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## EFFECT OF EXTRACTION METHOD OF SOME NATURAL EXTRACTS ON ENZYMATIC BROWNING OF APPLE JUICE

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### ABSTRACT

A comparative study concerning the inhibition of the enzymatic browning in apple juices stored for 24 hours at room temperature (25°C) was carried out between the natural extract of some vegetables and Oyster mushroom with different extraction methods (water, ultra-filtration and alcoholic). The colour changes were assayed quantitatively involved % inhibition using Hunter Lab colorimeter and polyphenoloxidase activities. The results showed that higher inhibition of enzymatic browning was obtained at the end of 24 hour storage period with the extracts of squash, cucumber and pepper followed by mushroom in apple juices. Meanwhile alcoholic and UF extracts were effective in maintaining the storage quality of apple juices. They were found as effective inhibitors. The obtained results confirmed the presence of a proportional relation between the natural vegetable extracts by different extraction methods and their inhibitory effect, whereas the Oyster mushroom extracts in storage time (24 hours) showed an opposite trend. The inhibitory effect of the natural squash extract was comparable with its different extraction method and being higher than pepper and cucumber under all experimental conditions. The natural extracts derived from vegetables were more active than their counterpart derived from mushroom. However, each of them could be considered as potential natural inhibitor having technological and commercial benefits to be applied in processed fruits.

**Key words:** Enzymatic browning, polyphenoloxidase, colour, apple, juices, cucumber, pepper, squash, Oyster mushroom, extracts, water, alcoholic, ultra-filtration.

### INTRODUCTION

Browning occurs usually in certain fruits and vegetables during post harvest, handling, processing operations and storage after harvest. The browning phenomenon is mainly due to the enzymatic oxidation of endogenous phenolic compounds catalysed by polyphenol oxidase (PPO, EC 1.14.18.1) or tyrosinase inherent in biological tissues (McEvily et al., 1992). The PPO is a copper-containing enzyme that catalyses, in the presence of oxygen, the oxidation of diphenols to o-quinones which further lead to polymerizing brown pigments. It is impossible to neglect this PPO-catalysed browning in color occurring in food and food stuffs because unpleasant appearance and concomitant generally result in loss of nutritional and market values (Lee 1992). However, apple is one of the most popular fruits, consumed all over the world and thus the susceptibility of apple and their products to enzymatic browning (PPO). The enzymatic browning of apple has been investigated by many researchers (Murata et al., 1995 and Mi et al., 2002), since enzymatic browning of this particular fruit is an important topic from the standpoint of food science and technology (Iyidogan and Bayindirh 2004 and Eissa et al., 2006).

The PPO-catalysed browning of food can be prevented by the addition of bisulfite (Regina and Goreti 2001), reduced glutathione and thiol-compounds (Sezgintürk and Dinçkaya 2004; Regina and Goreti 2001 and Eissa et al., 2006) as well as L-cysteine (Hülya and Ayten 2002; Emine and Sule 2004; Eissa et al., 2006). Thereby several studies have been devoted to the non-sulfite anti-browning agents, among these browning inhibitors, ascorbic acid and its derivatives and 4-hexyl resorcinol (4HR) have been used commercially with limited success (Cowan et al., 2000; Hülya and Ayten 2002; Emine and Sule 2004).

For instance, sulfites are commercially used as effective inhibitors for PPO but these compounds have been restricted by the Food and Drug Administration (FDA) due to the possibility of its associated potential hazards. Although the bisulfites are effective to prevent browning, they could be harmful for human health, especially in asthmatic patients. However, these compounds can have adverse health effects and can also react with other components in the food system, resulting in unwanted effects (Sapers et al., 1989 and McEvily et al., 1992). The need for a safe and effective PPO inhibitor has

focused the research in the finding of natural inhibitors for PPO.

From this concept, there have been a lot of reports on the PPO inhibitors occurring in natural extracts or resources like honey (Oszmiansky & Lee, 1990), some fruits such as Pineapple and lemon juices (Patricia et al., 1993), Some of the leafy vegetables extracts, such as cabbage, mallow, fenugreek leaves, fig leaves and celery, contain ascorbic acid and sulfur dioxide (SO<sub>2</sub>) (Eissa and Salama 2002 and Ohad et al., 2006), some of spices extracts such as cardamom, clove and ginger are considered to be anti-browning agents of apple products (Eissa et al., 2003 a, b) as well as numerous species of mushrooms, including *Agaricus bisporus* (Esp<sup>o</sup>n et al., 1999 and Sibel et al., 2004) and 'Enokitake' mushroom (Mi et al., 2002).

The use of natural extracts by different methods, a powerful anti-browning agent, is discussed more because of the potential hazards of chemical agents. Thus, an active field of research is currently under development to find non-sulfite anti-browning agents as natural extracts by different methods for food industry (Cowan 1999; Son et al., 2001; Mi et al., 2002; Eissa and Salama 2002; Iyidogan and Bayindirh 2004; Sibel et al., 2004; Eissa et al., 2006 and Ohad et al., 2006).

However, no reports have been found on the inhibitory effect of edible 'Oyster' mushroom and some vegetables (cucumber, squash and pepper) on PPO activity so far. Because of, there is an increasing demand of consumers for substituting synthetic compounds with natural substances as food ingredients. Therefore, the compounds inherent in natural origins are widely accepted by consumers in the market. This study was designed for the search of natural browning control agents, because PPO inhibitors isolated from regularly consumed foods may be safer compared to non-natural products.

Due to the properties of red delicious apple juices but higher rates of browning, it will be beneficial to investigate the effectiveness of different natural extracts (squash, cucumber, pepper and mushroom) with different extraction methods on enzymatic browning in the red delicious apple juice. The main objective of this work was to study the inter-related effects of squash, cucumber, pepper and Oyster mushroom extracts on enzymatic browning (PPO) in apple juices. Also, to evaluate the inhibitory pattern of the natural vegetables and 'Oyster' mushroom extracts that well documented to have anti-browning effects in vivo against the enzymatic browning of apple juices stored for 24 h at 25 °C in comparison with the different extraction methods.

## **MATERIALS AND METHODS**

### **MATERIALS**

#### **APPLE FRUITS**

Apples: Red delicious (*Malus domestica* c.v. *Borkh*) were purchased from local market at the commercial maturity stored at 4 °C until used in about 2-3 days. The Delicious cultivar was chosen because of its

wide popularity as a food and rapid browning of the juices after preparation. Apple fruits were washed with water before the treatment.

#### **PREPARATION OF MUSHROOM**

Fresh cultivated Oyster Mushroom (*P.Sajor-Caju*, strain 290) on rice straw was purchased from the Center of Mushroom service (Comet, Com. Cairo, Egypt). Oyster Mushroom was washed with cold water and blanched with steam for 7 min, dried by air drier oven heated to 60°C for 4.5 h. and milled. The use of heat during drying hardens the texture and completely inactivates the enzyme polyphenoloxidase (PPO) in mushrooms (Paeaeckkoenen and Kurbela (1987).

#### **PREPARATION OF SOME VEGETABLES**

Cucumber (*Cucumis sativus* L.), squash (*Cucurbita pepo* L., cv. summer, mainly zucchini) and green pepper (*Capsicum annum* L., cv. Golden Summer) were purchased from the store of the Ministry of Agriculture, Cairo, Egypt, at the commercial maturity and stored at 4 °C until used in about 2-3 days. All vegetables (cucumber, squash and green pepper) were prepared by washed with water, sliced, trimmed, the cores discarded and the seeds and interlobular material removed.

#### **PREPARATION OF APPLE JUICE**

Apple (*Red delicious*) samples representing common cultivars were obtained from local food stores and stored briefly at 4°C until needed. One hour prior to use, fruits were removed from the refrigerator and equilibrated to room temperature. Apple fruits were rinsed with water, sectioned to longitudinal slices. Apple juice samples were prepared from individual apples (*red delicious*) with a juicerator and filtered by 3 layers of cheese cloth. Juice was collected in a beaker containing 5mg ascorbic acid / 100ml juice with stirring. The amount of ascorbic acid used was not enough to prevent browning for more than 1 hr. However, the ascorbic acid was used to prevent instantaneous browning, thereby providing a short lag time to allow test materials (natural extracts) to be added and mixed (Sapers and Douglas, 1987).

### **PREPARATION METHODS OF NATURAL EXTRACTS**

#### **WATER EXTRACTION**

Samples of mushroom and vegetables were mixed by blender with hot water (55-60 °C) at the concentration of 20% (w/v) for 30s. The aqueous extract was allowed to cool to 25°C and filtered by three layers of cheese cloth, as described by the method of Asehrou, et al (1997). The solution was centrifuged (3000 rpm for 10 min) and the supernatant was retained. Under the above conditions, the extent of extraction of mushroom and vegetables reached up to 90%. The filtered material was used as a source of PPO inhibitors.

#### **ULTRA-FILTRATION EXTRACTION**

Ultra Filtration extraction of mushroom and

vegetables was carried out according to the method of Gandia-Herrero et al., (2003) with some modifications.

120 g of some vegetables and mushroom were homogenized in model MORAT-Motor-Stirrer R 270 (Franz MORAT KG, GmbH & Co., Frano® - Geratetechnik, Germany) with 240 ml of 50 mM sodium phosphate buffer (pH 7), at 4 °C. The homogenate was filtered through two layers of cheese cloths and centrifuged at 5000 rpm for 40min. The sediment was discarded and the supernatant was ultra filtered or passed through 62 mm diameter DIAFLO Ultra-filtration membrane (DIAFLO-UF membranes, AMICON, INC., Beverly, MA, USA) having molecular weight cut-off (MWCO) value of 10,000 Dalton under pressure of 60 Psi at 4 °C and N<sub>2</sub> 40 kg.cm<sup>-2</sup> (76 mm in diameter, Millipore corporation, Bedford, MA 01730, USA). The filtered material was used as a source of PPO inhibitors.

### ALCOHOLIC EXTRACTION

Alcoholic extraction of mushroom and some vegetables was carried out according to the method of Cowan (1999) with some modifications.

40 g of vegetables fruits and mushroom were homogenized in model MORAT-Motor-Stirrer R 270 (Franz MORAT KG, GmbH & Co., Frano® - Geratetechnik, Germany) with 100 ml of methyl alcohol (methanol) for 30 min. The homogenate was filtered through two layers of cheese cloths and centrifuged at 3000 rpm for 20min. The sediment was discarded and the supernatant was mixed with the same volume of distilled water. The methanol in the mixture was evaporated using Rotavapor (Model: RE121-type: B-461, Büchi, Lab. AG, CH 9230, FLAWIL / SCHWEIS) at 300 rpm and 50 °C with oil pump under vacuum (TEISTAR, CE, Spain) to evaporate all methanol from samples mixture extract. The extract after evaporation of methanol was used as a source of PPO inhibitors.

### APPLE JUICE TREATMENTS AND PROCESSING

Apple fruit were washed under water current and juiced with ordinary domestic food processor. Samples of juice (100 ml) were poured into beakers containing 1% concentration of the natural extracts (vegetables or mushroom) by water, UF and alcoholic extraction and immediately stirred with a magnetic stirrer for 24 hours. A juice sample containing no natural extracts was considered as control. Each prepared sample was immediately subjected to absorbance and colour measurements as well as estimation of polyphenoloxidase enzyme activity during storage at 4 °C for 24 hours.

### EVALUATION OF BROWNING CAPACITY IN APPLE JUICE

Portions of Cucumber, Squash, Green Pepper and Mushroom natural extracts treated juice and untreated sample (25ml in 50ml beakers containing magnetic stirrer bars, covered to prevent evaporation), were stirred at 400 rpm for as long as 24 hours at room temperature on a stirrer to accelerate enzymatic browning if it were to occur. Juices were held at room temperature with stirring during

which time tristimulus reflectance L\*, a\* and b\*-values for controls and treated juices were periodically measured during 24 hours, using a spectro colorimeter (Hunter, LabScan XE, USA) with White Tile of Hunter Lab Color Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14(L\*= 92.46; a\*= -0.86; b\*= -0.16) (Sapers and Douglas, 1987). Also, the tristimulus reflectance L\*, a\* and b\*-values for controls and treated juices were periodically measured at 1, 30, 60, 120, 180 and 360min., and after 24 hours during storage at 4°C for 24 hours.

### ANALYTICAL METHODS

#### POLYPHENOLOXIDASE ACTIVITY

Polyphenoloxidase (PPO) enzyme activity was determined using 0.1M catechol as substrate in sodium phosphate buffer (pH 7.0) according to the procedure given in the study of Özoğlu and Bayindirh (2002). One unit of enzyme activity for polyphenoloxidase was defined as 0.001 Δ A<sub>420</sub> S<sup>-1</sup> under the assay conditions.

#### ABSORBANCE MEASUREMENT (A<sub>420NM</sub>)

Absorbance values at A<sub>420nm</sub> were recorded with 4054 U.V/ visible spectrophotometer (LKB-Biochrom) according to the method of İyidoğan and Bayindirh, (2004).

#### COLOR MEASUREMENTS

Objective evaluations of apple juice samples were measured. Hunter a\*, b\* and L\* parameters were measured with a color difference meter using a spectro-colorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Color Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L\*= 92.46; a\*= -0.86; b\*= -0.16) (Sapers and Douglas, 1987).

The Hue (H)\*, Chroma (C)\* and Browning Index (BI) was calculated according to the method of Palou et al., (1999) as follows:

$$H^* = \tan^{-1} [b^*/a^*] \dots \dots \dots \text{Equation [1]}$$

$$C^* = \text{square root of } [a^{*2} + b^{*2}] \dots \dots \dots \text{Equation [2]}$$

$$BI = [100 (x-0.31)]^{10.72} \dots \dots \dots \text{Equation [3]}$$

Where:-

$$X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$$

#### ESTIMATION OF INHIBITION

The browning inhibition based on L-mesurments and A<sub>420</sub> was calculated using the equations of İyidoğan and Bayindirh (2004):

$$\text{Inhibition (\%)} = (\Delta A_{420, \text{ control}} - \Delta A_{420, \text{ treatment}}) \times 100 / \Delta A_{420, \text{ control}} \dots \dots \text{Equation [4]}$$

$$\text{Inhibition (\%)} = (\Delta L_{\text{ control}} - \Delta L_{\text{ treatment}}) \times 100 / \Delta L_{\text{ control}} \dots \dots \text{Equation [5]}$$

Where: Δ in Eqs. (4) And (5) describes the change in L or A<sub>420</sub> between time t (24 h) and the initial time t<sub>0</sub> at room temperatures.

Total colour change (Δ E) was also used to evaluate browning potential according to the following equation:

$$\Delta E = [(L_t - L_{t_0})^2 + (a_t - a_{t_0})^2 + (b_t - b_{t_0})^2]^{0.5} \dots \dots \dots \text{Equation [6]}$$

## STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS statistical package (Version 9.05) according to (Rattanathanalerk *et al.*, 2005), analysis of variance (ANOVA) and least significant difference (LSD) was chosen to determine any significant difference among various treatments.  $P < 0.05$  was selected as the level decision for significant differences.

## RESULTS & DISCUSSIONS

### EFFECT OF NATURAL EXTRACTS AND ITS EXTRACTION METHODS ON POLYPHENOLOXIDASE (PPO) ENZYME ACTIVITY OF APPLE JUICES

Red Delicious apple has one of the highest rates of enzymatic browning among several apple cultivars. The obtained results represents the enzymatic browning as polyphenoloxidase (PPO) enzyme activity of the untreated and treated apple juices with different extraction methods (Figs 1-3). The results showed that the polyphenoloxidase (PPO) enzyme activity of the untreated fresh apple juices increased sharply as compared to other treatments (Figs 1-3). These results are consistent with those reported by Dong *et al.*, (1995) who found that the PPO-activity in fresh apple juices increased sharply compared to treated juices. The vegetables (cucumber, squash and pepper) and mushroom extracts did not showed high browning result in high inhibition of enzymatic browning (PPO) in apple juice in all extraction methods.

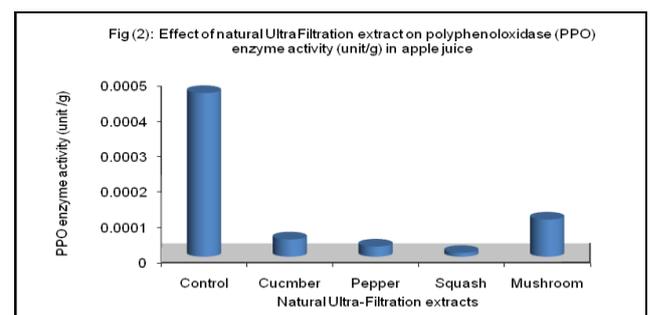
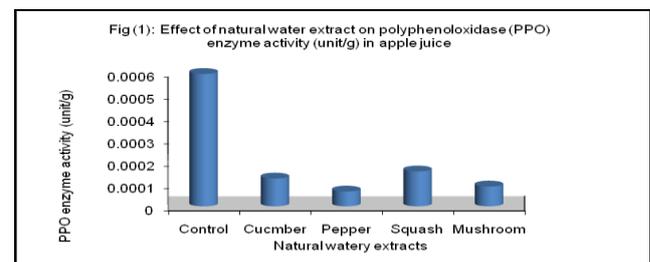
Effects of water, alcoholic and ultra-filtration extracts on the apple juices PPO activities are shown in Figs 1, 2 and 3, respectively. Under the experimental conditions described above, the control samples without added extracts showed an increase in PPO activities up to 0.00065 (unit / gram) in apple juices.

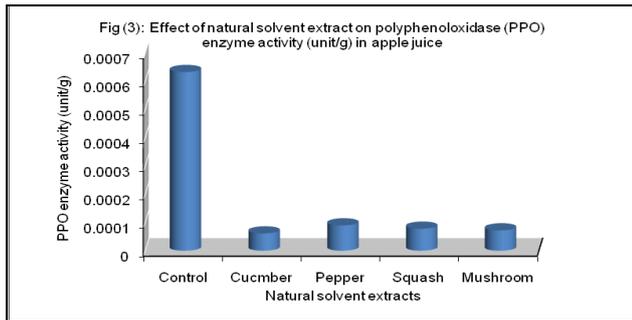
Watery extract of some vegetables and mushroom inhibited PPO enzyme activity but it was lower than natural ultra-filtration and alcoholic extracts in apple juice, as seen in Figs 1-3. The natural watery mushroom extract had higher inhibition effect of PPO activity than natural watery vegetables extract in apple juice because the watery extract of some vegetables was more viscous than mushroom extract, as shown in Fig 1. Ultra-filtration extract of cucumber had higher inhibition effect of PPO activity than natural ultra-filtration pepper and mushroom extracts in apple juice (Fig 2). Finally for alcoholic extract, of some vegetables and mushroom inhibited PPO enzyme activity but it had higher inhibition effect than natural ultra-filtration and watery extracts in apple juices (Figs 1-3). The natural alcoholic cucumber extract had higher inhibition effect of PPO activity than natural alcoholic pepper and mushroom extracts in apple juice (Fig 3). No PPO-activity was observed in apple juices treated with squash methanol extracts, as seen in Figs 3.

The decrease in PPO activity with different extraction methods probably resulted from the formation of oxidation reaction products derived from the natural compounds (such as ascorbic acid, sulfites, sulfur containing amino acids, organic acids and phenolic acids)

in vegetables and mushroom extracts which inhibit PPO activity and possibly the pH degradation after processing at 25°C. However, the combination of different compounds may prevent enzymatic browning (PPO) better than a specific compound alone (Mi *et al.*, 2002, Eissa and Salama 2002 and Iyidogan and Bayindirili 2004). These results are in agreement with those reported by Patricia *et al.* (1993). Eissa and Salama (2002) indicated that the treatments with cabbage, celery and fenugreek leaves water extracts inhibited the polyphenoloxidase activity in apple juices. Also, Wang & Lee (1996) reported that browning inhibitors in natural extracts of legumes that inhibited polyphenoloxidase had anti-browning activity in apple juice. However, a mean ascorbic acid content of raw vegetables (cucumber, squash and pepper) and mushroom extracts is about 36-40 mg/100 g (Franco-Vargas *et al.*, 1995). The results of Gandia-Herrero, (2003) supported the assumption that the enzyme PPO is present in the cucumber skin, but its activity is inhibited. On the basis of the obtained results, the inhibitory action of some squash, pepper and cucumber compounds against PPO activity may be an indication of the benefits of the use of squash, pepper and cucumber in food to prevent browning. These results clearly showed that the squash and 'Oyster' alcoholic extract had the potential to inhibit apple PPO activity. Inhibitory effects were found regarding the browning of apple juice.

The inhibition of browning in apple has been reported by several researchers (Friedman and Molnar-Perl, 1990). Discoloration correlated well with the PPO activity and the concentration of phenolics (Fraignier *et al.*, 1995; Murata *et al.*, 1995). Consequently, brown pigmentation has been attributed directly to the catalysed action of PPO (Underhill and Critchley, 1993). The alternative treatments in order to control enzymatic browning due to PPO are usually accomplished by the addition of ascorbic acid, citric acid, benzoic acid, calcium chloride, cinnamic acid, cysteine, glutathione, and various combinations of these compounds.





**EFFECT OF NATURAL EXTRACTS AND ITS EXTRACTION METHOD ON COLOR CHARACTERISTICS OF APPLE JUICE**

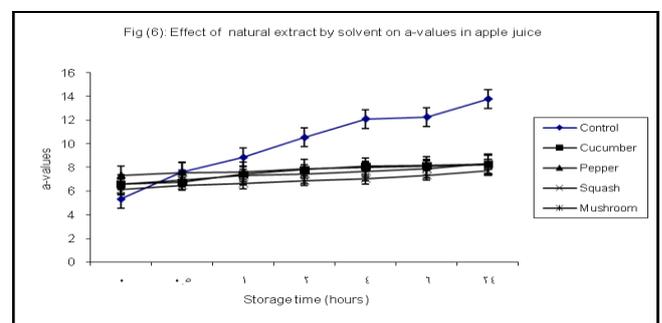
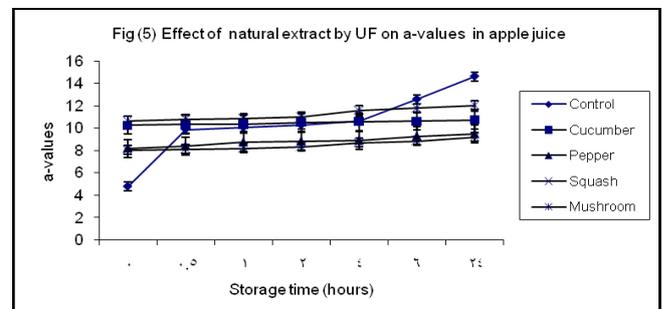
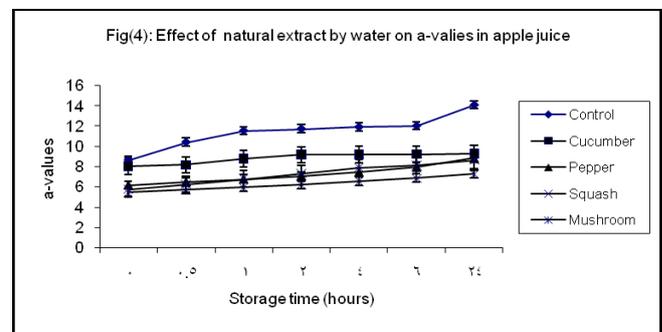
Figures (4-6) illustrates browning trends in the treated apple juices with different extraction methods (water, ultra-filtration and alcoholic) during storage up to 24 hours at room temperature. The results showed that the a\*-value of the untreated fresh apple juices increased sharply within the 24 hours period, as compared to other treated samples (Figs 4-6). Twenty-four hour observation is sufficient in order to see the end of enzymatic browning (no more colour change). The obtained results are consistent with those reported by Dong et al. (1995) who found that the PPO-activity in fresh apple juices showed a higher correlation to a\*-values than to L\*-values of Hunter colour. These results are also in agreement with the results of Patricia et al. (1993). However, Hunter L\* and a\* values were normally used as browning indicators in apple products (Sapers and Douglas 1987).

Changes in a\* values of the apple juices treated with water, ultra-filtration and alcoholic extracts are shown in Figs 4-6. After 24 hours of storage, untreated samples showed the highest a\* values while the samples treated with water extracts showed approx. 40-50% less than untreated samples. In case of the ultra-filtration and alcoholic extracts, little changes in a\* values were observed after 6 hour of storage at 25 °C. The natural squash extracts by all extraction methods had the highest inhibition effect of enzymatic browning (PPO) as decreasing of a\*-values in apple juices during 24 hours of storage at 25°C. But, the natural mushroom extracts by all extraction methods had the lowest inhibition effect of enzymatic browning (PPO) as decreasing of a\*-values in apple juice during 24 hours of storage at 25°C (Figs 4-6). The natural alcoholic and Ultra-filtration extraction methods had higher inhibition effect of enzymatic browning (PPO) as decreasing of a\*-values than water extraction method in apple juice compared with a\*-values of untreated samples during 24 hours of storage at 25°C (Figs 4-6). These results strongly supported that the addition of the vegetables and ‘Oyster’ mushroom extracts by water, ultra-filtration and alcoholic extraction was effective to prevent browning of apple juices. These results are in agreement with those of Patricia et al., (1993); Meza et al., (1995); Wang & Lee (1996) and Eissa & Salama (2002).

Hue angle and chroma were calculated using the pre-mentioned equation (eqn.1 and eqn. 2) Also, the browning index (BI) was calculated using the pre-

mentioned equation (eqn.3), for untreated and treated samples of apple juices, the results represented in Tables 1-3. It is clear that untreated samples markedly higher BI, H\* and C\* values.

The main colour change in untreated of apple juice and pre-treated by natural extracts was due to the increase in browning index (BI) and a\*-value, which were in high correlation to browning measurement. According to our results, the main color change in extracts treated apple juice was due to the decrease in chroma, hue angle, A<sub>420nm</sub> and a\*-value, which were in high correlation to browning measurement. Sapers and Douglas (1987) reported that decrease and increase in the CIE L\* value and a\* value respectively correlated well with increases in apple browning. Hunter hue angle and saturation index (chroma) remained almost constant in all samples other than natural untreated samples, which changed due to polyphenoloxidase activity.



**EFFECT OF NATURAL EXTRACTS AND ITS EXTRACTION METHOD ON THE BROWNING INHIBITION OF APPLE JUICE**

Quantification of enzymatic browning in apple juice stored for 24hours at room temperatures 25 °C was followed either by absorbance measurements (A<sub>420nm</sub>) or reflectance methods (L\*, a\* and b\*-measurements). The

inhibitory effect (%) of the natural vegetables and Oyster mushroom extracts by different extraction methods (water, UF and alcoholic) based on  $A_{420nm}$  measurements (Eq.4) and L-measurements (Eq. 5) were calculated and cited in Tables 1- 3. The total colour changes ( $\Delta E$ ) for all tested samples were calculated (Eq.6) from the hunter colour lab values and given also in the same tables 1-3. It is obvious that for all extracts there were some differences between percentage of inhibition based on measurement of  $A_{420nm}$  and L-values. Under all tested conditions, squash showed higher inhibition values based on L-values than  $A_{420nm}$  measurements compared with other extracts.

$\Delta E$  is the unit for computing the total color differences from the initial value. We found a good linear relation between  $\Delta E$  and the the extracts treated apple juices with different extraction methods, as seen in Tables 1-3. Similar relation between  $\Delta E$  and % inhibition of  $\Delta A_{420}$  and  $\Delta L^*$  in apple juices after storage for 24 hours at room temperature (25°C) revealed by the obtained results.

For all extracts treated samples the decrease in  $a^*$ -values of the apple juices revealed an increase in the inhibition percentage than untreated samples. Such trend is in agreement with those of Son et al., (2001) and Ozoglu and Bayindirh (2002). The inhibitory effect of various natural vegetables and Oyster mushroom extracts with different extraction methods (water, UF and alcoholic) based on %  $\Delta A_{420nm}$  and %  $\Delta L$ -measurements as well as  $\Delta E$  are shown in tables 1-3 for apple juices, stored at 25 °C in the following decrease order squash > mushroom > cucumber > pepper > control in the case of treated apple juices. Also, The inhibitory effect of various extraction methods (water, UF and alcoholic) for natural some vegetables and Oyster mushroom extracts based on %  $\Delta A_{420nm}$  and %  $\Delta L$ -measurements as well as  $\Delta E$  are shown in tables 1-3 for apple juices, stored at 25 °C in the following decrease order UF > alcoholic > water in the case of treated apple juice compared to control samples. The results are in accordance with those of Meza et al. [1995], Eissa and Salama (2002), Mi et al., (2002) and Eissa et al (2006). The treatments especially squash and pepper extracts can prevent browning and colour changes in apple juices because they contain the sulfhydryl groups, phenolic compounds, organic acids and ascorbic acid (Roshita et al., 2004 and Iyidogan and Bayindirili 2004).

The aforementioned order showed noticeable variations at UF, watery and alcoholic extracts. It is of great importance to refer to the opinion of Cowan 1999 who investigated the use of natural extracts by different methods, a powerful anti-browning agent, is discussed more because of the potential hazards of chemical agents and their influence on PPO activity. No uniform correlation was found between the natural extracts and inhibition activity. Who attributed these findings to the possibility that, the natural extracts may undergo significant side reaction in preventing browning of fruits and vegetables.

As shown in Figures 1-6, all tested anti-browning natural of some vegetables and Oyster mushroom extracts exhibited higher inhibition activity compared with untreated samples. Extracts have been used as anti-browning agents for processing fruits and vegetables. They prevent enzymatic browning by reducing the quinone products to their original polyphenol compounds (Son et al., 2001).

Under tested storage conditions, the squash extract exhibited higher effect than other extracts regarding inhibitory effect on the basis of L- values- measurements (Tables 1-3), whereas the highest inhibitory effect of squash by alcoholic extraction being of higher effective by 100 % than other extraction methods (water and UF). Such aforementioned level decreased to 90 % by UF for the same sample .These results are in accordance with those of Son et al ., 2001, who evaluated the effect of various anti-browning agents on apple juices. The obtained results revealed that extracting by alcoholic and UF could be effective regarding anti-browning agents under investigated conditions. However, any natural extracts contains L-cysteine and sulfur-containing amino acid considered to be effective inhibitors of PPO (Buta et al., 1999; Gacche et al., 2004). These compounds prevent enzymatic browning by reacting with o-quinone to produce stable, colourless adducts instead of the brown pigments (McEvily et al., 1992). These results confirm those reported by Ozoglu and Bayindirh, (2002) who compared the effectiveness of a series of compounds for inhibition of browning in the apple juice.

**Table 1. Effect of natural vegetables and Oyster mushroom extracts by water on color characteristics and parameters in apple juice after 24 hours storage at room temperature**

Color characteristics & parameters	Control or Untreated samples	Cucumber extract	Pepper extract	Squash extract	Oyster Mushroom extract
	Apple juice				
L*	17.85	26.57	25.16	26.71	24.18
a*	14.11	9.29	8.83	7.26	8.63
b*	22.98	25.71	24.52	22.99	23.88
A420	0.66	0.87	0.94	1.17	0.89
H*	58.45	70.13	70.2	72.47	70.13
C*	26.97	27.34	26.06	24.11	25.39
BI	732.40	398.43	403.56	319.66	413.76
% $\Delta A_{420}$	0.00	37.31	43.28	53.73	32.84
% $\Delta L^*$	0.00	80.59	74.48	83.03	62.76
$\Delta E$	11.1	2.97	3.67	2.5	4.57

**Table 2. Effect of natural vegetables and Oyster mushroom extracts by solvent on color characteristics and parameters in apple juice after 24 hours storage at room temperature**

Color characteristics & parameters	Control or Untreated samples	Cucumber extract	Pepper extract	Squash extract	Oyster Mushroom extract
	Apple juice				
L*	22.76	25.37	24.86	27.84	24.94
a*	13.77	8.26	8.24	7.7	8.32
b*	25.96	27.3	24.55	25.65	27.03
A420	0.76	1.61	1.46	1.48	1.14
H*	62.05	73.17	71.45	73.29	72.89
C*	34.52	37.27	34.94	37.85	36.78
BI	564.24	482.87	410.95	358.12	490.62
% Δ A420	0.00	72.09	69.77	75.19	42.64
% Δ L*	0.00	79.15	75.39	100	73.65
Δ E	22.72	7.11	4.19	5.42	6.46

**Table 3. Effect of natural vegetables and Oyster mushroom extracts by UF on color characteristics and parameters in apple juice after 24 hours storage at room temperature.**

Color characteristics & parameters	Control or Untreated samples	Cucumber extract	Pepper extract	Squash extract	Oyster Mushroom extract
	Apple juice				
L*	23.11	33.84	22.28	23.82	32.00
a*	14.62	10.71	9.5	9.2	12.03
b*	28.94	31.37	22.48	22.67	29.89
A420	0.53	1.73	1.0	1.19	1.45
H*	63.2	71.15	67.09	67.91	68.08
C*	32.42	33.15	24.40	24.47	32.22
BI	686.99	366.65	437.08	391.31	378.19
% Δ A420	0.00	78.72	58.51	70.21	50.00
% Δ L*	0.00	83.21	81.93	88.69	77.37
Δ E	14.39	4.37	4.62	2.82	4.44

Recently, Eissa and Salama (2002) showed that a natural extract of some leafy vegetables was efficient in preventing browning of dried apple rings. Also, Mi et al., (2002) suggested that juice and sliced apple could be protected from browning using 'Enokitake' mushroom extracts. The obtained results revealed that vegetables and 'Oyster' mushroom extracts were effective regarding inhibition of PPO activity, that is might be because they contain certain compounds (such as ascorbic acid, organic acids, phenolic compounds, L.cysteine and bisulphates) which are effective in inhibition of PPO-activity in apple juice. However, Ascorbic acid has received particular attention because of its multiple effects: it chelates copper, reduces o-quinones and acts as a competitive inhibitor of PPO (Pizzocaro *et.al.*, (1993). Also, L-Cysteine and sulphite compounds in all extracts prevent brown pigment formation by reacting with the quinone intermediates to form stable colourless compounds (Vamos-Vigyazo, 1995). The extracts also prevented the development of browning occurring in the apple juice. Moreover, the present study clearly showed that treatment of apple juice using some vegetables and 'Oyster' mushroom extracts prevented the browning. The adverse health effects of sulphites as well as increased regulatory scrutiny (McEvily et al., 1992) have created needs for alternatives chemical agents (George et al., 1999). The result showed that the ultra-filtration and alcoholic extract effectively inhibited

the browning of apple juices, so it demonstrated that (1) vegetables and 'Oyster' mushroom extracts contain certain potential inhibitors of PPO; (2) these inhibitors are stable under room temperature (25 °C) for a certain time (24 hours); and (3) there are potentials of commercial application. A number of other antibrowning treatments, including reducing agents, acidulants, chelating agents, polyphenoloxidase inhibitors, inorganic salts and enzymes have been investigated but are not applied in commercial use (Sapers et al., 1989). Utilization of the the vegetables and 'Oyster' mushroom extract will be promised as a natural food additive for the prevention of color browning caused by PPO. Although effective compounds in some vegetables and 'Oyster' mushroom extracts in terms of a PPO inhibitor have not been identified, it's potential as an inhibitor of apple PPO. Further research will be required for the identification of effective compounds in natural extracts.

## CONCLUSION

There are numerous of natural compounds capable to reduce the enzymatic browning; therefore the use of natural extracts is still stimulated to meet the demands for production of healthy food products having high quality. However, a natural extracts (squash, pepper, cucumber and Oyster mushroom) could also be used for substitution for chemical agents to delay browning of minimally processed

apple juices by inhibition the PPO-catalyzed browning. Besides being able to retain the colour and enzyme activity of the samples almost as good as the natural extracts treatment, is cheaper and is normally considered as a common item in the most of the households. Also, tested extracts were found to be good anti-browning agents in maintaining the storage quality of apple juice at room temperature. On the other hand, the different methods of extraction showed comparable results with squash, cucumber, pepper and Oyster mushroom extracts, so the use of these natural extracts is more economical.

## REFERENCES

- Asehraou A., Mohieddine S., Faid M., Serhrouchni M. "Use of antifungal principles from garlic for the inhibition of yeasts and molds in fermenting green olives" *Grasas Aceites*. 1997; 48 (2): 68-73.
- Buta, G. J., Moline, H. E., Spaulding, D. W., Wang, C. Y. Extending storage life of fresh-cut apples using natural products and their derivatives. *J. of Agric. and Food Chem.* 1999; (47) 1–6.
- Cowan M. M. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*. 1999; 12 (4): 564–582.
- Cowan M. M., Horst E. A., Luengpailin S., Doyle R. J. Inhibitory Effects of Plant Polyphenoloxidase on Colonization Factors of *Streptococcus sobrinus* 6715. *Antimicrob Agents Chemother.* 2000; 44(9): 2578–2580.
- Dong K., Kil K., Smith N., Chang Y. Changes in flesh color and PPO activity by apple cultivars. *Foods and Biotechnology*. 1995; 4, (4): 222-225.
- Eissa H. A., Salama M. F. Inhibition of enzymatic browning by Natural Leafy Vegetables Extracts and keeping Quality of fresh and dried apple rings. *Polish J. of Food and Nutrition Sciences*. 2002;11/52 (3): 27-32.
- Eissa H. A., Hoda H.M.Fadel, Ibrahim G.E., Hassan I.M., and Elrashid A.Abd. Thiol containing compounds as controlling agents of enzymatic browning in some apple products. *Food Research International* 2006, 39, 855 – 863.
- Eissa H. A.; Nadir A. M., Ibrahim K.E. Effect of Anti-browning and Anti- microbial Constituents of Some Spices on The Quality and Safety of Apple Juice. *A.M.S.E. France*,2003a; 64 (2): 43-57.
- Eissa H. A.; Nadir A. M., Ibrahim K.E. Effect of anti-browning and anti-microbial constituents of some spices on the quality and safety of apple slices. *A.M.S.E. France*, 2003; 64 (No 4) 15-33.
- Emine Ziiyan and Sule Pek Yardimci . Purification and Characterization of Pear (*Pyruscommunis*) Polyphenol Oxidase *Turk J Chem.* 2004; 28, 547 – 557.
- Esp' n J. C., Jolivet S., Overeem, A. Wichers, H. J. Agaritine from *Agaricus bisforus* is capable of preventing melanin formation. *Phytochemistry*, 1999; 50, 555–563.
- Fraignier, M. P., Marque` s, L., Fleuriet, A., Macheix, J. Biochemical and immunochemical characteristics of polyphenol oxidase from di.erent fruits of *Prunus*. *Journal of Agricultural and Food Chemistry*, 1995; 43, 2375–2380.
- Franco-Vargas E.; Diaz A.; Orzaez M. Influence of cooking on hydro-soluble vitamins in Cole. *Alimentaria*, 1995; 261, 119-123.
- Friedman, M., Molnar-Perl, I. Inhibition of browning by sulfur amino acids. I. Heated amino acid-glucose systems. *J. of Agric. and Food Chem.* 1990; (38): 1642–1647.
- Gacche, R.N., Wargkar, S.C., Ghole, V.S. Glutathione and cinnamic acid: natural dietary components used in preventing the process of browning by inhibition of polyphenol oxidase in apple juice. *J. of Enzyme Inhibition and Medicinal Chem.* 2004; (19): 175-179.
- Gandia-Herrero F., Jimenez M., Cabanes J. Tyrosinase inhibitory activity of cucumber compounds: Enzyme responsible for browning in cucumber. *Journal Agriculture Food Chemistry*. 2003; 51, 7764-7769.
- George, J.B., Harold, E.M., David, W.S., Chien, Y.W. Extending storage life of fresh-cut apples using natural products and derivatives. *Agricultural and Food Chemistry* 1999; 47: 1-6.
- Hülya Yagar, Ayten Sagiroglu. Partially Puri\_ cation and Characterization of Polyphenol Oxidase of Quince *Turk J Chem.* 2002; 26, 97 - 103.
- İyidoğan N., Bayindirh A. Effect of L-cysteine, kojic acid and 4-heyresorcinol combination on inhibition of enzymatic browning in Amasya apple juice. *Journal of Food Engineering*. 2004; 62, 299-304.
- Lee C. Phenolic compounds in food and their effects on health I, *ACS Symposium Series* 506; Ho, C.-T., Lee, C.Y., Huang, M.-T., Eds.; American Chemical Society: Washington, DC, 1992; pp 305.
- McEvily, A. J., Iyengar, R., Otwell, W. S. Inhibition of enzymatic browning in foods and beverages. *CRC Critical Reviews in Food Sci. and Nutri.* 1992; (32): 253–273.
- Meza J, Lozano P, Anzaldúa A, Torres J., Jimenez J. Addition of pineapple juice for the prevention of discoloration and textural changes of apple slices. *IFT Annual Meeting*, 1995; pp. 68.
- Mi Soon Jang, Aiko Sanada, Hideki Ushio, Munehiko Tanaka, Toshiaki Ohshima Inhibitory E.ects of 'Enokitake' Mushroom Extracts on Polyphenol

- Oxidase and Prevention of Apple Browning. *Lebensm.-Wiss. u.-Technol.*, 2002; 35, 697–702.
- Murata, M., Tsurutani, M., Tomita, M., Homma, S., Kaneko, K. Relationship between apple ripening and browning: changes in polyphenol content and polyphenol oxidase. *J. of Agric. and Food Chem.* 1995; (43): 1115–1121.
  - Ohad Nerya, Ruth Ben-Arie, Tal Luzzatto, Ramadan Musa, Soliman Khativ, Jacob Vaya. Prevention of *Agaricus bisporus* postharvest browning with tyrosinase inhibitors. *Postharvest Biology and Technology.* 2006; 39, 272–277.
  - Oszmiansky, J., Lee, C. Y. Inhibition of polyphenol oxidase activity and browning by honey. *J. of Agric. and Food Chem.* 1990; (38), 1892-1895.
  - Özoğlu H., Bayindirh A. Inhibition of enzymatic browning in cloudy apple juice with selected anti-browning agents. *Food Control.* 2002; 13, 213-221.
  - Paeaeckoenen K., Kurbela R., Effect of drying method on the water sorption of blanched Northern milk cap mushrooms (*Lactarius trivialis*) at different temperatures. *Lebensmittel-Wissenschaft-und-Technologie.* 1987; 20: (3): 158-161.
  - Palou E., Lopez-Malo A., Barbosa-Canovas G., Chanes-Welti J., Swanson W. "Polyphenoloxidase and colour of blanched and high hydrostatic pressure treated banana puree" *J. Food Sci.* 1999; 64: 42-45.
  - Patricia G., Diane M., Ronald E., Robert W. Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. *J. of Food Science.* 1993; 58 (2): 399-404.
  - Pizzocaro, F., Torreggiani, D., Gilardi, G. Inhibition of apple polyphenol oxidase (PPO) by ascorbic acid, citric acid and sodium chloride. *Journal of Food Processing and Preservation*, 1993, 17: 21–30.
  - Rattanathanalerk, M., Naphaporn, C., Walaiporn, S. Effect of thermal processing on the quality loss of pineapple juice. *J. of Food Eng.* 2005; (66): 259-265.
  - Regina Maria Araújo Gomes, Goreti Maria de Almeida Oliveira. Prorried Ades Físico-Químicas De Polifenoloxidase Ase De FEIJÃO (*Phaseolus Vulgaris* L.) *Ciênc. Tecnol. Aliment.*, Campinas, 21(1): 69-72, jan.-abr. 2001.
  - Roshita Ibrahim, Azizah Osman, Nazamid Saari, Russly Abdul Rahman. Effects of anti-browning treatments on the storage quality of minimally processed shredded cabbage. *Food, Agriculture & Environment.* 2004; 2 (2): 54-58.
  - Sapers, G., Douglas, F. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. *J. Food science*, 1987; 52, (5): 1258.
  - Sapers, G. M., Hicks, K. B., Phillips, J. G., Garzarella, L., Pondish, D. L., Matulaitis, R. M., McCormack, T. J., Sondey, S. M., Seib, P. A., El-Atawy, Y. S. Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. *J. of Food Sci.* 1989; (54): 997-1012.
  - Sezgintürk M. K., Dinçkaya E. An amperometric inhibitor biosensor for the determination of reduced glutathione (GSH) without any derivatization in some plants. *Biosensors and Bioelectronics* 2004; 19, 835–841.
  - Sibel Topçu, Mustafa Kemal Sezgintürk., Erhan Dinçkaya. Evaluation of a new biosensor-based mushroom (*Agaricus bisporus*) tissue homogenate: investigation of certain phenolic compounds and some inhibitor effects. *Biosensors and Bioelectronics.* 2004; 20, 592–597.
  - Son, S. M., Moon, K. D., Lee, C. Y. Inhibitory effects of various antibrowning agents on apple slices. *Food Chem.* 2001; (73): 23–30.
  - Underhill, S. J. R., Critchley, C. Lychee pericarp browning caused by heat injury. *Horticultural Science*, 1993; 28, 721–722.
  - Vamos-Vigyazo, L. Prevention of enzymatic browning in fruits and vegetables. In C. Y. Lee, & J. R. Whitaker (Eds.), *ACS Symposium Series 600, Enzymatic browning and its prevention*, 1995, pp. 49–62). Washington: American Chemical Society.
  - Wang L., Lee C. Split green pea fraction as a potential natural source of enzymatic browning inhibitor. IFT annual meeting: book of abstracts, 1996, pp. 22 ISSN 1082 - 1236.