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Utilization of Some Natural Medical Plant (NMP) Extracts as Antibacterial, Antifungal and Antibrowning in Red Apple Juice Preservation

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ABSTRACT

Six essential oils (EO) extracted from Shieh, kafoor and Neem at 0.5% concentration in water or solvent extracts were investigated for their inhibitory effect against Salmonella typhimurium, Staphylococcus aureus, Escherichia coli, Aspergillus flavus, A.parasiticus, A.niger and Penicillium digitatum as well as their effects on polyphenoloxidase (PPO) enzymatic browning. The well agar diffusion method was used for determining the inhibitory effect of each EO on the inhibition zone of the fungus and bacterial growth. Very low antibacterial activity was observed by using 0.5% Shieh, kafoor or Neem in solvent extracts dose between 0% and 3%, while the EO extracts for antifungal activity from 0.5% Shieh, kafoor or Neem showed less inhibitory. Such effects against food spoilage and producing fungi indicated that each essential oil was a potential food preservative. Also, the results showed that apple juices treated with EO extract from 0.5% Shieh or kafoor in water or solvent extracts had a positive effect towards the inhibition of PPO activity and reducing browning. Using Neem in water or solvent extracts treated juices, at room temperature (25°C) and at refrigerator (4°C), increased the shelf life of apple juice up to 4 weeks. Therefore, 0.5% Shieh or kafoor in water or solvent extracts proved to be efficient extractives for reducing both the enzymatic browning (PPO) and antimicrobial activity during the preservation of apple juice by refrigeration at 4°C.

Key words: Apple, juice, medical plant, extracts, Shieh, kafoor, Neem, antimicrobial, antifungal, enzymatic browning (PPO), colour, anti-browning.

Introduction

The international fruit juice market has been growing rapidly over the last few years. This is partly due to the rise of the standard of living, the increased demand for natural product, and the introduction of many new products formulated with fruit juices.

Fruits and their products are preserved by different methods to inactivate degradative enzymes and kills spoilage microorganisms. Most recent reviews have concentrated on technology for improving the fruits and their products quality without adding chemicals (Jeong et al., 2008 and Krzysztof et al., 2010). Fresh apple juice is a most unstable material from both a chemical and a microbiological point of view. Consequently, the distinct types of apple juice that are available on the market largely reflect the preservation techniques that have been used for their production. Application of plant extracts and essential oil have been extensively evaluated plant application on fruits as antimicrobial and antibrowning agents. Natural plant extracts can successfully replace synthetic chemical agents to control enzymatic browning and microorganisms in apple juice and apple products (Eissa et al., 2003, 2008 and 2010). A wide range of foods are prone to deterioration by moulds and other pathogenic microorganisms during post-harvest, transport and storage (Chauhan, 2004), of these moulds A. flavus and A. parasiticus represent a unique group that are able to produce aflatoxins in food (Guo et al., 1996). Therefore, the presence of toxigenic moulds in foods stored for long periods of time presents a potential hazard to human health (Soliman and Badeaa, 2002). Thus, numerous studies have been conducted to inhibit growth of pathogenic microorganisms. Many researchers used either natural antimicrobial agents such as essential oils and phenolic compounds (Bluma and Etcheverry, 2008; Al-Bayati, 2008 and Kumar et al., 2008). Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic and cardio protective effects (Balasundram et al., 2006). On the other hand flavonoids Artemisia herba-alba is a perennial shrub in the Artemisia genus that grows commonly on the steppes of the Mediterranean regions in Northern Africa and the Middle East e.g. Egypt (GRIN 2010). It is an herb used in traditional medicine. It is called "White dessert wormwood" in english, “Armoise blanche” in french and “Shīḥb”, Gallisai (2002). Essential oil of A. herba-alba, from the Sinai Desert, has been detected in the methanol extract of aerial parts, collected in Egypt (Yashphe et al., 1987 and Ahmed et
Azadirachta indica (Neem) is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to India growing in tropical and semi-tropical regions. Recent research showed that Neem's affects fungi and bacteria. Cinnamomum camphora (commonly known as Camphor tree, Camphor is a white crystalline substance, obtained from the tree Cinnamomum camphora. Camphor has been used for many centuries as a culinary spice, a component of incense, and as a medicine. shajarol-kafoor (trees of kafoor). Today, camphor is widely used in cooking in India, it is labeled as "edible camphor". Camphor laurel contains volatile chemical compounds in all plant parts, also the wood and leaves are steam distilled for the essential oils. Kumar et al., (2010) revealed that the possible exploitation of Ocimum sanctum (EO) and eugenol as plant based safe preservatives against fungal spoilage of food stuffs during storage. The Ocimum sanctum (EO) and eugenol also completely inhibited the aflatoxin B$_1$ production at 0.2 and 0.1 μl ml$^{-1}$, respectively. Products containing fruits, vegetables, nuts, seeds, stems, flours, and tea have shown antifungal, antiviral and antibacterial activity (Cushine and Lamb 2005).

The objective of this study was to extend the hurdle approach to the preservation of red apple juice by using three common medical plant (Shieh, Neem and Camphor) in water and solvent extracts at low concentration at room temperature and refrigeration (4 ºC). Moreover, to investigate the effect of these medical plant extracts on the enzymatic browning and safety of red apple juice as antibacterial, antifungal and anti-browning constituents. Also, developing a technology to produce a high quality and long shelf life of apple juice by controlling enzymatic browning was aimed.

Materials and Methods

1. Source Of Fruit Samples:

Good selected imported red apples (Red delicious) used for this study was obtained from a retail market in Cairo, Egypt. Fruits were placed in refrigerator at 4 ºC before using.

2. Preparation Of Fruit Material:

One hour prior to use, fruits were removed from the refrigerator and equilibrated to room temperature. Each red apple fruit was rinsed with water and sectioned to longitudinal slices.

3. Extraction Of Natural Medical Plants (NMP):

Natural Medical Plants (NMP) collected from the Faculty of Agriculture farm, Cairo University, Cairo, Egypt were air dried, milled to a size of 1 mm and stored at room temperature (25 ºC) until use. Plants were subjected to extraction at a ratio 10:1 using water and ethanol. Extractions were carried out using a shaking incubator at room temperature for 24 hours, followed by filtration through Whatman No.1 filter paper. The residues were re-extracted in the same manner and the two filtrates were combined. The extracts were concentrated using a rotary evaporator (BUCHI-Rota vapor R-205 Switzerland) at 55 ºC to near dryness (Mohsen and Ammar, 2009). The final extracts contained 0.18% TSS for ethanolic extracts and %20 TSS for water extracts, respectively.

4. Preparation Of Juice Samples:

Apple (Red delicious) samples representing common cultivars were obtained from local food stores during the fall and winter of 2011 and stored briefly at 4 ºC 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. 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 medical plant (NMP) to be added and mixed (Sapers and Douglas, 1987). Apple juice was used as a basal medium in this study. 0.5 % natural medical plant [Shieh in water (SW), Shieh in solvent (SS), kafoor in water (KW), kafoor in solvent (KS), Neem in water (NW) and Neem in solvent (NS)] extracts was added to red apple juice. Each assay was performed by triplication.

5. Evaluation Of Capacity Of Browning In Red Apple Juice And Colour Assessment:

Portions of 0.5% NMP extracts treated and untreated control apple juices (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 any present enzymatic browning. Apple 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 at room temperature (25°C) during which time tristimulus reflectance measurements were carried out at intervals, using a spectrophotometer (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). Values of the tristimulus coordinates in the L*, a* and b* values were recorded at 1, 10, 30, 60, 120 and 180 min; and after 24 hours. The tristimulus coordinates were plotted against time, and the slopes of linear portions of these curves were obtained by linear regression as described by the method of Sapers and Douglas (1987).

6. Colour Determinations:

Hunter a*, b* and L* parameters were measured with a colour difference meter using a spectrophotometer (Tristimulus Colour Machine) with the CIE lab colour 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) as mentioned by Hunter (1975) and as shown by Sapers and Douglas (1987). The ∆E-values, Hue-Angle (H)*, Chroma (C)* and Browning Index (BI) were calculated according to the method of Palou et al. (1999).

7. Physicochemical Analyses:

The pH of apple juice samples was measured using a digital pH-meter (HANNA, HI 902 meter, Germany). The percent Total Soluble Solids (TSS), expressed as °Brix (0-32) or g/Kg, was determined with a Hand refractometer (ATAGO, Japan). Titratable acidity was determined according to the method reported by Tung-Sun et al. (1995).

8. Polyphenol Oxidase (PPO) Enzyme Activity Determination:

Extraction of polyphenoloxidase enzyme (PPO, E.C. 1.14.18.1.) was carried out using the method described by Galeazi et al. (1981). Crude enzymes extracts were prepared from the tested samples by extracting with sodium phosphate buffers as follows: 10 g of fresh juices were mixed for 30 sec. with 100 ml of a 0.2 M sodium phosphate buffer at pH 7.0, the suspension was centrifuged at 4 °C for 15min at 5000 rpm, HERMLE Z 323 K Germane. The enzyme activity remained in the supernatant as crude of different enzymes.

Assay of polyphenoloxidase enzyme activity: Polyphenol oxidase (PPO) reaction was started by adding 1 ml of 0.2 M catechol into the mixture containing 1 ml of NMP treated and untreated apple juice and 2 ml of 50 mM phosphate buffer (pH 6.5). Where, PPO enzyme activity was determined by measuring the increase of absorbance at 420 nm and 25 °C with, Spectrophotometer UVD-3500, Labomed, USA. The absorbance at 420 nm was recorded every 30sec., from the recorded time the enzyme extract was added for 3 min at room temperature. One unit of PPO activity was defined as 0.001 DA420/min (Ozoglu & Bayindirli, 2002).

9. Non-Enzymatic Browning Determination:

Non-enzymatic browning was measured spectrophotometrically by Spectrophotometer, UVD-3500, Labomed, USA, as absorbance at 420nm using ethanol as blank according to the method of Stamp & Labuza (1983) and Birk et al. (1998).

10. Antimicrobial Activity Of The Natural Medical Plants (NMP) Extracts:

The antimicrobial activity of 3 ethno medicinal plants was determined using the well agar diffusion method according to (Kalalou et al., 2004) as follows: Suspensions of microorganisms containing 106 CFU/ml were inoculated onto a plate surface. Test plates (diameter of 10 cm) were prepared with 20 ml potato dextrose agar and nutrient agar, and holes of ø5 mm in diameter were punched in the agar plates. One hole was filled with 100 μl ethanolic each plant extract and the other with 100 μl water plant extracts separately; also wells were filled with 100 μl 95% ethanol and 100 μl water as control. The diameter of the inhibition zone (mm) around the holes was measured after 72 hrs at 37°C for pathogenic bacteria and after 5 days at 25 °C for moulds. Tests were performed in duplicates.

The unit of antifungal and antibacterial activity per ml of filtrate was calculated using the equation according to (Batish et al., 1990).
11. Statistical Analysis:

However, the experiment was performed in triplicate. The results were analyzed statistically using standard deviations, analysis of variance and Least Significant Difference (LSD) as described by Richard and Gouri (1987).

Results and Discussion

1. Antibacterial Activity Of The Natural Medical Plants (NMP) Extracts (Shieh, Kafoor And Neem):

Inhibition zones for all the natural medical plants (NMP) extract (Table 1) ranged from 2 to 22 mm against the test organisms of bacteria. *E. coli* showed sensitivity to all medical plant in solvent extract and in water extract except NW 0.5%. Also, *E. coli* showed the highest activity against *SW 0.5%* with inhibition zone of 22 mm, while it showed a zone of <12 mm against all other NMP in water extracts. *Salmonella typhimurium* and *Staphylococcus aureus* showed sensitivity to all medical plant in solvent extract and in water extract except NW 0.5%. *Salmonella typhimurium*, *Staphylococcus aureus* and *E. coli* were the most inactive hence their inhibition zones were less than 3 mm against 0.5% KS, SS and NS, while they showed inhibition zones of 6 - 22 mm against 0.5% KW, SW and NW.

The results of determination of inhibition zones for all the natural medical plants (NMP) extract (Table 1) ranged from 4-36 mm against the tested fungal; *A. flavus* showed more sensitivity to all medical plant in solvent extract than in water extract. Also, *A. flavus* was the most active against NW 0.5% (inhibition zone: 33 mm), while it showed a zone of 0 mm against NS 0.5%. *A. parasiticus*, *A. niger* and *Penicillium digitatum* showed sensitivity to all medical plant in solvent extract from 9 to 29 mm greater than those recorded for water extract (14 – 36 mm). However, *A. flavus*, *A. parasiticus*, *A. niger* and *Penicillium digitatum* were the most inactive with inhibition zones of 5, 11, 13 and 13 mm against SS 0.5%, while active with zone of inhibition of 25, 36, 35 and 28 mm against KW 0.5%, respectively. These observations showed that the natural medical plants (NMP) extracts have reducing characteristics hence can act as antimicrobial, antioxidants, reducing agent, hydrogen donor and oxygen quenchers as previously showed by NCCLS, 2002 and Habila et al., 2011.

<table>
<thead>
<tr>
<th>Natural Medical Plant Extracts</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. typhimurium</em></td>
<td><em>Staph. aureus</em></td>
</tr>
<tr>
<td>KW</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>KS</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>SW</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>SS</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>NW</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

2. Effect Of Some Medical Plant Extracts On Enzymatic Browning And Physicochemical Properties Of Red Apple Juices:

The results of physicochemical evaluations (Table 2) indicate that there was no difference for pH, TSS and acidity before and after 4 weeks storage of apple juice at refrigerator (4°C). The % titratable acidity in untreated and natural medical plant (NMP) extracts treated apple juices at zero time ranged from 0.31 to 0.33 (Table 2). With long-term storage at 4°C, total acidity decreased slightly after 4 weeks storage of apple juices ranged from 0.29 to 0.32. A similar decrease in acidity has been reported for apple juice by Moller and Palmer, (1984). All natural medical plant (NMP) extracts treated and untreated apple juice had closely similar TSS/acidity ratio at zero time and after storage for 4 weeks at 4 oC, as shown in Table (1). This indicates that natural medical plant extracts treated apple juice may be advisable for fresh use. However, it might be processed into acceptable quality juice. Fellers et al. (1988) reported that grapefruit juice with TSS / acidity ratios 7.0 had lower consumer preference scores than juice with TSS / acidity ratios above 11.0. As acidity decreased during storage, the pH decreased slightly. The pH value of all treatments ranged from 3.31 to 3.60, whereas it was 3.60 in the untreated sample. The pH of the untreated juice decreased from 3.60 to 3.23 up to 4 weeks. Also, pH decreased in all NMP extract treated juice up to 4 weeks. These results are in agreement with those reported by Kim et al, (1993). This decrease in pH value could be attributed to the action of natural medical plant (NMP) extracts. Most of the bacteria could not grow at low pH value and hence good keeping quality of the juice should maintained (Ranganna 1986 and Uma et al., 2011). Evaluation of pH in foods is important as it influences palatability. Total soluble solids content (TSS) of fruit juices indicates maturity of fruits procured for juice extraction. Total soluble solids of the apple juice were in the range of 12.2 to 13.3 Brix up to 4 weeks and later
concentration the maximum activity of polyphenoloxidase (PPO) enzyme was 0.163, 0.345, 0.394 and 0.579
pulp treated by SW, SS, KW and KS (0.5%), respectively. At the same natural medical plants extracts and same
seen that the activity of polyphenoloxidase (PPO) of fresh apple juice (control) was 1.420 units / ml. The
apple juice was evaluated. The obtained results are recorded in Tables (3). From the obtained results, it could be
0.5% concentration on polyphenoloxidase (PPO) enzyme activity (unit/ml), % activation and % inhibition in
The effect of natural medical plant in water and solvent extracts treatments (Shieh, kafoor and Neem) at
Apple Juice:
3. Effect Of Natural Medical Plants (NMP) Extracts On Polyphenoloxidase (PPO) Enzyme Activity In Red Apple Juice:

The effect of natural medical plant in water and solvent extracts treatments (Shieh, kafoor and Neem) at
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seen that the activity of polyphenoloxidase (PPO) of fresh apple juice (control) was 1.420 units / ml. The
maximum percent of inhibition of polyphenoloxidase PPO enzymes was 88.52, 75.70, 72.25 and 59.23 in apple
pulp treated by SW, SS, KW and KS (0.5%), respectively. At the same natural medical plants extracts and same
concentration the maximum activity of polyphenoloxidase (PPO) enzyme was 0.163, 0.345, 0.394 and 0.579
(unit / ml) in apple juice treated with 0.5% of SW, SS, KW and KS, respectively. As shown in Table (3), both
Shieh and kafoor in water and solvent extracts could significantly reduce PPO activity in fresh sapple juice and
the effect of water extraction pretreatments with 0.5% concentration pretreatments was much more significant
than solvent extraction pretreatments. PPO activity in untreated fresh apple juice was about hundredfold higher
than that from NMP treated apple juice. On the other hand, addition of 0.5% SW and 0.5% SS caused 11.48 and
24.30% decrease of PPO activity. SW and SS with 0.5% pretreatment caused the lowest PPO activity, which
was hardly detected throughout the storage. The highest PPO activity 1.420 (Unit / ml juice) was observed in the
control juice, hence there were high changes of PPO activity in all treated samples. Results in Table (3) showed
that the percent of activation of PPO was high in 0.5% of control, NW and NS treated apple juice (100%) versus
in 11.48, 24.30, 27.75 and 40.78 SW, SS, KW and KS treated juices respectively. It was also observed that the
SW, SS and KW extracts 0.5% treated apple juices had a positive effect controlling or retarding color changes
and inhibition of polyphenoloxidase (PPO) when applied to natural apple juices. Therefore, the use of SW, SS,
KW and KS extracts for increasing the inhibition of oxidative enzyme browning (PPO) could be suggested to
improve quality of the red apple juices.

As shown in Figures (1 and 2), the a*-values of using natural medical plant, 0.5% shieh and kafoor in
water and solvent extracts for fresh apple juice reduced its enzymatic browning more than 0.5% neem pretreatment. Enzymatic browning of untreated fresh apple juice was about hundred fold higher than that of 0.5% shieh and kafoor in water and solvent extracts treated juice. On the other hand, addition of 0.5% shieh and kafoor in water and solvent extracts increased inhibition percentage to 88.5 to 72.25%, respectively, as shown in Table (4). These inhibitory effects of shieh and kafoor 0.5% in water and solvent extracts pretreatments on

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### Table 2: Effect of some natural medical plant (NMP) extracts on the inhibition of polyphenoloxidase (PPO) enzyme and characteristics of red apple juice.

<table>
<thead>
<tr>
<th>Plant extracts (0.05%)</th>
<th>After 24 hours at room temperature (25 °C)</th>
<th>After four weeks at refrigerator (4 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PH</td>
<td>TSS</td>
</tr>
<tr>
<td>Control</td>
<td>3.6</td>
<td>12.5</td>
</tr>
<tr>
<td>KW</td>
<td>3.44</td>
<td>13.2</td>
</tr>
<tr>
<td>KS</td>
<td>3.36</td>
<td>13.1</td>
</tr>
<tr>
<td>SW</td>
<td>3.31</td>
<td>13</td>
</tr>
<tr>
<td>SS</td>
<td>3.41</td>
<td>13.3</td>
</tr>
<tr>
<td>NW</td>
<td>3.45</td>
<td>13.1</td>
</tr>
<tr>
<td>NS</td>
<td>3.46</td>
<td>12.7</td>
</tr>
</tbody>
</table>

**Total acidity expressed as malic acid (mg/100g).**

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**3. Effect Of Natural Medical Plants (NMP) Extracts On Polyphenoloxidase (PPO) Enzyme Activity In Red Apple Juice:**

The effect of natural medical plant in water and solvent extracts treatments (Shieh, kafoor and Neem) at
0.5% concentration on polyphenoloxidase (PPO) enzyme activity (unit/ml), % activation and % inhibition in
apple juice was evaluated. The obtained results are recorded in Tables (3). From the obtained results, it could be
seen that the activity of polyphenoloxidase (PPO) of fresh apple juice (control) was 1.420 units / ml. The
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enzymatic browning were related with the prevention of PPO enzyme activity. Browning is mostly the result of the activity of PPO enzyme acting on phenolic compounds to produce dark coloured polymers when apple juice is extracted to release the juice (Uma et al., 2011 and Krzysztof et al., 2010).

**Table 3:** Effect of some natural medical plant (NMP) extracts on polyphenoloxidase (PPO) enzyme activity (unit / ml) and percentage of the activation and inhibition for red apple juice.

<table>
<thead>
<tr>
<th>Medical Plant Extracts (0.05%)</th>
<th>PPO enzyme activity (unit / ml)</th>
<th>% activation</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.420 (±0.04)*</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>KW</td>
<td>0.394 (±0.07)*</td>
<td>27.75</td>
<td>72.25</td>
</tr>
<tr>
<td>KS</td>
<td>0.579 (±0.10)*</td>
<td>40.78</td>
<td>59.23</td>
</tr>
<tr>
<td>SW</td>
<td>0.163 (±0.09)*</td>
<td>11.48</td>
<td>88.52</td>
</tr>
<tr>
<td>SS</td>
<td>0.345 (±0.11)*</td>
<td>24.30</td>
<td>75.70</td>
</tr>
<tr>
<td>NW</td>
<td>1.428 (±0.06)*</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>NS</td>
<td>1.579 (±0.12)*</td>
<td>111</td>
<td>0</td>
</tr>
</tbody>
</table>

* Standard Deviations

4. Effect Of Natural Medical Plants (NMP) Extracts On Enzymatic Browning In Red Apple Juice Stored At Room Temperature And Refrigeration:

Raw red apple juice might represent a more useful system than the cut surface of plugs for the comparison of multilevel treatments to inhibit browning since it would be homogeneous and more easily manipulated. However, preliminary experiments with adding three kinds of natural medical plants (NMP) in water and solvent extracts (Shieh, kafoor and Neem) to apple juice (Red Delicious) indicated that browning in the freshly prepared juice occurred quite rapidly to permit sample treatment and evaluation. Reflectance measurements and a*-values were increased in the browning juices (Fig. 1). Browning of the apple juices was measured by a* (green-red). An increase in a*-value is indicative of browning (Monsalve-Gonzales et al., 1993). No heat treatment was given to the apple juices; thus enzymatic activity of polyphenoloxidase was assumed. The results that show the effect of treating apple juices with the different NMP extracts as anti-browning agents during storage at room temperature (25°C) for 24 h or for 4 weeks at 4°C on inhibiting the browning reactions are graphically represented in Figs 1 and 2. These figures illustrate the changes in the colour of apple juices in terms of a*-values over 24 h after adding Shieh, kafoor and Neem in water and solvent extracts at a concentration of 0.5% for each one. Also, the colour a*-values were recorded for the untreated apple juice over 24 h directly after preparing. It can be observed that the red apple juices treated with Shieh in solvent extract have no browning or the lowest a*-value (<2.47) followed by those treated with kafoor in water and solvent extract (<3.68) then with Shieh in water extract (<5.5) after 24 hours stored at room temperature (25°C). While browning or a*-value of neem in water and solvent extract and untreated apple juices was quite high (>9.9) after 24 hours storage at room temperature. On other hand, both shieh and kafoor in water and solvent extracts are considered to be anti-browning agents than other NMP (neem) extracts, in controlling enzymatic browning reactions, as shown in fig. (1). Furthermore, for long-term storage at 4°C, the results showed that the red apple juices treated with 0.5% SS Shieh in solvent extract have no browning or the lowest a*-value (<3.24) followed by those treated with 0.5% KS and 0.5% SW Kafoor in solvent and Shieh in water extracts (<9.62 and <10.45) then with 0.5%NW Neem in water and 0.5%NS Neem in solvent extracts (<10.33 and <13.84) after 4 weeks of storage at refrigeration (4°C); while the a*-value of untreated apple juices was quite high (>13.24) after 4 weeks of storage at refrigeration, as shown in Fig. (2). The result showed that application of a browning inhibitor extracts containing 0.5% Shieh and Kafoor in solvent (0.5% SS and 0.5%KS) extracts could control the enzymatic browning of red apple juices. The use of these treatments, especially 0.5% Shieh and Kafoor in solvent (0.5% SS and 0.5%KS) extracts and refrigeration at 4°C for 4 weeks constitutes an effective method of quality improvement and shelf life extension. However, refrigerating and 0.5% Shieh and Kafoor in solvent (0.5% SS and 0.5%KS) extracts treatments caused inhibition of enzymatic browning up to 4 weeks at 4°C and preservation for the quality of red apple juice. These results are consistent with the results of Eissa et al. (2003) and Ejechi et al. (1998), who revealed that the tropical spices extracts might prove useful in preservation of red apple and fruit juices by hurdle technology.

The percentage inhibition of polyphenoloxidase (PPO) activity in apple juice treated with 0.5% SS, 0.5% SW, 0.5% KW, 0.5% NS, 0.5% KS or 0.5% NW after storage at refrigeration (4°C) for 4 weeks was 96.32, 83.72, 73.64, 73.26, 68.80 and 60.46 %, respectively (Fig. 2 and Table 2). These results indicate that addition of NMP extracts to apple juice inhibited polyphenoloxidase (PPO) activity, therefore the NMP extracts of all treatments were higher than those of the control apple juice throughout storage periods at 4 °C. The most effective natural medicine plants (NMP) extracts pre-treatments on enzymatic browning (a*-value) were 0.5% Shieh and 0.5% Kafoor in water and solvent extracts of apple juices stored for 24 hours at 25 °C as well as for 4 weeks at 4°C (Table 2 and Figs 1 & 2). These results indicate that the essential oil extracts in NMP extracts treatment inhibited browning of refrigerated red apple juices as compared with that of the untreated samples. These results nearly[ consistent with those given by Vijay-Sethi (1991), Monsalve-Gonzales et al. (1993) and
Eissa et al. (2003), who proved the effectiveness of some essential oil in spices extracts and 4-hexylresorcinol as anti-browning agents in tomato juice and apple slices, respectively. Fig. 2 shows that refrigeration could enhance only the inhibitory effect of 0.5% SS, 0.5% SW and 0.5% KW. The use of essential oil extracts from NMP as a preservative might be due to the fact that they contain aldehydes and volatile compounds that have efficient on the inhibition of enzymatic browning and growth microorganisms. These results are confirmed by the findings of Kumar et al. (2010), who found that the essential oil of NMP extracts like eugenol and isoeugenol in Kafoor and Neem extracts exhibited appreciable effects in spite of they have quite strong characteristic odors to be used as food additives during storage.

Fig. 1: Effect of some natural medical plant (NMP) extracts treatments on colour characteristics and non-enzymatic browning (A420nm) in red apple juice stored at room temperature (25°C).

5. Effect Of Some Natural Medical Plant (NMP) Extracts Treatments On Colour Characteristics And Non-Enzymatic Browning (A420nm) In Red Apple Juice Stored At Room Temperature And Refrigeration:

The colour of apple juice was measured with a colour difference meter (Hunter Lab colour scale). Under all tested conditions, Shieh and kafoor in water and solvent extracts treatment had higher efficient values based on a* values than A420nm measurements, where the other natural extracts behaved an opposite trend. For all tested samples the increase in the concentration of the natural extracts revealed an increase in the inhibition efficient. Such trend is in agreement with previous studies of Janovitz-Klapp (1989) and Ozoglu and Bayindirh, (2002). The inhibitory effect of the studied natural extracts on apple juice was based on measurements at their minimum
concentrations as shown in Figs (1 and 2). Both figures showed a decrease at the following order kafoor in solvent extract > kafoor in water extract > Shieh solvent extract > Shieh in water extract > neem in solvent extract > neem in water extract > untreated apple juice. It is obvious that neem in water and solvent extracts of apple juice increased the development of red colour $a^*$ value as enzymatic browning. The Hunter colour values of solvent extracts samples in apple juice were lower than those of water extracts samples. Also, the Hunter colour value of Kafoor in water and solvent pre-treatment in apple juice was lower than that of shieh and neem in water and solvent extracts pre-treatments. These results indicated that the browning (redness) increased in neem in water and solvent extracts samples than in kafoor and shieh in water and solvent extracts samples for apple juice. According to the present results, the main colour change in untreated and pre-treated apple juice of natural medical plant extracts treatments was due to the increase in browning index (BI) and $a^*$-value, which were in high correlation to browning measurement. Also, other colour parameters such as Hue angle and chroma indicated that heat pre-treatment caused a slight colour change. Shieh and kafoor in water and solvent extracts treatment showed that, Shieh and kafoor in water extracts had a BI lower than that of Shieh and kafoor in solvent extracts in apple juice. Meanwhile, BI values in Shieh and kafoor in water and solvent extracts samples were lower than those of neem in water and solvent extracts samples as shown in Figs (1 and 2). These results are in good agreement with those of Eissa et al. (2003 and 2010).

![Graphs showing colour characteristics and enzymatic browning](image)

Fig. 2: Effect of some natural medical plant (NMP) extracts treatments on colour characteristics and non-enzymatic browning (A420nm) in red apple juice stored at refrigerator (4 oC).
Generally, pre-treatments of shieh and kafoor in water and solvent natural extracts improved the colour of apple juice (Figs 1 and 2). From the above mentioned results, it could be concluded that, the pretreated apple juice with natural medical plant shieh and kafoor in water and solvent extracts had the best colour values ($a^*$ and BI) and lower enzymatic browning compared to the other natural medical plant neem in water and solvent extracts pre-treatments, as shown in figs (1 and 2). The optimum conditions of natural medical plant neem, shieh and kafoor in water and solvent extracts pre-treatment for improving the quality of apple juice (inhibition enzymatic browning - good colour) were found in case of using 0.5 % shieh and kafoor in water and solvent extracts, as seen in Figs (1 and 2). The colour of treated apple juice was measured with a colour difference meter, using the Hunter Lab colour scale. The Hunter colour values of apple juice were determined immediately after treating. Changes in $L^*$ values were inversely proportional to the changes in $a^*$values of the Hunter colours. The CIE $L^*$, $a^*$, $b^*$ colour parameters, hue angle, chroma and BI were found to be suitable indicators for the brown pigment formation because of non-enzymatic browning after processing, as seen in figs (1 and 2). The $a^*$ values and BI for 0.5% shieh and kafoor in water and solvent extracts were low in contrast to those high values for untreated and other extracts treated apple juice. The CIE $a^*$ and colour parameters like $\Delta E$-values, hue angle ($H^*$), chroma ($C^*$), browning index (BI) and nonenzymatic browning ($A_{420\text{nm}}$) of apple juice samples had the lowest values in shieh and kafoor 0.5% in water and solvent extracts treated samples compared with the untreated and other extracts treated samples in apple juice, as seen in Figs (1 and 2). Also, it was generally found that 0.5% shieh and kafoor in water and solvent extracts treatments improved the colour of apple juice. However, 0.5% shieh and kafoor in water and solvent extracts samples had the highest increase in colour as optical density ($A_{420\text{nm}}$) compared with the untreated and other extracts treated samples in apple juice. The increase in colour (browning as $A_{420\text{nm}}$) could be attributed to the reaction occurred between amino groups and active carbonyl groups (Maillard reaction) after treatments. From the above mentioned results, it could be concluded that the pretreated apple juice with 0.5% shieh and kafoor in water and solvent extracts had the best colour values and lower nonenzymatic browning as compared with the other treatments of apple juice, as seen in Figs (1 and 2). The results are in accordance with those of Eissa et al. (2003 and 2010).

Conclusions:

Using 0.5% shieh and kafoor in water and solvent extracts as a pretreatment for apple juice was able to optimize the quality of apple juice during storage at room temperature (25 °C) for 24 hrs and at refrigerator (4 °C) for 4 weeks regarding to $L^*$, $a^*$, $C^*$, BI, $\Delta E$-values, non-enzymatic browning ($A_{420\text{nm}}$) and enzymatic browning (PPO). Also, using shieh and kafoor 0.5% in water and solvent extracts as a pretreatment for apple juice showed an inhibitory effect against polyphenoloxidase (PPO) enzymatic browning, bacterial activity, Salmonella typhimurium, Staphylococcus aureus and E. coli as well as an effect on spoilage fungi, Aspergillus flavus, A. parasiticus, A. niger and Penicillium digitatum. It is also concluded that there was no browning (inhibition of PPO) and the highest antibacterial (Salmonella typhimurium, Staphylococcus aureus and E.coli) and no microbial spoilage caused by the above mentioned microorganisms were observed in red apple juices pre-treated with 0.5% shieh and kafoor in water and solvent extracts during storage at room temperature (25 °C) for 24 hours and refrigerator (4°C) for 4 weeks. In general, the results showed that 0.5% shieh and kafoor in water and solvent extracts with refrigeration at 4 °C are considered to be anti-browning and anti-microbial agents than other NMP extracts, in controlling both enzymatic browning reactions and microorganisms, with potential antifungal properties. Therefore, it can be concluded that 0.5% shieh and kafoor in water and solvent extracts treatments with refrigeration at 4 °C may serve as an alternative to conventional chemical preservatives in the preservation of fruit juices by hurdle technology. A practical application of natural medical plant (NMP) in food as natural inhibitors against browning and microorganisms activity should be further studied.

References


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