Utilization of hydro-alcoholic extracts of peel and rind and juice of pomegranate as natural antioxidants in cotton seed oil

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The 5th Arab and 2nd International Annual Scientific Conference on:
Recent Trends of Developing Institutional and Academic Performance in Higher Specific Education Institutions in Egypt and Arab World

Faculty of Specific Education
Mansoura University - Egypt
April, 14-15, 2010
Utilization of hydro-alcoholic extracts for peel and rind and juice of pomegranate
Utilization of Hydro-Alcoholic Extracts for Peel and Rind and Juice of Pomegranate as Natural Antioxidants in Cotton Seed Oil

Ramadan, Afaf- haniem*, El-badrawey, S.*, Abd el-ghany, M.*, and Nagib, R.M.*

Abstract

The present study was carried out to investigate the utilization of pomegranate fruit as natural antioxidant and to estimate the chemical composition of peel, rind and juice, which include moisture, ash, protein, fat and carbohydrate. The active substances such as, alkaloid, resins, cardenolides, flavonoids and tannins were identified and also evaluation the effect of peel and rind hydroalcoholic extracts and juice of pomegranate fruit on peroxide, TBA, p-anisidine, carbonyl values and DPPH was evaluated.

Data indicated that, the peel of pomegranate contains the high value of ash, protein, fat and carbohydrate (4.49±0.07, 1.01±0.03, 0.89±0.02 and 86.52±0.18 %) respectively. It also contains tannin, terpenes, alkaloids, saponins, flavonoids, carbohydrates, cardenolides, gallic, chlorogenic, caffeic and catechol. The antioxidant activity of peel hydro-alcoholic extract showed significant decrease in peroxide value, malondealdehyde, p-anisidine and carbonyl values at 7, 14, 21, and 28 day of storage at 60 °C and also DPPH at 200 µg in comparing with BHT.

The rind of pomegranate contains moisture, ash, protein, fat and carbohydrate (8.15±0.47, 3.18±0.18, 0.96±0.06, 0.81±0.02 and 86.89±0.54 %) respectively. It also contains tannin, alkaloids, flavonoids, carbohydrates, cardenolides, gallic, chlorogenic, vanillic and synergetic. The antioxidant activity of rind hydro-alcoholic extract showed significant decrease in peroxide value, malondealdehyde, p-anisidine and carbonyl value at 7, 14, 21, and 28 day of storage and also DPPH at 1000, 500, 250, 200, 100 µg in comparing with BHT.

The juice of pomegranate contains moisture, ash, protein, fat and carbohydrate (85.0±0.67, 0.43±0.06, 0.12±0.03, 0.06±0.03 and 14.39±0.69 %) respectively. It also contains tannin, alkaloids, flavonoids, carbohydrates, cardenolides, gallic, chlorogenic, vanillic and synergetic. The antioxidant activity of juice of pomegranate resulted in significant decrease in

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peroxide value, malondialdehyde, p. anisidine and carbonyl value at 7, 14, 21, and 28 day of storage at 60°C and also DPPH at 1000, 500, 250, 200, 100 µg in comparing with BHT.

**Key words:** pomegranate peel – rind and juice – natural antioxidants - BHT - cotton seeds oil

**A-INTRODUCTION**

The pomegranate *Punica granatum* is one of the oldest known edible fruits. This fruit is one of the species mentioned in the Bible and Koran and is often associated with fertility. It is native to Persia and perhaps some surrounding areas and cultivated in ancient Egypt, Greece and Italy. Pomegranate spreads into Asia (Turkmenistan, Afghanistan, India, China, etc.), North Africa and Mediterranean Europe including Turkey. The domestication process took place independently in various regions (Salaheddin and Kader, 1984 and Sarkhosh et al., 2006).

The edible parts of pomegranate fruits are consumed fresh. They are also used in the preparation of fresh juice, canned beverages, jelly, jam and paste and for flavoring and coloring drinks (Hodgson, 1971; Ewaida, 1987 and Nagy et al., 1990) and have potent antioxidant properties. (Lanskey and Newman, 2007).

The major phenolic constituents of pomegranate fruit juice and pericarp used as feedstock and dry extracts by using high-performance liquid chromatography were analysed. Ellagic acid, a common botanical constituent that is currently used to standardize pomegranate extracts, as well as ellagitannin punicalin, were found to be only minor constituent (Jimenez et al., 2006).

Shahid et al., (2008) reported that pomegranate peel can stabilize sunflower oil very effectively at all the concentrations. Pomegranate peel extract at concentration of 800-850 ppm has stabilization efficiency comparable to conventional synthetic antioxidant. BHT at its legal limit. It improves resistance of sunflower oil against thermal deteriorative changes. Besides, polyunsaturated fatty acid content is saved appreciably by creating resistance in oil against oxidative rancidity. Pomegranate peel can be recommended as a potent source of antioxidants for the stabilization of food systems, especially unsaturated vegetable oils.

Zhang et al., (2007) cleared that the antiperoxidant activity of the pomegranate peel extracts on lard was studied by peroxide value method. All
the extracts showed enhanced inhibitory effect on lard peroxidation with the increase of phenolic concentrations.

**B-Materials and methods**

**I- Materials :**

1- Pomegranate fruits were purchased from the local market, EL Mansoura city, Egypt.

2- Cotton seed oil obtained from Misr Oil and Soap Company, Mansoura, Egypt.

3- Trichloroacetic acid (TCA) and ethylenediamine tetraacetic acid (EDTA) were obtained from Sigma Chemical Co. (USA) Products.

**II- Methods:**

2-1- Preparation of Pomegranate peel and rind:-

The peel and rind were manually removed, dried at 60°C and powdered.

2-1-1 Preparation of Fresh Pomegranate Juice:-

Fruits were washed in cold tap water and drained. They were manually cut up, and the outer leathery skin was removed. The juice in the sacs was manually pressed and extracted. The juice obtained was deep red. It was stored at 4°C over night for the settling of suspended particles, then filtered.

2-1-2 Hydro-alcoholic crude extract:-

The extraction procedure to obtain the hydro-alcoholic pomegranate extract was carried out according to Charles et al., (1993).

2-2 Chemical methods of pomegranate peel, rind and juice:-

Approximate chemical composition of pomegranate (moisture, ash, crude protein, and fat) were determined according to the methods of the (A.O.A.C. 1995), while total carbohydrates were estimated by subtracting from initial weight of the samples as follows:

\[
\text{Carbohydrates}\% = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \%\text{ ash})
\]

2-3. Screening of crude extracts of different pomegranate samples:

2-3-1 Detection of tannins: Tannins were detected in the plant samples according to the method of Gouzalez and Delgado (1962).

2-3-2. Detection of terpenes: Terpenes substances were detected in different crude extracts under investigation according to the method of Finar (1968).
Utilization of hydro-alcoholic extracts for peel and rind and juice of pomegranate

2-3-3. Detection of alkaloids: Alkaloid substances were detected in different crude extracts according to the method of Kiang (1961).

2-3-4. Detection of saponins: Saponin substances were detected in crude extracts of different samples according to the method of Trease (1961).

2-3-5. Detection of flavonoids: Flavonoid substances were detected in extracts of different samples using the method of Geissman (1962).

2-3-6. Detection of carbohydrates and glycosides: Carbohydrates and glycosides were treated by Molish test according to the method of Balbaa et al., (1976).

2-3-7. Detection of cardenolide and resins: Cardenolide and resin substances were detected according to A.O.A.C. (2000).

2-4 Fractionation and identification of phenolic compounds of peel, rind and juice:

Phenolic acid was determined in Central Laboratory of Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt. An HP 1100 HPLC system equipped with an alpha Bond C18 125A column (4.6 × 250 mm, particle size 5 μm) and coupled with Agilent 1100 series Chem Station software was used for quantifying the individual phenolic compounds.

Antioxidant effectiveness: Peel and rind hydro extracts and juice of pomegranate as natural antioxidants and BHT as synthetic antioxidants were added at the concentration of 400 ppm to the oil to examine the following properties.

2-5 Peroxide value was determined according to the method published in (AOCS 1998b), which is a modified oven test (Bandoniene et al., 2002).

2-6- Thiobarbituric acid value (TBA): The test was performed according to the methods previously stated by (Sidwell et al., 1954). TBA value was expressed as mg. malonaldehyde/kg sample using the following equation: TBA=7.8 × O.D.

O.D. is the absorbance at 530 nm.

2-7p Anisidine value: The test was performed according to the method of (AOCS 1998a), and absorbance (As) was measured at 350 nm using the solution from the second test tube as a blank using a UV-VIS spectrophotometer.
The P-anisidine value (P-A.V.) is calculated by the formula:

$$P\text{-A.V.} = \frac{25 \times (1.2 \text{As-Ab})}{m}$$

where, \( \text{As} \) = absorbance of the fat solution after reaction with the p-anisidine reagent, \( \text{Ab} \) = absorbance of the fat solution, \( m \) = mass of the test portion, g.

2-8 Total carbonyl value: Carbonyl value was evaluated according to the methods as reported earlier (Frankel, 1998).

2-9. Scavenging effect on DPPH: Scavenging effect on D-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by the method reported (Miller and Rice, 1997 and Sanchez et al., 1998) with minor modifications. The extracts (100, 200, 250, 500 and 1000 µg) in methanol (1 mL) was mixed with 4 mL of 0.004 % methanolic solution of DPPH. The mixture was shaken vigorously and left to stand for 30 min in dark at 30°C, and the absorbance was then measured at 517 nm in a spectronic 20 D Milton Roy spectrophotometer.

The percent of DPPH discoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100.$$ 

C-Results and discussions

Gross Chemical composition of pomegranate peel, rind and juice: Moisture, protein, fat, ash and carbohydrates of pomegranate peel, rind and juice were determined and results tabulated in table (1). It can be noticed that moisture content in peel, rind and juice 7.06 ± 0.07, 8.15 ± 0.47 and 85 ± 0.67 %, respectively. It was clear that protein, fat, ash and carbohydrates contents were 1.01 ± 0.03, 0.89 ± 0.02, 4.49 ± 0.07 and 86.52 ± 0.18 g/100 g for peel while they were 0.96 ± 0.06, 0.81 ± 0.02, 3.18 ± 0.18 and 86.89 ± 0.54 g/100 g for rind in D.W. In juice values were 0.12 ± 0.03, 0.06 ± 0.03, 0.43 ± 0.06 and 14.39 ± 0.69 g/100 g in w.w, respectively. The moisture content of juice was higher than moisture content of peel and rind, but the protein content of peel was higher than the protein content of rind and juice. The fat content of peel was higher than the fat content of rind and juice. The ash content of peel was higher than ash content of rind and juice. The content of carbohydrate in rind was somewhat lower than the content of carbohydrate in juice.
The results of elementary chemical composition of pomegranate peel, rind and juice were agreed with those reported by Morton et al., (1987); El-Nemr et al., (1990) and Ibrahim (1992).

Salah et al., (2002) found that fresh juice contained 84.57% moisture, 14.1% sugar, 1.05% protein and 0.33% ash. While El-Nemr et al., (1992) reported a higher moisture value (85.4%) in fresh juice. Aviram et al., (2000) cleared out that pomegranate juice (PJ) contained 85% water, 10% total sugars, 1.5% pectin, ascorbic acid, and polyphenolic flavonoids.

Table (1): Chemical composition of peel, rind and juice of pomegranate

<table>
<thead>
<tr>
<th>Chem. Comp Samples</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peel*</td>
<td>7.06</td>
<td>1.01</td>
<td>0.89</td>
<td>4.49</td>
<td>86.52</td>
</tr>
<tr>
<td></td>
<td>± 0.07</td>
<td>± 0.03</td>
<td>± 0.02</td>
<td>± 0.07</td>
<td>± 0.18</td>
</tr>
<tr>
<td>Rind *</td>
<td>15</td>
<td>0.96</td>
<td>0.81</td>
<td>3.18</td>
<td>86.89</td>
</tr>
<tr>
<td></td>
<td>± 0.47</td>
<td>± 0.06</td>
<td>± 0.02</td>
<td>± 0.18</td>
<td>± 0.54</td>
</tr>
<tr>
<td>Juice**</td>
<td>85.00</td>
<td>0.12</td>
<td>0.06</td>
<td>0.43</td>
<td>14.39</td>
</tr>
<tr>
<td></td>
<td>± 0.67</td>
<td>± 0.03</td>
<td>± 0.03</td>
<td>± 0.06</td>
<td>± 0.69</td>
</tr>
</tbody>
</table>

(d.w) *: in dry weight           ** (w.w): in wet weight

Data in table (2) concerns with the phytochemical screening (flavonoids, tannins, saponins, terpenes, alkaloids, carbohydrates, resins and cardenolides) of peel and rind hydro-alcoholic extract and juice of pomegranate fruit. The phytochemical screening of peel and rind hydro-alcoholic extracts and juice of pomegranate fruit indicated that tannins, terpenes, alkaloids, saponins, flavonoids, carbohydrates and cardenolides were found in peel hydro-alcoholic extract while resins were not detected in it. Tannins, alkaloids, flavonoids, carbohydrates and cardenolides were found in rind hydro-alcoholic extract while terpenes, saponins and resins were not detected in it. Also, Tannins, terpenes, alkaloids, saponins, flavonoids, carbohydrates, cardenolides and resins were found in juice of pomegranate fruit. These results agreed with Trease and Evans., (1989); Kokate et al., (1996) and Prashanth et al., (2001) who found that methanolic extracts of Punica granatum Preliminary phytochemical screening gave positive tests for sterols, alkaloids, tannins, flavonoids, carbohydrates and cardenolides.
It could be considered that, pomegranate rind is a good source of secondary products such as tannins and alkaloids (Anonymous, 1982). Possibly, members of these two phytochemical classes could account for the genotoxic properties of *P. granatum* extract. In addition, the tannins in pomegranate fruit should be taken into consideration for their genotoxic potential. Tannins, a class of naturally occurring plant phenols, have been recognized as antioxidants, but have also been reported to exert a certain genotoxic activity (Ferguson, 2001).

Arils of pomegranate are rich in sugars, vitamins, polysaccharides, polyphenols and minerals, and seeds are rich in polyunsaturated (n-3) fatty acids. The three major anthocyanidins (polyphenols) present in arils in pomegranate fruit are delphinidin, cyanidin and pelargonidin. Punicalagin (an ellagic tannin) is the major chemical constituent of pith and rinds (Melgarejo and Artes, 2000 and Noda et al., 2002).

Table (2): Preliminary phytochemical screening of peel and rind hydro-alcoholic extracts and juice of pomegranate fruit

<table>
<thead>
<tr>
<th>Chem. Comp</th>
<th>Samples</th>
<th>Tannins</th>
<th>Terpenes</th>
<th>Alkaloids</th>
<th>Saponins</th>
<th>Flavonoids</th>
<th>Carbohydrates</th>
<th>Cardenolides</th>
<th>Resins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peel</td>
<td>Peel</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rind</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Juice</td>
<td>Juice</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Positive (+)  
Negative (-)

Whole fruit hydro-alcoholic extract of *Punica granatum* contained saponins, alkaloids and flavonoids Vidal et al., (2003)

Data in table (3) present the identified phenolic acid of pomegranate peel, rind and juice (µg/g).

The examined phenolic acids of pomegranate peel showed gallic, chlorogenic, caffeic and catechol as the value being 6208.168, 24659.06, 228.586 and 2423.92 µg/g, respectively. Vanillic and synerigic acids were not detected in peel samples. The phenolic acids of pomegranate rind were gallic, chlorogenic, vanillic and synerigic the values being 7587.624, 28377.53, 935
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The phenolic acids of pomegranate juice were gallic, chlorogenic, vanillic, caffeic and catechol as the value being 63.801, 259.326, 32.587, 13.257 and 61.509 µg/g, respectively. The synergic acid was not detected.

Peel sample have the higher value in caffeic and catechol but the rind sample showed highest value in gallic, chlorogenic and vanillic acids.

The results were in parallel with the fact that pomegranate (Punica granatum) is a good source of anthocyanins (delphinidin, cyanidin, and pelargonidin) and other phenolic compounds (including hydrolyzable tannins such as punicalin, pedunculagin, punicalagin, gallagic, and ellagic acid), organic acids, and antioxidant activity (Gil et al., 2000; Noda et al., 2002 and Poyrazoglu et al., 2002). So, The phenolic components extracted from pomegranate (Punica granatum L.) have been shown to possess antioxidant property in several instances (Noda et al., 2002; Negi et al., 2003; Kulkarni et al., 2004; Sudheesh and Vijayalakshmi, 2005 and Li et al., 2006).

Aviram et al.,(2000) reported that the edible portion of pomegranate fruit (50% of total fruit weight), called arils, originates from the pith (anchoring tissue), is well protected by carpellary membranes (rinds), and is a natural potent antioxidant present in fruit waste. The most popular and commercially valuable pomegranate derivative (PDER) is pomegranate juice (PJ) which contains 85% water, 10% total sugars, 1.5% pectin, ascorbic acid, and polyphenolic flavonoids. In PJ, fructose and glucose are present in similar quantities; the soluble polyphenol content varies within the limits of 0.2–1.0%, depending on the variety, and includes anthocyanins, catechins, ellagic tannins, and gallic and ellagic acids.

Table (3): Phenolic acid of pomegranate peel, rind and juice (µg/g).

<table>
<thead>
<tr>
<th>Chem. Comp</th>
<th>Gallic</th>
<th>Chlorogenic</th>
<th>Vanillic</th>
<th>Synergic</th>
<th>Caffeic</th>
<th>Catechol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peel</td>
<td>6208.168</td>
<td>24659.06</td>
<td>-----</td>
<td>-----</td>
<td>228.586</td>
<td>2423.92</td>
</tr>
<tr>
<td>Rind</td>
<td>7587.624</td>
<td>28377.53</td>
<td>935.742</td>
<td>294.305</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Juice</td>
<td>63.801</td>
<td>259.326</td>
<td>32.587</td>
<td>-----</td>
<td>13.257</td>
<td>61.509</td>
</tr>
</tbody>
</table>
2- Antioxidative effectiveness of peel and rind hydro-alcoholic extracts and juice of pomegranate fruit on cotton seed oil:

a- peroxide value:

Data in table (4) and Fig (1) show the effect of peel and rind hydro-alcoholic extracts and juice of pomegranate fruit on peroxide value of cottonseed oil. Peroxide value (PV) as the measure of degree of initial oxidation of oils and fats was increased slowly during the first days of storage at 60°C, the increase of PV was observed at the 7th day reaching the maximum value after 28 days.

The peroxide value (m. eq./kg) of cottonseed oil which treated with BHT was gradually increased with advancement of the storage period, it was 2.25±0.29 at zero time and 9.24±0.89 (m. eq./kg) after 28 days of storage, but these values were significantly lower at 7, 14, 21 and 28 day of storage in comparing with control samples.

The PVs of cottonseeds oil which treated with peel hydro-alcoholic extract was gradually increased during the period of storage and it begin with 2.25±0.29 at zero time and ended by 7.17±0.49 (m. eq./kg) at 28 day of storage, but these values were significantly less at 7, 14, 21 and 28 day of storage in comparing with control.

The peroxide values of cottonseed oil which treated with rind hydro-alcoholic extract was gradually increased with increasing of storage and it started by 2.25±0.29 at zero time and ended by 8.19±0.26 (m. eq./kg) at 28 day of storage, but these values were significantly decreased at 7, 14, 21 and 28 day of storage in comparing with control.

The peroxide value of cottonseed oil which treated with juice was gradually increased by the increase of storage and it started with 2.25±0.29 at zero time and ended by 8.44±0.24 (m. eq./kg) at 28 day of storage, but these values were significantly lower at 7, 14, 21 and 28 day of storage in comparing with control.

These results agreed with Liu and White, (1992) who reported that pomegranate peel can stabilize sunflower oil very effectively at all the concentration used. Pomegranate peel extract at concentration of 800–850 ppm has a stabilization effect in comparison with at of synthetic antioxidants namely BHT at its legal limit. It improves resistance of sunflower oil against thermal deteriorative changes. Besides, polyunsaturated fatty acid content is saved appreciably by creating resistance in oil against oxidative rancidity.
Pomegranate peel can be recommended as a potent source of antioxidants for the stabilization of food systems, especially unsaturated vegetable oils (Shahid et al., 2008).

Table (4): peroxide value of cotton seed oil treated with hydro-alcoholic extracts and juice of pomegranate fruit

<table>
<thead>
<tr>
<th>Samples</th>
<th>Days</th>
<th>Zero time</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td>2.25 ± 0.29</td>
<td>5.86 ± 0.29</td>
<td>8.13 ± 0.15</td>
<td>10.48 ± 0.28</td>
<td>13.74 ± 0.21</td>
</tr>
<tr>
<td>BHT</td>
<td></td>
<td>2.25 ± 0.29</td>
<td>2.91 ± 0.10</td>
<td>5.14 ± 0.29</td>
<td>7.59 ± 0.67</td>
<td>9.24 ± 0.89</td>
</tr>
<tr>
<td>Peel</td>
<td></td>
<td>2.25 ± 0.29</td>
<td>3.61 ± 0.16</td>
<td>4.39 ± 0.33</td>
<td>5.34 ± 0.53</td>
<td>7.17 ± 0.49</td>
</tr>
<tr>
<td>Rind</td>
<td></td>
<td>2.25 ± 0.29</td>
<td>3.60 ± 0.36</td>
<td>5.43 ± 0.65</td>
<td>7.25 ± 0.25</td>
<td>8.19 ± 0.26</td>
</tr>
<tr>
<td>Juice</td>
<td></td>
<td>2.25 ± 0.29</td>
<td>3.87 ± 0.13</td>
<td>5.60 ± 0.92</td>
<td>6.54 ± 0.26</td>
<td>8.44 ± 0.24</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates ±SD.

Significant with control group  
*P<0.05  **P<0.01  ***P<0.001
(Fig. 1) Peroxide value of cottonseed oil as affected by blending with peel, rind hydro-alcoholic extracts and juice of pomegranate.

**b- Thiobarbitruic acid values (TBA):**

Data in table (5) show the effect of peel and rind hydro-alcoholic extracts and juice of pomegranate fruit on the malonaldehyde formation using thiobarbituric acid (TBA) value of cottonseed oil.

The Thiobarbutric acid (TBA) test is one of the more commonly used methods for detection of lipid oxidation (Gray, 1987).

The malonaldehyde formation of cottonseed oil which treated with BHT was gradually increased by increasing the period of storage and it started by 0.54 ± 0.02 at zero time and reached to 4.47 ± 0.10 (mg.mal./kg) at 28 day of storage, but these values were significantly low at 7, 14, 21 and 28 day of storage in comparison with control.

The malonaldehyde formation of cottonseed oil which treated with peel hydro-alcoholic extract was gradually increased by with increase of storage period and it started by 0.54 ± 0.02 at zero time and ended by 3.60 ± 0.09 (mg.mal./kg) at 28 day of storage, but these values were significantly less at 7, 14, 21 and 28 day of storage in comparison with control.
The malonaldehyde formation of cottonseed oil which treated with rind hydro-alcoholic extract was gradually increased by increasing the period of storage and it started by 0.54± 0.02 at zero time and ended by 4.07 ± 0.07 (mg.mal./ kg) at 28 day of storage, but these values were significantly decreased at 7, 14, 21 and 28 day of storage as compared with control.

The malonaldehyde formation of cottonseed oil which treated with juice was also gradually increased by increasing the period of storage and it started with 0.54± 0.02 at zero time and end with 4.25 ± 0.03 (mg.mal./ kg) at 28 day of storage, but these values were significantly lower at 7, 14, 21 and 28 day of storage in comparison with control. The results of table (5) agreed with that of Kelawala and Ananthanarayan (2004) who reported that pomegranate peel showed the maximum antioxidant activity due to its rich polyphenolic content. Pomegranate peel is a potential source of antioxidant phytochemicals with associated health benefits. Total antioxidant activity of pomegranate could be measured by the TBA.

Table (5): TBA value of cotton seed oil treated with peel and rind hydro-alcoholic extracts and juice of pomegranate fruit (mg.mal./ kg)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Zero time</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.54 ± 0.02</td>
<td>2.31 ± 0.05</td>
<td>5.32 ± 0.10</td>
<td>7.25 ± 0.11</td>
<td>9.61 ± 0.90</td>
</tr>
<tr>
<td>BHT</td>
<td>0.54 ± 0.02</td>
<td>1.05 ± 0.03</td>
<td>2.15 ± 0.07</td>
<td>3.54 ± 0.08</td>
<td>4.47 ± 0.10</td>
</tr>
<tr>
<td>Peel</td>
<td>0.54 ± 0.02</td>
<td>0.52 ± 0.03</td>
<td>1.10 ± 0.05</td>
<td>2.36 ± 0.09</td>
<td>3.60 ± 0.09</td>
</tr>
<tr>
<td>Rind</td>
<td>0.54 ± 0.02</td>
<td>1.32 ± 0.01</td>
<td>2.38 ± 0.05</td>
<td>3.22 ± 0.06</td>
<td>4.07 ± 0.07</td>
</tr>
<tr>
<td>Juice</td>
<td>0.54 ± 0.02</td>
<td>0.72 ± 0.03</td>
<td>1.75 ± 0.05</td>
<td>2.50 ± 0.11</td>
<td>4.25 ± 0.03</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates ±SD. Significant with control group *P<0.05  **P<0.01  ***P<0.001
c- The P-anisidine value :

Data in table (6) showed the effect of peel and rind hydro-alcoholic extracts and juice of pomegranate fruit on the P-anisidine value of cottonseed oil. The P-anisidine value of cottonseed oil which treated with BHT was gradually increased by increases the period of storage and it started by $11.76 \pm 0.50$ at zero time and ended by $36.48 \pm 0.72$ at 28 day of storage, but these values were significantly less at 7, 14, 21 and 28 day of storage in comparison with control.

The P-anisidine value of cottonseed oil which treated with peel hydro-alcoholic extract was gradually increased with the increase of the period of storage and it started by $11.76 \pm 0.50$ at zero time and ended by $26.37 \pm 0.64$ at 28 day of storage, but these values were significantly lower at 7, 14, 21 and 28 day of storage as compared with control.

The P-anisidine value of cottonseed oil which treated with rind hydro-alcoholic extract was gradually increased as the period of storage increased and it started by $11.76 \pm 0.50$ at zero time and ended by $32.53 \pm 1.69$ at 28 day of storage, but these values were significantly less at 7, 14, 21 and 28 day of storage in comparison with control.

The P-anisidine value of cotton oil which treated with juice was gradually increased with increasing of the period of storage and it started by $11.76 \pm 0.50$ at zero time and ended by $27.59 \pm 1.40$ at 28 day of storage, but these values were significantly lower at 7, 14, 21 and 28 day of storage in comparing with control.

The results of table (6) agreed with that of Changjiang et al., (2008) who reported that pomegranate juice is more effective in improving antioxidant function and reducing oxidative damage.
Table (6): P-anisidine value of cottonseed oil. Treated with peel and rind hydro-alcoholic extracts and juice of pomegranate fruit

<table>
<thead>
<tr>
<th>Days</th>
<th>Zero time</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>11.76 ± 0.50</td>
<td>30.32 ± 1.44</td>
<td>45.11 ± 2.91</td>
<td>56.63 ± 1.71</td>
<td>69.04 ± 2.28</td>
</tr>
<tr>
<td>BHT</td>
<td>11.76 ± 0.50</td>
<td>18.13 ± 1.77</td>
<td>23.45 ± 0.90</td>
<td>30.70 ± 2.52</td>
<td>36.48 ± 0.72</td>
</tr>
<tr>
<td>Peel</td>
<td>11.76 ± 0.50</td>
<td>12.79 ± 0.63</td>
<td>14.00 ± 0.94</td>
<td>19.22 ± 2.56</td>
<td>26.37 ± 0.64</td>
</tr>
<tr>
<td>Rind</td>
<td>11.76 ± 0.50</td>
<td>22.42 ± 1.49</td>
<td>25.16 ± 1.43</td>
<td>28.89 ± 1.86</td>
<td>32.53 ± 1.69</td>
</tr>
<tr>
<td>Juice</td>
<td>11.76 ± 0.50</td>
<td>15.70 ± 0.37</td>
<td>18.02 ± 1.71</td>
<td>22.75 ± 0.97</td>
<td>27.59 ± 1.40</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates ±SD.

Significant with control group

*P<0.05  **P<0.01  ***P<0.001

**D- Carbonyl value:**

Data in table (7) showed the effect of peel and rind hydro-alcoholic extracts and juice of pomegranate fruit on carbonyl value of cottonseed oil. The carbonyl values of cottonseed oil which treated with BHT was gradually increased as the period of storage increased, and it started by 2.45±0.68 at zero time and ended by 28.87±0.99 (mg/100 g) at 28 day of storage, but these values were significantly lower at 7, 14, 21 and 28 day of storage as compared with control.

The carbonyl value of cottonseed oil which treated with peel hydro-alcoholic extract was gradually increased by increasing of the period of storage and it started by 2.45±0.68 at zero time and ended by 22.58±0.75 (mg/100 g) at 28 day of storage, but these values were significantly less at 7, 14, 21 and 28 day of storage when compared with the control.

The carbonyl value of cottonseed oil which treated with rind hydro-alcoholic extract was gradually increased by increasing of the period of storage
and it started by $2.45 \pm 0.68$ at zero time and ended by $30.37 \pm 0.82$ (mg/100 g) at 28 day of storage, but these values were significantly lower at 7, 14, 21 and 28 day of storage in comparing with control.

The carbonyl value of cottonseed oil which treated with juice was gradually increased with advancement of the period of storage and it started by $2.45 \pm 0.68$ at zero time and ended by $35.08 \pm 2.37$ (mg/100 g) at 28 day of storage, but these values were significantly less at 7, 14, 21 and 28 day of storage in comparison with control.

The highest of carbonyl value of treatments appeared in cottonseed oil treated with rind at 7 and 14 day of storage and in cottonseed oil which treated with juice at 21 day and 28 day of storage. These results were agreed with Guo et al., (2003) reported that juice made from pomegranate pulp has superior antioxidant capacity to apple juice, and in addition subjects consuming juice made from pomegranate pulp exhibited significantly decreased plasma carbonyl content.

Table (7): Effect of peel and rind hydro-alcoholic extracts and juice of pomegranate fruit on carbonyl value of cotton seed oil (mg/100 g).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Days</th>
<th>Zero time</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.45 ± 0.68</td>
<td>22.25 ± 1.98</td>
<td>37.29 ± 2.00</td>
<td>45.54 ± 0.88</td>
<td>55.37 ± 0.76</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td>2.45 ± 0.68</td>
<td>10.37 ± 0.94</td>
<td>15.74 ± 0.81</td>
<td>19.87 ± 1.06</td>
<td>28.87 ± 1.09</td>
</tr>
<tr>
<td>BHT</td>
<td></td>
<td>2.45 ± 0.68</td>
<td>7.16 ± 1.18</td>
<td>9.70 ± 0.31</td>
<td>16.70 ± 2.43</td>
<td>22.58 ± 0.75</td>
</tr>
<tr>
<td>Peel</td>
<td></td>
<td>2.45 ± 0.68</td>
<td>12.95 ± 1.82</td>
<td>15.87 ± 1.07</td>
<td>20.62 ± 1.10</td>
<td>30.37 ± 0.82</td>
</tr>
<tr>
<td>Rind</td>
<td></td>
<td>2.45 ± 0.68</td>
<td>11.54 ± 1.64</td>
<td>13.91 ± 1.28</td>
<td>21.41 ± 1.01</td>
<td>35.08 ± 2.37</td>
</tr>
<tr>
<td>Juice</td>
<td></td>
<td>2.45 ± 0.68</td>
<td>11.54 ± 1.64</td>
<td>13.91 ± 1.28</td>
<td>21.41 ± 1.01</td>
<td>35.08 ± 2.37</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates ±SD.
Data in table (8) show the scavenging effect of peel and rind hydroalcoholic extracts and juice of pomegranate fruit on 1,1-diphenyl-2-picrylhydrazyl % (DPPH).

The value of DPPH of peel hydro-alcoholic extract show nonsignificant difference at 1000, 500, 250 and 100 µg and significant decrease at 200 µg in comparing with BHT. The values of DPPH of rind hydro-alcoholic extract showed significant decrease at 1000, 500, 250, 200 and 100 µg in as compared with BHT. Also, The value of DPPH of juice of pomegranate showed significant decrease at 1000, 500, 250, 200 and 100 µg in comparing with BHT. The free radical scavenging activity of pomegranate fruit product (PFP) was measured by the DPPH scavenging methods proposed by Brand-Williams et al., (1995) and Re et al., (1999) respectively. DPPH radical scavenging activity was also measured by electron spin resonance (ESR) spectrometer.

Finally, Pomegranate juice displays potent antiatherogenic action in atherosclerotic mice and humans (Aviram et al., 2000 and Kaplan et al., 2001). All these activities may be related to diverse phenolic compounds present in pomegranate juice, including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3,5-diglucosides).

So, These compounds are known for their properties in scavenging free radicals and inhibiting lipid oxidation in vitro (Gil et al., 2000 and Noda et al., 2002). For investigation of the antioxidant activity of PFP, different free radical scavenging methods were tested. PFP of rind showed a higher inhibitory effect of 69% for DPPH radical (Rout and Banerjee, 2007).
Table (8): Scavenging effect of peel and rind hydro-alcoholic extracts and juice of pomegranate on 1,1-diphenyl-2-picrylhydrazyl (DPPH)%

<table>
<thead>
<tr>
<th>Variables</th>
<th>Samples</th>
<th>500 µg/ml</th>
<th>1000 µg/ml</th>
<th>100 µg/ml</th>
<th>200 µg/ml</th>
<th>250 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHT</td>
<td></td>
<td>48.50 ± 3.18</td>
<td>72.50 ± 2.56</td>
<td>75.60 ± 2.94</td>
<td>89.00 ± 6.07</td>
<td>95.90 ± 6.51</td>
</tr>
<tr>
<td>Peel</td>
<td></td>
<td>43.60 ± 1.90</td>
<td>66.80 ± 2.88</td>
<td>72.91 ± 2.31</td>
<td>80.83 ± 3.34</td>
<td>94.99 ± 2.09</td>
</tr>
<tr>
<td>Rind</td>
<td></td>
<td>31.52 ± 3.25</td>
<td>39.99 ± 4.82</td>
<td>62.21 ± 2.95</td>
<td>67.35 ± 4.10</td>
<td>83.33 ± 5.20</td>
</tr>
<tr>
<td>Juice</td>
<td></td>
<td>34.58 ± 3.70</td>
<td>43.88 ± 2.77</td>
<td>53.47 ± 4.79</td>
<td>70.97 ± 3.54</td>
<td>87.35 ± 1.87</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates ± SD
Significant with control group *P<0.05  **P<0.01  ***P<0.001

References


Utilization of hydro-alcoholic extracts for peel and rind and juice of pomegranate


Changjiang, Guo; Jingyu, Wei; Jijun, Yang; Jing, Xu; Wei, Pang and Yugang, Jiang (2008): Pomegranate juice is potentially better than apple juice in improving antioxidant function in elderly subjects. Nutrition Research 28, 72:77.


الاستفادة من مستخلصات الفضور واللحاء وعصير ثمرة الرمان

كمضادات أكاسدة طبيعية في زيت بذرة القطن

عفاف هانم محمود رمضان، السيد البدراوي، عبدالله محمود عبد الغني، رشا محمد نجيب

ملخص البحث

الرمان هو نبات معروف باسم Punica granatum ولعائلة Punicaceae حيث يوجد العصير في أطعمة أو حويصلات عصيرية تحتوي بالبذور، ويستخدم العصير الحلو في تحضير مشروبات نباتية و بعض المشروبات الكحولية كما إن القشرة الخارجية لثمرة الرمان تحتوي على مواد فينولية و التي منها التانينات و هي ذات فائدة كبيرة لعلاج الكثير من الأمراض.

هدفت هذه الدراسة إلى إمكانية الاستفادة من إضافة مستخلصات القشرة واللحاء والعصير

الرمان كمضاف مضادة للأكاسدة مقارنة ب BHT كمضاد أكاسدة صناعي.

أجريت الدراسة بتجفيف قليل من القشرة الخارجية واللحاء الداخلي لثمرة الرمان و طحنهم و استخدام العصير كعكا و تم تقدير-rate الآتي:

البروتين، والدهون، والكربيوهيدرات والرمان والرطوبة كما تم تقدير المواد الفينولية باستخدام جهاز HPLC لدراسة الخواص المضادة للأكاسدة لتلك المستخلصات من خلال تحضير مستخلص مائي صحوالي من قشرة الرمان واللحاء الداخلي كما تم إجراء بعض الاختبارات على هذه المستخلصات كمواد مضادة للأكاسدة خارجية.

وقدت النتائج الكيميائية لتحليل قليل من القشرة الخارجية واللحاء الداخلي والعصير لثمرة الرمان على وجود بروتين ودهون وكربيوهيدرات ورمان ورطوبة حيث سكك في القشرة الخارجية (101) -0.89 - 0.87 - 0.44 - 0.06/100جم و على التوالي، بينما سككن في اللحاء الداخلي (100) -0.81 - 0.18 - 0.16 - 0.05/100جم.

أما العصير فكان يحتوي على (100) -0.81 - 0.18 - 0.16 - 0.05/100جم.

وكما أثبتت النتائج المتحصل عليها أن مستخلص القشرة الخارجية ومستخلص اللحاء الداخلي لثمرة الرمان يوجد فيه حمض فينلوقينويدين، التانينات والصوديوم، والكربوهيدرات والإستروالا، والكولتيد، والجلوكوزيدات فيما عدا الصمو المذ كليا، كما لم يثبت عليه بينما يوجد في عصير الرمان سم الفينولات

وما يثبت أن العصير له أثر قوي كمضاد للأكاسدة.

قسم الاقتصاد المنزلي – كلية التربية النوعية – جامعة المنصورة – مصر

Faculty of Specific Education

Mansoura University - Egypt April, 8-9, 2009.
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