ml concentrations of rosemary treatments offering 90% antioxidant activity in the quince. Alikhani et al. (2009) reported that usage of thyme

Table 3. Group comparison of applying essential oils as a peroxidase reducing agent in the golden delicious, red delicious and quince.

C	Treatment							Sum of square		
	Thyme *in vivo*	Thyme *in vitro*	Coriander *in vivo*	Coriander *in vitro*	Rosemary *in vivo*	Rosemary *in vitro*	Control	Golden delicious	Red delicious	Quince
C_1	+1	+1	+1	+1	+1	+1	-6	0.92**	0.10*	0.44**
C_2	+1	+1	-1	-1	0	0	0	1.74**	0.32**	0.61**
C_3	+1	+1	0	0	-1	-1	0	2.61**	0.30**	0.49**
C_4	0	0	+1	+1	-1	-1	0	0.17^{ns}	0.0007^{ns}	0.38**

** ($p<0.01$), ^{ns} ($p>0.05$) .C, Comparison; C_1, comparison *in vivo* and *in vitro* applying of thyme, coriander and rosemary essential oils with control; C_2,comparison between *in vivo* and *in vitro* applying of thyme with coriander; C_4, comparison between *in vivo* and *in vitro* applying of thyme with rosemary; C_3, comparison between *in vivo* and *in vitro* applying of coriander with rosemary.

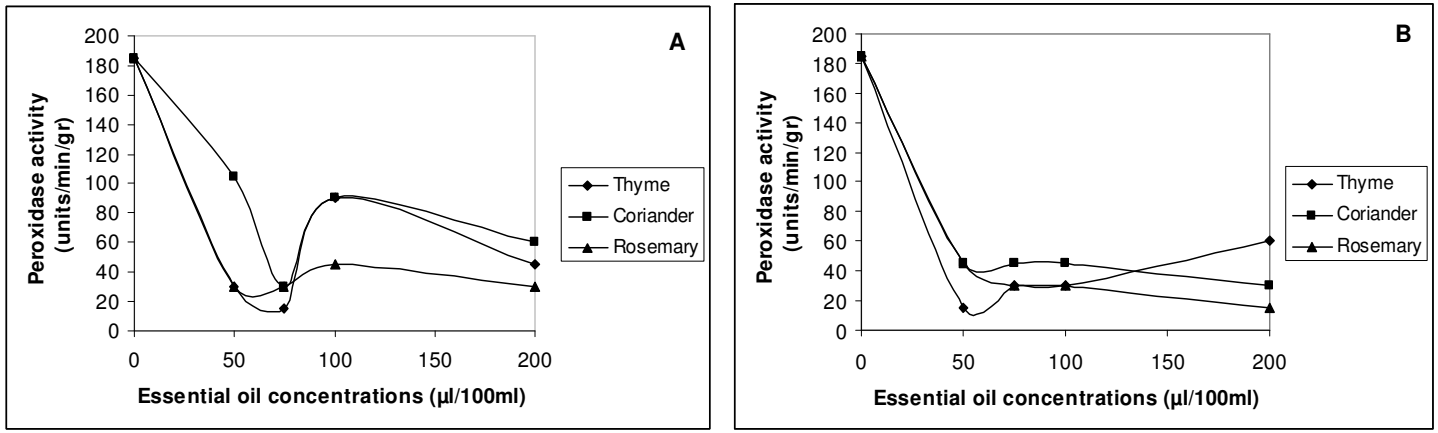

Figure 1. Change of peroxidase activity in the presence to applying of thyme, coriander and rosemary essential oils *in vivo* (A) and *in vitro* (B) of golden delicious ($LSD_{0.01}$ = 30.3).

essential oil on pear can minimize the peroxidase. In this study, *in vitro* and *in vivo* thyme essential oil exerted the highest reduction of peroxidase activity on the quince and apple cultivars. Some reports concluded that there is a significant difference between vegetables as essential oils antioxidant activities (Alikhani et al., 2009; Ponce et al., 2004) as well as the effectiveness of the essential oils as natural antioxidants varied with the enzyme sources (Ponce et al., 2004, 2008). Hemeda and Klein (1990) revealed the differences in the peroxidase activity against antioxidant agents could be related to the presence of isoenzymes. The loss of the activity of these isoenzymes is associated with the origin of the enzyme (Valderrama and Clemente, 2004). So, Valderrama and Clemente (2004) purified various peroxidase isoenzymes from peel and

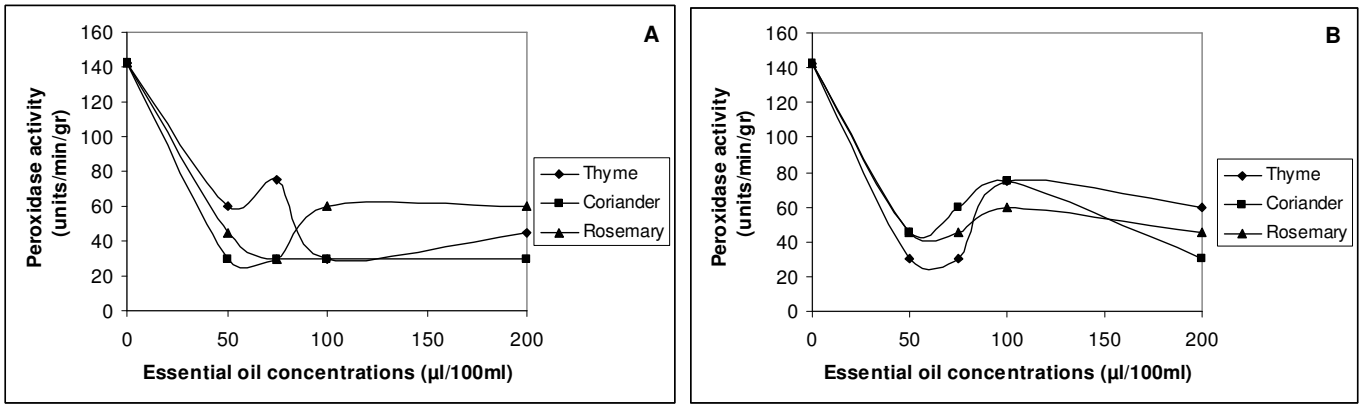

Figure 2. Change of peroxidase activity in the presence to applying of thyme, coriander and rosemary essential oils *in vivo* (A) and *in vitro* (B) of red delicious ($LSD_{0.01}$ = 24.57).

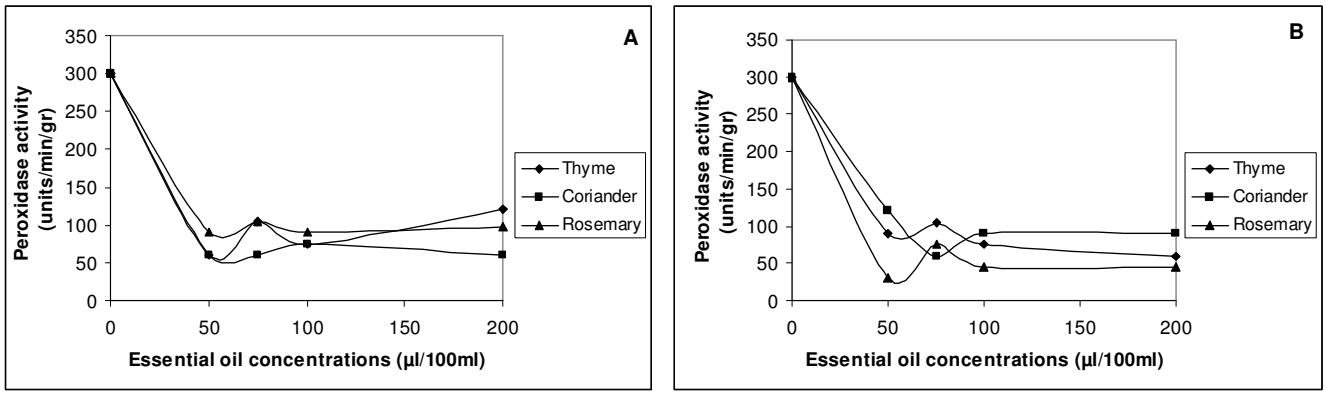

Figure 3. Change of peroxidase activity in the presence to applying of thyme, coriander and rosemary essential oils *in vivo* (A) and *in vitro* (B) of quince ($LSD_{0.01}$ = 33.51).

pulp of apple cultivars.

Ascorbic acid antioxidant activity

Ascorbic acid has been widely used as an antibrowning agent for processing of fruits and vegetables (Son et al., 2001). The results revealed that *in vitro* and *in vivo* application of ascorbic acid decreased the peroxidase activity on the fruits and its antioxidants activities were high (Table 2). The best studied function of ascorbic acid is its participation in the detoxification of active oxygen species in chloroplasts, cytosol and peroxisomes through its oxidation by ascorbate peroxidase activity (Talano et al., 2008). Some reports indicated that ascorbic acid delayed browning more effectively in apple (Baldwin et al., 1996). Also, ascorbic acid in 1% solution effectively inhibits browning of apple slices (Son et al., 2001). Yurdugul (2005) found that ascorbic acid provides high quality for the quince, leading to an extension in shelf life. Likewise addition of various antibrowning agents such as ascorbic acid to coating solutions was advantageous in maintaining color of Fuji apples (Lee et al., 2003). Alikhani et al. (2009) concluded that usage of ascorbic acid minimized the peroxidase activity in pear extract.

Conclusions

The use of essential oils applied to golden delicious, red delicious and quince improved the antioxidant protection of the fruits thereby offering a great advantage in the prevention of peroxidase activity. *In vitro* and *in vivo* studies comparing the thyme, coriander and rosemary antioxidant properties did not observed great different behaviors. This indicates that *in vitro* and *in vivo* studies exert essential oils effect over peroxidase. However, when essential oils were used *in vivo* as a coating and applied directly to this fruits, it enhanced its antioxidant properties over peroxidase enzyme. This result would indicate that as respects to antioxidants properties of essential oils, this natural compound, as a coating, could be use to minimize the peroxidase activity and enzymatic browning in the golden delicious, red delicious and quince.

REFERENCES

Alikhani M, Sharifani M, Azizi M, Hemmati K, Mousavizadeh SJ (2009). The effect of some natural compounds in shelf-life and quality of Pear fruit (Esfahan Shah mive cultivar) (In Persian). J. Agric. Sci. Natur. Resour., 16: 158-171.

Baldwin EA, Nisperos MO, Chen X, Hagenmaier RD (1996). Improving storage life of cut apple and potato with edible coating. Postharvest Biol. Technol., 9(2): 151-163.

Ball JA (1997). Evaluation of two lipid-based edible coatings for their ability to preserve post harvest quality of green bell peppers. A thesis of Master of Science in Human Nutrition, Foods, and Exercise. Virginia State University, Polytechnic Institute. p. 89.

Conesa A, Punt PJ, vanden-Hondel CAMJJ (2002) Fungal peroxidases: molecular aspects and applications. J. Biotechnol., 93: 143-58.

Eisenmenger MJ, Corcuera JIR (2009) High pressure enhancement of enzymes: A review. Enz. Microbiol. Technol., 45: 331-347.

Fu Y, Zhang K, Wang N, Du J (2007) Effects of aqueous chlorine dioxide treatment on polyphenol oxidases from Golden Delicious apple. LWT. 40: 1362-1368.

Hemeda HM, Klein BP (1990) Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. Food Sci. 55: 184-186.

Khan AA, Robinson DS (1993) The thermostability of purified mango soperoxidases. Food Chem. 47: 53-59.

Lanciotti R, Gianotti A, Patrignani F, Belletti N, Gverzoni ME, Gardini F (2004) Use of natural aroma compound to improve shelf life and safety of minimally processed fruit. Trend. Food. Sci. Technol., 15: 201-208.

Lee JY, Park HJ, Lee CY, Choi WY (2003) Extending shelf-life of minimallyprocessed apples with edible coatings and antibrowning agents. LWT. 36: 323-329.

Leja N, Mareczek A, Ben J (2003) Antioxidant properties of two apple cultivars during long-term storage. Food Chem. 80: 303-307.

Moreira MR, Ponce AG, Delvalle CE, Roura SI (2005). Inhibitory parameters of essenial oils to reduce a foodborne pathogen. LWT. 38: 565-570.

Nicoli MC, Anese M, Severini C (1994). Combined effects in preventing enzymatic browining reaction in minimally processed Fruit. J. Food Qual. 17: 221-229.

Nicoli MC, Elizalde BE, Pitotti A, Lerici CR (1991). Effect of sugares and maillard reaction products on polyphenol oxidase and peroxidase activity in food. J. Food Biochem. 15: 169-184.

Omiadze N, Parlar H, Leupold G, Mchedlishvili N, Gulua L, Akhvlediani K, Abutidze M, Sadunishvili T, Rodriguez-lopez JN, Kvesitadze G (2004). Inhibition of apple phenoloxidase and peroxidase by natural phenolics of tea leaves. Adv. Food Sci. 26: 26-31.

Ponce AG, Delvalle CE, Roura SL (2004). Natural essential oils as reducing agents of peroxidase activity in leafy vegetables. LWT. 37: 199-220.

Ponce AG, Fritz R, Delvalle CE, Roura SI (2003). Antimiribial activity of essential oils on native mirobial population of organic Swiss chard. LWT. 36: 679-684.

Ponce AG, Roura SI, DelValle CE Moreira MR (2008). Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: *In vitro* and *in vivo* studies. Postharvest Biol. Technol. 49: 294-300.

SAS Institute (2001) SAS/StaT user´s guide. Version 9. SAS Institute, Cary, N.C.

Soltani A (2007) Application SAS in statistical analysis. Mashhad University Press. p. 182.

Son SM, Moon KD, Lee CY (2001) Inhibitory effects of various antibrowning agents on apple slices. Food Chem. 73: 23-30.

Talano AM, Agostini E, Oller WLA, Medina IM, Forchetti MRS (2008) Modulator role of ascorbic acid on coniferyl alcohol oxidation by a basic peroxidase from tomato hairy roots. Plant Sci. 175: 724-730.

Valderrama P, Clemente E (2004) Isolation and thermostability of peroxidase isoenzymes from apple cultivars Gala and Fuji. Food Chem. 87: 601-606.

Wang SY, Jiao HJ, Faust M (1991) Changes in the activities of catalase, peroxidase and polyphenol oxidase in apple buds during break induced by thidiazuron. J. Plant Growth Regul. 10: 33-39.

Yurdugul S (2005). Preservation of quinces by the combination of an edible coating material, Semperfresh, ascorbic acid and cold storage. Eur. Food Res. Technol. 220: 579-586.