

## CHEMICAL AND BIOLOGICAL STUDIES ON SOME POMEGRANATE PRODUCTS

(Received: 3.12.2013)

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### ABSTRACT

Pomegranate fruit is widely used in food and processing industries due to its excellent nutritional and health value. Therefore, the aim of the present study was to produce pomegranate molasses by different methods and jam from pomegranate fruit arils, and measure the main quality characteristics of these products. Also, to study the effect of these products on general antioxidative status and some immune parameters in healthy young rats.

It was found that pomegranate molasses produced by rotary with sugar and pomegranate jam achieved the highest scores for overall acceptability compared with other samples. Total soluble solids of pomegranate molasses produced by rotary with sugar were higher than the other pomegranate molasses as a result of sugar addition. Meanwhile, pomegranate molasses produced by heating method showed the lowest values for all color indices compared with other products as a result of severe heat treatment. Pomegranate molasses contained higher levels of Fe, Zn and Ca than juice. Pomegranate molasses produced by rotary without sugar contained the highest polyphenols ( $18.93 \pm 0.63$  mg/100g) and total flavonoids ( $44.71 \pm 3.54$  mg/100g) levels with the highest antioxidant activity.

Feeding healthy young rats on different pomegranate products caused significant reduction in liver marker enzymes AST and ALT activities but significant increase in glutathione peroxidase activity (GPx) in serum of rats relative to the control. The highest elevation in GPx activity was achieved by feeding group rat on pomegranate molasses produced by rotary without sugar at 11.75%. Also, feeding rats on different pomegranate products caused significant increase in the levels of serum iron, IgM and IgG levels. From the present results it could be concluded that, pomegranate molasses produced by rotary methods achieved the highest quality characteristics compared with other pomegranate products. Pomegranate products could be used in many foods and products to improve general antioxidative status and immune functions due to its many bioactive compounds.

**Key words:** *antioxidant activity, bioactive compounds, healthy rats, immune functions, jam, molasses, pomegranate.*

### 1. INTRODUCTION

Considerable importance is given to functional foods which a part from their basic nutritional functions, provide physiological benefits and play an important role in disease prevention or slow the progress of chronic diseases. There has been much more interest in pomegranate as a medicinal and nutritional product because of its multi-functionality and its great benefit in the human diet due to its several groups of substances which are useful in disease risk treatment (Jaiswal *et al.*, 2010).

Pomegranate (*Punica granatum* L., Punicaceae) is one of the oldest edible fruits native to the area of Iran to the Himalayas in northern India, and has been cultivated and

naturalized over the entire Mediterranean region. The edible parts of pomegranate fruit are consumed fresh or used for the preparation of fresh juice, canned beverages, jelly, jam, paste and also for flavoring and coloring beverage products. The edible part of the fruit (50%) consists of 40% arils, which are used to obtain the juice, and 10% seeds. Arils contain 85% water, 10% total sugars, mainly fructose and glucose, 1.5% pectin, organic acids such as ascorbic, citric and malic acids, bioactive compounds such as phenols and flavonoids, principally anthocyanins (Mousavinejad *et al.*, 2009 and Tezcan *et al.*, 2009).

Pomegranate aril juice is primarily considered as an important source of phenolic compounds:

the soluble polyphenol content varies from 0.2 to 1.0 g/100 g, dry weight being anthocyanins one of the most important. Together with lignans, gallayl-type tannins, ellagic acid derivatives and other hydrolysable tannins which contribute to the antioxidant activity of the juice. Pomegranate juice possessed a 3-folds higher antioxidant activity than that of green tea, and 2-, 6- and 8-folds higher levels than those detected in grape or cranberry, grapefruit and orange juices, respectively (Gil *et al.*, 2000 and Rosenblat and Aviram, 2006). Seeram *et al.* (2008) reported that, pomegranate juice had the greatest antioxidant potency composite index among such beverages as apple, black cherry, blueberry, cranberry, grape, orange juices and iced tea and the antioxidant activity was at least 20% greater than any of the other beverages tested.

Pomegranate molasse is a thick syrup made from cooked-down pomegranate juice, which is a slightly astringent, sweet-sour condiment that is deep and dark (and slightly ruby) in color. Production of pomegranate molasses typically includes cleaning and crushing of pomegranates, extraction, filtration, clarification and concentration of pomegranate juice in open vessel or molasses have had various applications as a flavoring agent, a salad dressing or soft drink ingredient. Pomegranate molasses may have 2 to 3 fold higher mineral components and antioxidant content than fresh pomegranate juice (Maskan, 2006 and Yilmaz *et al.*, 2007).

Pomegranate fruit could be considered a functional food because it has valuable phytochemicals that display medicinal effects. These can act as antioxidant, anti-hepatotoxic, antimicrobial, anti-inflammatory and anti-diabetic and improve cardiovascular health (Duman *et al.*, 2009 and Lee *et al.*, 2010). Polyphenols as dietary antioxidants may affect various aspects of both innate and adaptive wings of the immune system by shifting pro-oxidant/antioxidant balance. Complement system, for instance, has been shown to be inhibited by polyphenols and this complement inhibitory effect may have some role in anti-inflammatory properties of polyphenols. Pomegranate rich concentration of diverse, free-radical scavenging bioflavonoids, made it recommended in the treatment of acquired immune deficiency syndrome (Moneim *et al.*, 2011).

Therefore, the aim of this study was to produce pomegranate juice, molasses by different methods and jam from pomegranate fruit. Evaluation of the new products through measuring the main

quality of these products. Also, to study the effect of these products on general antioxidant status and some immune parameters in healthy young rats.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Fresh pomegranate fruits (*Punica granatum* L., Punicaceae, variety Sukary) were purchased from the local market of Giza Governorate, Egypt. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and common commercial suppliers.

### 2.2. Methods

#### 2.2.1. Technological studies

##### 2.2.1.1. Preparation of pomegranate juice

Pomegranate fruits were washed in tap water and drained. They were manually cut-up and the outer leathery skin (peel) which encloses hundreds of fleshy arils was removed. The juice that is localized in the arils was manually pressed and extracted. The obtained aril juice had a deep-red color was stored at 4°C overnight for settling suspended particles, then filtered and stored at -20°C for further analysis.

##### 2.2.1.2. Preparation of pomegranate molasses

Two different (heating/evaporation) processes were employed for the production of pomegranate juice concentrate (molasses). The juice was concentrated to a final 60.5° Brix from an initial 17.5° Brix by the following evaporation processes:

- 1- Evaporation at atmospheric pressure: The pomegranate juice was concentrated by using an electromagnetic heater (PYRO MUI Ti-Magnestir). The juice sample (500 ml) was placed in a beaker and replaced on the heater open to atmosphere. The sample was continuously heated and stirred during this process. Samples were taken for measurement of °Brix and replaced again after use.
- 2- Evaporation by using rotary vacuum evaporator: The juice sample (500 ml) was concentrated in a laboratory rotary vacuum evaporator (RE 100 Model, Bibby Sterilin Ltd., England) rotating at 66 rpm and 40°C. Samples were taken from the bulk of juice periodically for measurement of °Brix and replaced again after used. Sugar was added to a part of pomegranate juice before production of pomegranate molasses produced by rotary evaporator to obtain pomegranate molasses with or without sugar.

##### 2.2.1.3. Preparation of pomegranate low-sugar jam

Pomegranate jam was prepared according to

the procedure described by Poiana *et al.* (2012) with some modifications. Pomegranate juice (450g) was blended and then cooked under atmospheric pressure with the addition of sucrose (247g). Pectin was added at a concentration of 1% at the final stage of jam cooking. Citric acid was added towards the end of cooking for adjusting pH value to pH 3. Time of thermal processing was about 25 min. at 80°C until the final product reached 45° Brix. The jam was filled into hot glass jars, capped and pasteurized at 80°C for 10 min., it was allowed to cool at room temperature and stored in the dark until analysis.

#### **2.2.1.4. Physical analysis**

Total soluble solids (T.S.S.) content of pomegranate juice, molasses and jam were determined by using an Abbe refractometer (Bellingham Stanley Limited) at 20°C and expressed in degrees of Brix. pH was measured by means of a pH meter (Jenway, Staffordshire, United Kingdom). Titratable acidity was measured by titration to an endpoint of pH 8.2 using a Metrohm 862 compact titrosampler (Herisau, Switzerland). ACR-300 chroma meter (Minolta, Osaka, Japan) was used for color measurements. The instrument was calibrated with a white standard tile ( $L^* = 97.43$ ,  $a^* = -0.01$ ,  $b^* = 1.64$ ). A glass beaker containing the samples was placed below the light source.

#### **2.2.1.5. Chemical analysis**

Protein and ash contents of pomegranate products were determined according to A.O.A.C. (2006). Total and reducing sugars were measured as described by Melgarejo *et al.* (2000) with some modifications. Total phenolic contents of pomegranate juice, molasses and jam were determined according to Singelton *et al.* (1999) using Folin-Ciocalteu reagent and gallic acid as standard and the results expressed as mg gallic acid equivalent/100 ml. Free-radical scavenging activity of the tested samples against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to the method described by Karioti *et al.* (2004). Total flavonoids were measured spectrophotometrically at 510 nm and expressed as mg gallic acid equivalents/100 ml according to Yang *et al.* (2009). Mineral elements (K, Ca, Mg and Fe) were determined in the samples using a Perkin Elmer Optima 2100 DV model (Shalton, USA) coupled with plasma spectrometer according to Barnes (1997).

#### **2.2.1.6. Sensory evaluation**

Pomegranate juice, molasses and jam samples were evaluated by ten trained panelists from Food Technology Research Institute, Giza, Egypt. The

evaluated sensory characteristics included appearance, texture, taste, color and odor using a 10-point hedonic scale ranging from extremely dislike (1) to extremely like (10) according to Saka *et al.* (2007).

### **2.2.2. Biological studies**

#### **2.2.2.1. Animals and experimental diets**

A total of 36 Sprague-Dawley male rats were obtained from Ophthalmology Research Institute, Giza, Egypt. The rats weighing  $150 \pm 5$  g were housed in plastic cages and fed on basal diet and water for one week (adaptation period). The basal diet composed of casein (15%), cellulose (5%), vitamin mixture (1%), salt mixture (4%), corn oil (10%) and corn starch (65%) according to Zamora *et al.* (1991). The rats were divided into 6 groups ( $n=6$ ). The first group was fed on basal diet and considered as a control. The other five groups of rats were orally administered with 3 ml/kg of pomegranate juice, molasses and jam samples according to the results mentioned in animal studies (Moneim *et al.*, 2011) and given for each rat once daily before meals. Blood samples were taken from orbital plexus venous from food-deprived rats for 12 h. and the serum was separated and stored at -20°C for analysis.

**2.2.2.2. Biochemical analysis:** Serum glutathione peroxidase (GPx) activity was estimated according to the method described by Daret and Ching (1996). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined colorimetrically according to the method described by Bergmeyer and Harder (1986). Iron levels in serum were measured by the method mentioned by Seigin, (1977). Serum samples for measurement of antigen-specific IgM and IgG titers were prepared and analyzed by an immulon 4 enzyme linked immunosorbent assay (ELISA) kits (Dynex Technologies, Inc., Chantilly) as described by Deisenhammer *et al.* (1996). Hemoglobin concentration and blood cell counts were performed on the EDTA-treated blood according to the method of Craven *et al.* (1992).

#### **2.2.2.3. Statistical analysis**

The standard analysis of variance procedure in a completely randomized design was applied for the present data according to Gomez and Gomez (1984).

Least significant difference (LSD) test was to compare a pair of group means. The level of statistical significance was set at  $P < 0.05$ .

## **3.RESULTS AND DISCUSSION**

### **3.1. Technological studies**

### 3.1.1. Sensory evaluation of pomegranate products

The sensory characteristics, *i.e.*, appearance, texture, taste, color and odor of pomegranate products were evaluated by ten panelists. The overall acceptability was calculated and the obtained data were statistically analyzed and shown in Table (1). Pomegranate molasses obtained from the local market as a control were used for the comparison with the research pomegranate products. However there was no pomegranate jam in the local market to be used as a control for pomegranate jam produced in the present study. Pomegranate molasses produced by rotary with sugar achieved the highest scores for different sensory attributes compared with all other products examined in the present study. There were non - significant differences between the control pomegranate molasses by rotary without sugar and pomegranate juice for appearance, texture and color. Control pomegranate molasses were not significantly different for texture compared with pomegranate molasses produced by rotary with sugar. The highest score was recorded for the control pomegranate molasses and pomegranate molasses produced by rotary with sugar which were significantly different compared with other products, followed by pomegranate juice, pomegranate molasses produced by rotary without sugar and pomegranate molasses produced by heating, respectively.

Astringency is known to play an important role in pomegranate juices with elevated phenolic contents. The consumption of strongly astringent food causes saliva protein denaturation and may markedly impair taste bud sensation. In the present study, all products produced from pomegranate juice obtained from pomegranate arils had intermediate phenolic contents than peels and seeds. The juices were assessed with regard to

their visual appearance, bitterness and typical pomegranate flavor. Pomegranate juices showed significant differences among them for all sensory parameters except for aroma, which might point out that aroma is not a distinctive sign of pomegranate juice, while color and taste have been reported as crucial for the attractiveness of red fruits (Fischer *et al.*, 2011).

### 3.1.2. Physical characteristics of pomegranate products

Data presented in Table (2) show the physical characteristics of pomegranate juice, pomegranate molasses and pomegranate jam. These characteristics including: total soluble solids, acidity, pH and color. It could be observed that, there was significant difference between pomegranate products in total soluble solid contents but pomegranate jam contained the highest total soluble solids ( $55.4 \pm 2.95^\circ$  Brix) as a result of high concentration during processing, while fresh pomegranate juice contained the lowest total soluble solids ( $13.5 \pm 0.98^\circ$  Brix). Total soluble solids for pomegranate molasses produced by rotary with sugar were higher compared with other pomegranate molasses as a result of sugar addition. Acidity was significantly higher in pomegranate jam compared with other products. On the other hand, fresh pomegranate juice and molasses produced by rotary with sugar were not significantly different compared with each other for acidity characteristic. Regarding pomegranate products, pH value of pomegranate jam was significantly lower than those of the other pomegranate products.

From Table (2) it could be found that, pomegranate juice and molasses produced by rotary with sugar were not significantly different for color indices of lightness ( $L^*$ ), while pomegranate molasses produced by heating and by rotary without sugar were not different for ( $L^*$ ) value. Redness ( $a^*$ ) values were significantly

**Table (1): Sensory evaluation of pomegranate products.**

Samples	Appearance (10)	Texture (10)	Taste (10)	Color (10)	Odor (10)	Overall acceptability (50)
Pomegranate juice	8.9 <sup>ab</sup>	8.2 <sup>c</sup>	8.7 <sup>b</sup>	8.9 <sup>ab</sup>	8.3 <sup>b</sup>	43.0 <sup>b</sup>
Control PM	8.6 <sup>b</sup>	9.3 <sup>a</sup>	9.1 <sup>a</sup>	8.3 <sup>c</sup>	8.6 <sup>ab</sup>	43.9 <sup>b</sup>
PM by heating method	7.1 <sup>c</sup>	8.4 <sup>bc</sup>	6.8 <sup>c</sup>	6.4 <sup>d</sup>	6.7 <sup>c</sup>	35.4 <sup>c</sup>
PM by rotary with sugar	9.4 <sup>a</sup>	9.3 <sup>a</sup>	9.3 <sup>a</sup>	9.5 <sup>a</sup>	9.0 <sup>a</sup>	46.5 <sup>a</sup>
PM by rotary without sugar	8.6 <sup>b</sup>	8.7 <sup>b</sup>	8.6 <sup>b</sup>	8.9 <sup>b</sup>	7.4 <sup>cd</sup>	42.2 <sup>b</sup>
pomegranate jam	8.5 <sup>b</sup>	8.8 <sup>b</sup>	7.9 <sup>c</sup>	9.1 <sup>a</sup>	8.5 <sup>ab</sup>	42.8 <sup>b</sup>
L.S.D.	0.53	0.43	0.52	0.55	0.61	2.06

\*PM = pomegranate molasses produced.

\*Numbers in the same column with the same letters were not significantly different at  $P < 0.05$ .

**Table (2): Total soluble solids, acidity, pH and color measurements of pomegranate products.**

Physical properties	Total soluble solids (%)	Acidity	pH	Color parameters		
				L*	a*	b*
Pomegranate juice	13.5 ± 0.98 <sup>e</sup>	0.679±0.15 <sup>d</sup>	3.44±0.06 <sup>b</sup>	17.59±0.83 <sup>a</sup>	7.10±0.22 <sup>a</sup>	11.32±0.74 <sup>b</sup>
PM by heating method	30.5±1.06 <sup>d</sup>	1.091±0.19 <sup>c</sup>	3.50±0.08 <sup>b</sup>	14.19±0.74 <sup>b</sup>	1.82±0.21 <sup>d</sup>	6.55±0.36 <sup>c</sup>
PM by rotary with sugar	38.0±2.53 <sup>b</sup>	0.627±0.13 <sup>d</sup>	3.67±0.10 <sup>a</sup>	17.89±0.95 <sup>a</sup>	6.44±0.36 <sup>b</sup>	12.09±0.55 <sup>a</sup>
PM by rotary without sugar	34.0±1.48 <sup>c</sup>	1.360±0.28 <sup>b</sup>	3.81±0.07 <sup>a</sup>	14.47±0.62 <sup>b</sup>	4.21±0.29 <sup>c</sup>	10.77±0.42 <sup>b</sup>
Pomegranate jam	55.4±2.95 <sup>a</sup>	2.100±0.32 <sup>a</sup>	3.05±0.06 <sup>c</sup>	N.D.	N.D.	N.D.
L.S.D.	2.36	0.24	0.15	0.46	0.52	0.63

\*PM = pomegranate molasses produced.

\*Numbers in the same column with the same letters were not significantly different at P<0.05.

N.D. = not detected.

different between different pomegranate products. The present results revealed that pomegranate molasses produced by rotary evaporation with sugar, achieved the highest values for color attributes (L\*, a\* and b\*) compared with other pomegranate molasses. Meanwhile, pomegranate molasses by heating method showed the lowest values for all color indices as a result of severe heat treatment. Color index values were not detected in pomegranate jam because of high viscosity and concentration of the product.

The present results are in good agreement with the results mentioned by Rinaldi *et al.* (2013) who found that, pomegranate juice obtained from 100 g/100 g of arils showed pH value of 3.46 ± 0.01, acidity of, 0.68 ± 0.01g/100ml and 13.75 ± 0.06° Brix total soluble solids. The pH of pomegranate molasses comes predominantly from the organic acids of pomegranate fruits. The present results are higher than those reported by Vardin *et al.* (2008) who determined pH of the pomegranate juice concentrate as 1.34 to 2.90. The variation in the acidity values of pomegranate molasses may be due to the evaporation methods applied in the production of molasses.

The red, blue and orange color in flowers, fruits and vegetables is due to anthocyanins, these have to be accounted for in processing. These compounds must be preserved during fruit juice processing, because they exert health protective effects for human (Vardin and Fenercioglu, 2003). Since (L\*) value is a measure of the color in the light–dark axis, the reduce in this value indicates that the pomegranate molasses of the present study were turning darker. The decreases in (L\*) value correlated well with the increases in the browning of food materials and pigment destruction (Ahmed *et al.*, 2004). Our results are in agreement with the results of Patras *et al.* (2010), who observed

decreasing values in Hunter (a\*) parameter as a result of the thermal treatment of strawberry and blackberry purees at 70°C. Because pomegranate molasses are concentrated products of pomegranate juice, lab color values correspond to dark, slightly red and blue in color.

### 3.1.3. Chemical composition of pomegranate products

Table (3) shows the chemical composition of pomegranate juice, molasses and jam products including: total sugars, reducing sugars, protein and ash contents. Total sugars were significantly different in all pomegranate products with the highest content found in pomegranate molasses produced by the heating method followed by pomegranate jam. Fresh pomegranate juice contained a lower value of total sugars than those of other products. Reducing sugars content was not significantly different for pomegranate molasses produced either by heating or rotary without sugar, while fresh pomegranate juice and pomegranate jam were not significantly different in reducing sugar content. Evaporation of water during the production of molasses increases the concentration of carbohydrates in the final product.

Suh *et al.* (2003) reported that, sugar and sugar degradation products have been found to be effective on accelerating anthocyanin (pomegranate pigment) breakdown and enhance non–enzymatic browning during thermal processing. Poyrazoglu *et al.* (2002) found total sugars of 148.75 g/l by analyzing freshly squeezed pomegranate juice of 13 different types. A significant part of the total sugars content was subjected to inversion through thermal processes. There was non- significant difference between pomegranate molasses produced by heating or rotary with sugar in protein content. Meanwhile

protein content of fresh pomegranate juice and pomegranate molasses produced by rotary without sugar was not significantly different (Table 3). Ash content was not statistically different between pomegranate molasses produced by rotary either with or without sugar. Filtration and clarification of pomegranate juice during commercial processing may be the main reason for low protein and ash contents of pomegranate molasses. According to Anonymous (2006). The protein content of pomegranate was 0.95 g/100g and the protein content of some samples increased through concentration of the products, while some decreased through denaturation of proteins during heating treatments.

#### **3.1.4. Mineral composition of pomegranate products**

Data presented in Table (4) show the mineral composition (Fe, Ca, Mg and K) of pomegranate products. Fresh pomegranate juice contained lower Fe, Ca, Mg and K contents compared with other pomegranate products. Pomegranate molasses produced by rotary without sugar contained the highest mineral contents followed by pomegranate molasses with sugar. The present results are in agreement with those reported by Fadavi *et al.* (2005) who found that, pomegranate juice contained lower levels of Fe, Zn and Ca than pomegranate molasses, which is a good source of various minerals. It was revealed that pomegranate molasses was richer in minerals than the fruit itself. However, the results showed that in the concentrated product the mineral contents of pomegranate juice were variously affected by the concentration process. Incedayi *et al.* (2010) found that, pomegranate molasses were rich in minerals, especially potassium (450-4700 mg/100g), calcium (71.88-1803.63 mg/100 g), magnesium (7.48-409.10 mg/100 g) and iron (1.05- 22.99 mg/100 g).

#### **3.1.5. Polyphenols, total flavonoids and antioxidant values of pomegranate products**

Polyphenolic compounds give the color, astringency and bitter taste to pomegranate juice. Also these compounds cause cloudiness during the concentration process and storage (Alper *et al.*, 2005). Since pomegranate molasses is a concentrated product, it was anticipated that the phenolic substances would increase in percentage despite the losses during thermal processing. Pomegranate molasses produced by heating method contained the lowest polyphenols and total flavonoids contents compared with other products, while pomegranate molasses produced by rotary

without sugar contained the highest polyphenols and flavonoid contents (Table 5). There was non-significant difference between pomegranate molasses produced by rotary with sugar and pomegranate jam in polyphenol content but there was a significant difference between fresh pomegranate juice and the produced molasses by rotary with sugar or jam in total flavonoid contents. Also, non significant difference was found between fresh pomegranate juice and molasses produced by rotary with sugar or jam in total flavonoid contents. The increase in total phenols and total flavonoids content of pomegranate molasses is mostly due to the evaporation of water during processing. From the technological point of view and owing to their biological properties, the greatest importance among all phenol compounds present in pomegranate juices is considered to be due to ellagic acid derivatives, punicalagins and anthocyanins. Total phenol content was indicating considerable variations among pomegranate juices since the levels differed between 1500 and 4500 mg gallic acid equivalent/l (Mena *et al.*, 2011).

Antioxidant activity of pomegranate products measured by DPPH method revealed that, pomegranate molasses produced by rotary with and without sugar were not significantly different in antioxidant activity and both achieved the highest antioxidant activity compared with other products. The lowest antioxidant activity was found in pomegranate molasses produced by traditional heating method as a result of the long term thermal processing which caused lower antioxidant activity. Orak (2008) determined the antioxidant activities of pomegranate juice and molasses which were 79.06% and 85.91%, respectively, these are in accordance with the present results. The high value of antioxidant activity of pomegranate molasses may be due to the phenolic content in spite of anthocyanin being destroyed by the effect of heating. Pomegranate fruit is a rich source of polyphenols which ranged from 290-450 mg/100 ml juice and the total flavonoids contents were significantly different between pomegranate cultivars with approximately 1.6-folds difference between the highest and the lowest contents (Fawole *et al.*, 2011). Vrhovsek *et al.* (2004) recommended the daily intake (RDI) of polyphenols as 1 g/day, which means that the consumption of approximately 300 ml of the pomegranate juice will meet the RDI for polyphenols. Results of the present study showed that a few grams of pomegranate juice and molasses (with total

**Table (3): Chemical composition of pomegranate products.**

Chemical composition	Total sugars (%)	Reducing sugars (%)	Protein (%)	Ash (%)
Pomegranate juice	9.08±0.67 <sup>c</sup>	13.50±0.92 <sup>c</sup>	0.21±0.09 <sup>c</sup>	0.33±0.08 <sup>d</sup>
PM by heating method	43.29±2.69 <sup>a</sup>	20.56±1.75 <sup>a</sup>	0.37±0.11 <sup>b</sup>	2.65±0.33 <sup>a</sup>
PM by rotary with sugar	34.15±2.24 <sup>c</sup>	16.87±1.53 <sup>b</sup>	0.32±0.09 <sup>b</sup>	1.54±0.29 <sup>b</sup>
PM by rotary without sugar	25.15±1.83 <sup>d</sup>	19.87±1.82 <sup>a</sup>	0.27±0.08 <sup>c</sup>	1.53±0.19 <sup>b</sup>
Pomegranate jam	40.73±2.55 <sup>b</sup>	13.06±1.46 <sup>c</sup>	0.59±0.26 <sup>a</sup>	1.37±0.17 <sup>c</sup>
L.S.D.	2.18	1.84	0.17	0.15

\*PM = pomegranate molasses produced.

\*Numbers in the same column with the same letters were not significantly different at P<0.05.

**Table (4): Mineral composition of pomegranate products (mg/100 g) dry weight.**

Mineral composition	Fe	Ca	Mg	K
Pomegranate juice	1.36±0.25 <sup>d</sup>	23.3±1.34 <sup>d</sup>	26.12±1.30 <sup>d</sup>	86.35±3.69 <sup>c</sup>
PM by heating method	13.57±0.70 <sup>c</sup>	68.99±4.03 <sup>b</sup>	38.57±1.60 <sup>c</sup>	143.52±16.73 <sup>c</sup>
PM by rotary with sugar	16.64 ±1.34 <sup>b</sup>	69.20±4.67 <sup>b</sup>	45.96±1.74 <sup>b</sup>	169.55±19.60 <sup>b</sup>
PM by rotary without sugar	19.47±1.63 <sup>a</sup>	82.89±4.96 <sup>a</sup>	51.10±2.04 <sup>a</sup>	183.47±18.90 <sup>a</sup>
Pomegranate jam	12.98±0.92 <sup>c</sup>	53.64±3.44 <sup>c</sup>	39.96±1.68 <sup>c</sup>	98.37±3.84 <sup>d</sup>
L.S.D.	2.13	6.45	3.50	10.76

\*PM = pomegranate molasses produced.

\*Numbers in the same column with the same letters were not significantly different at P<0.05.

**Table (5): Polyphenols, total flavonoids and antioxidant values of pomegranate products.**

Characters	Total Polyphenols (mg/100 g)	Total flavonoids (mg/100 g)	DPPH (%)
Pomegranate juice	7.82±0.35 <sup>c</sup>	39.99±3.53 <sup>b</sup>	81.90±5.87 <sup>b</sup>
PM by heating method	5.27±0.14 <sup>d</sup>	27.04±3.19 <sup>d</sup>	65.50±4.81 <sup>c</sup>
PM by rotary with sugar	12.62±0.44 <sup>b</sup>	37.19±3.04 <sup>b</sup>	92.15±5.56 <sup>a</sup>
PM by rotary without sugar	18.93±0.63 <sup>a</sup>	44.71±3.54 <sup>a</sup>	94.20±6.86 <sup>a</sup>
Pomegranate jam	13.64±0.56 <sup>b</sup>	37.47±3.54 <sup>b</sup>	82.60±4.39 <sup>b</sup>
L.S.D.	1.42	3.52	5.18

\*PM = pomegranate molasses produced.

\*Numbers in the same column with the same letters were not significantly different at P<0.05.

flavonoids and phenolic contents of 39.99 ± 3.53 mg/100g and 18.93 ± 0.63 mg/100 g, respectively), are able to make a significant contribution to the total daily intake of dietary phenols in the human diet.

### 3.2. Biological studies

#### 3.2.1. Effect of pomegranate products on liver enzyme activities and antioxidative status in rats

Data presented in Table (6) show the effect of pomegranate products on the activity of liver marker enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) as well as

glutathione peroxidase (GPx) as a biomarker of oxidative status in rats. Liver enzymes (ALT and AST) were not significantly different at zero time between control and pomegranate product groups and during the experimental period for the control rat group. A significant decrease in ALT and AST activities was observed at the end of the experimental period for pomegranate products fed rats than that at zero time. The highest decrease was achieved by feeding rats on pomegranate molasses produced by rotary without sugar compared with the other groups. Meanwhile, there were non - significant differences between other

pomegranate products at the end of the experimental period for ALT activity. AST activity was not statistically different between pomegranate juice and molasses produced by heating groups but it was significantly different for pomegranate molasses produced by rotary with sugar and jam products which were not significantly different compared to- each other. Kaur *et al.* (2006) evaluated antioxidant and hepatoprotective activities of pomegranate flowers extract *in vivo* and it was found to exhibit a potent protective activity in acute oxidative stress in tissue injury in animal model such as hepatotoxicity in mice. The antioxidant capacity is probably responsible for the hepatoprotective property of pomegranate extract. The increase in the activity of liver marker enzymes in serum is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream.

From the data presented in Table (6) it could be observed that, glutathione peroxidase activity (GPx) significantly increased in all pomegranate product groups compared with the control group. The highest increase was achieved by feeding on pomegranate molasses produced by rotary without sugar group (11.75%) followed by pomegranate molasses with sugar group (7.81%). Feeding healthy mice on pomegranate juice for 28 days resulted in a protective effect of antioxidative enzymes including glutathione peroxidase (GPx). Also. The administration of pomegranate pulp juice (250 ml/d) to healthy humans for 28 days increased plasma antioxidant capacity and decreased plasma carbonyl content (Faria *et al.*, 2007 and Guo *et al.*, 2008). The production of reactive oxygen species (ROS) was strongly diminished in the presence of pomegranate molasses but less in the presence of juice. The effect of molasses or juice was dose dependent of the product used. Thus, the strong antioxidant substances found in molasses are acting as reactive oxygen species scavengers. SOD activity increased significantly in the group of mice given molasses, but slightly in the group given juice. The phenolic core can act as a buffer and capture electrons from ROS to render them less reactive. Polyphenols can interfere with the cellular detoxification systems such as SOD, catalase and glutathione peroxidase (Mounayar *et al.*, 2012).

### **3.2.2. Effect of pomegranate products on serum iron, hemoglobin and blood picture in rats**

Table (7) shows the effect of pomegranate juice, molasses and jam on serum iron levels and hemoglobin concentrations as well as red blood cells, white blood cells and platelets counts in the

blood at the experimental rats. Serum iron levels were significantly increased as a result of feeding on different pomegranate products compared with either the control rats or pomegranate product groups at zero time. The highest increase in serum iron levels was recorded for pomegranate molasses produced by rotary without sugar followed by pomegranate jam groups. There were non significant differences in iron levels between pomegranate juice, molasses produced by rotary with sugar and jam groups.

While pomegranate molasses produced by heating method were statistically different compared with other groups. Also, hemoglobin concentration showed a significant increase in groups of rats fed on pomegranate products with the highest increase achieved by using pomegranate molasses produced by rotary without sugar and with sugar groups (16.91% and 16.18%, respectively). The lowest increase in hemoglobin concentration was noticed for the group fed on pomegranate jam (3.68%).

Red blood cells, white blood cells and platelets counts were significantly increased by feeding on different pomegranate products compared with the control and also between pomegranate product groups at zero time and at the end of the experimental period. Pomegranate molasse groups without and with sugar were not significantly different but achieved the highest counts for red blood cells and white blood cells, respectively. All pomegranate products were found to improve blood picture measurements in healthy rats with the highest records achieved by using pomegranate molasses produced by rotary without sugar. The present results in Table (7) of serum iron and hemoglobin values as well as RBCs count confirmed each other.

The mechanisms by which chronic synthesis of the red cell pigment heme involve at least two enzymes, the first one in the cytoplasm (delta-amino leuvinic acid) at the beginning of heme synthesis and the second enzyme in mitochondrial (ferrochelatase) at the end of heme synthesis. Ferritin is both a very efficient iron trap and a readily an available source of iron for metabolic requirements and for the formation of hemoglobin and other heme proteins. The minute concentration of ferritin in the serum is an indicator of body storage iron. Liver injury results in release of relatively enormous amounts of ferritin into plasma, resiging the serum ferritin concentration and serum iron concentration several hundered times (shibutani *et al.*, 2001).

**Table (6): Effect of pomegranate products on liver marker enzymes and antioxidative status in the experimental rats.**

Groups	ALT (IU/L)		AST (IU/L)		GPx (nmol/ml)	
	Zero	End	Zero	End	Zero	End
Control rats	24.5 <sup>a</sup>	24.3 <sup>a</sup>	29.1 <sup>a</sup>	28.5 <sup>a</sup>	243.6 <sup>a</sup>	248.4 <sup>d</sup>
Pomegranate juice	24.4 <sup>a</sup>	23.1 <sup>b</sup>	28.5 <sup>a</sup>	25.5 <sup>c</sup>	240.9 <sup>a</sup>	261.9 <sup>b</sup>
PM by heating method	24.3 <sup>a</sup>	23.5 <sup>b</sup>	28.3 <sup>a</sup>	24.7 <sup>cd</sup>	241.7 <sup>a</sup>	266.7 <sup>b</sup>
PM rotary without sugar	24.6 <sup>a</sup>	21.4 <sup>c</sup>	28.3 <sup>a</sup>	23.3 <sup>d</sup>	240.5 <sup>a</sup>	277.6 <sup>a</sup>
PM by rotary with sugar	24.2 <sup>a</sup>	23.4 <sup>b</sup>	28.4 <sup>a</sup>	26.4 <sup>b</sup>	241.3 <sup>a</sup>	267.8 <sup>b</sup>
Pomegranate jam	24.5 <sup>a</sup>	23.2 <sup>b</sup>	29.0 <sup>a</sup>	26.7 <sup>b</sup>	242.5 <sup>a</sup>	259.5 <sup>c</sup>
L.S.D.	0.74		0.88		8.51	

\*PM = pomegranate molasses produced.

\*Numbers in the same column with the same letters were not significantly different at P<0.05.

**Table (7): Effect of pomegranate products on serum iron, hemoglobin and blood picture in rats.**

Groups	Serum iron levels (µg/dl)		Hemoglobin concentrations (µg/dl)		Red blood cells X10 <sup>6</sup> count		White blood cells x10 <sup>3</sup> count		Platelets x10 <sup>3</sup> count	
	Zero	End	Zero	End	Zero	End	Zero	End	Zero	End
Control rats	90.0 <sup>a</sup>	92.5 <sup>d</sup>	12.91 <sup>a</sup>	13.40 <sup>c</sup>	4.6 <sup>a</sup>	4.7 <sup>c</sup>	12.0 <sup>a</sup>	12.3 <sup>c</sup>	329.1 <sup>a</sup>	340.4 <sup>c</sup>
Pomegranate juice	88.5 <sup>a</sup>	106.5 <sup>b</sup>	13.02 <sup>a</sup>	14.50 <sup>b</sup>	4.4 <sup>a</sup>	5.8 <sup>b</sup>	12.2 <sup>a</sup>	12.9 <sup>b</sup>	330.3 <sup>a</sup>	369.6 <sup>b</sup>
PM by heating method	89.2 <sup>a</sup>	99.6 <sup>c</sup>	12.87 <sup>a</sup>	14.80 <sup>b</sup>	4.3 <sup>a</sup>	6.2 <sup>b</sup>	11.9 <sup>a</sup>	13.3 <sup>b</sup>	331.7 <sup>a</sup>	365.3 <sup>b</sup>
PM by rotary without sugar	91.5 <sup>a</sup>	114.7 <sup>a</sup>	13.08 <sup>a</sup>	15.90 <sup>a</sup>	4.5 <sup>a</sup>	6.8 <sup>a</sup>	12.1 <sup>a</sup>	14.3 <sup>a</sup>	326.5 <sup>a</sup>	397.2 <sup>a</sup>
PM by rotary with sugar	88.3 <sup>a</sup>	108.3 <sup>b</sup>	12.90 <sup>a</sup>	15.80 <sup>a</sup>	4.6 <sup>a</sup>	6.7 <sup>a</sup>	11.8 <sup>a</sup>	14.1 <sup>a</sup>	324.1 <sup>a</sup>	371.8 <sup>b</sup>
Pomegranate jam	90.4 <sup>a</sup>	109.5 <sup>b</sup>	12.88 <sup>a</sup>	14.10 <sup>b</sup>	4.5 <sup>a</sup>	5.9 <sup>b</sup>	12.2 <sup>a</sup>	13.4 <sup>b</sup>	328.5 <sup>a</sup>	378.6 <sup>ab</sup>
L.S.D.	4.56		0.73		0.46		0.54		17.6	

\*PM = pomegranate molasses produced.

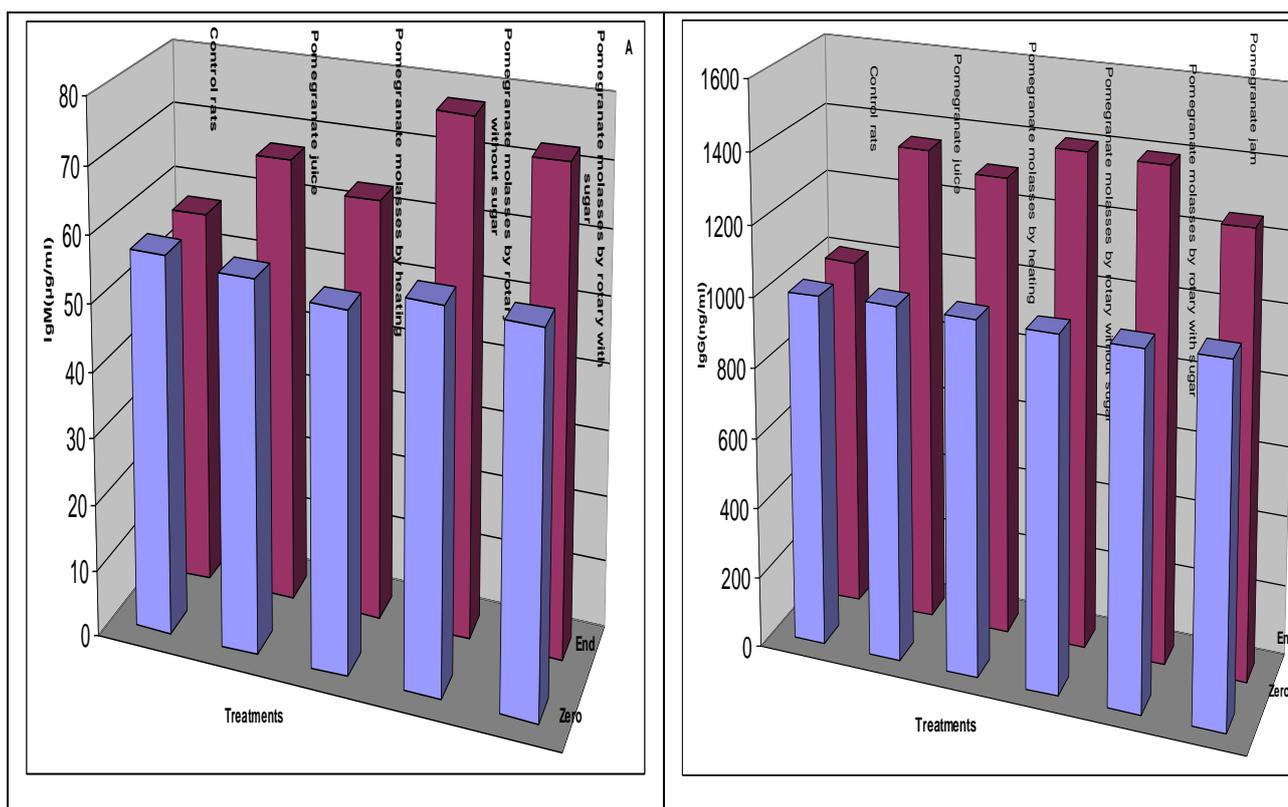
\*Numbers in the same column with the same letters were not significantly different at P<0.05.

**3.2.3. Effect of pomegranate products on immunoglobulins in rats**

Fig. (1) shows the effect of pomegranate products on aspects of immune function (immunoglobulins: IgM and IgG) in rats. Feeding rats on different pomegranate products caused a significant increase in IgM and IgG levels with the highest increase achieved by feeding on pomegranate molasses produced by rotary without sugar followed by pomegranate molasses produced by rotary with sugar groups. IgM levels were increased by 35.25% and 27.05%, while IgG levels were increased by 39.85% and 38.88% relative to the control, respectively. The lowest increase in IgM was achieved by feeding on pomegranate molasses produced by heating method (10.65%) which was not significantly different compared with pomegranate juice and jam groups. For IgG, the lowest increase was recorded in pomegranate jam group (24.89%) which was significantly different compared with the other groups. Feeding on pomegranate molasses produced by rotary and pomegranate

juice caused an improvement in immune function in rats compared with the control group. IgM is frequently associated with the immune response to antigenically complex, blood borne infections agents and is used as a diagnostic screening antibody. IgG functions as an antiallergic factor by competing with the allergen-specific IgE to bind to the receptor on the surface of the target cells such as mast cells and basophils. Therefore, both IgA and IgG exert a preventive effect on IgE-mediated allergy and viral infections (Roche *et al.*, 2001 and Dempsey *et al.*, 2003).

The pro-oxidant/antioxidant balance is an important approval of the immune cell function, not only for maintaining of integrity and functionality of membrane lipids, cellular proteins and nucleic acids of the immune cell, but also for the control of signal transduction and gene expression as well. The immune cells are particularly sensitive to oxidative stress because of the rather high percentage of polyunsaturated fatty acids in their cell membranes. To overcome this problem, the immune cells usually contain higher



**Fig(1): Effect of Pomegranate aril products on immunoglobulins.**

amounts of antioxidants than the other cells. Polyphenols as dietary antioxidants may affect various aspects of the immune system by shifting pro-oxidant/antioxidant balance towards antioxidant. Immunoglobulin production may be enhanced in rats fed on quercetin. The effect on Ig production by polyphenols can be complex and class specific. Daidzein enhance IgM and IgE levels at concentrations above 10 µM, moreover, quercetin and luteolin enhanced medium IgE level at all concentrations tested. These effects indicate that polyphenols induce general increase in immunoglobulin concentrations (Han *et al.*, 2002).

Pomegranate is among the richest fruit in polyphenols. It is logical to hypothesize that, fresh pomegranate juice should have a more considerable amounts of polyphenols than that found in molasses obtained after 6 h. of heating. One can consider that high temperature should deteriorate polyphenols in molasses as severe thermal treatment can alter the bioavailability of polyphenols by decreasing the absorption of active compounds due to the formation of oxidized products. On the contrary, polyphenols in molasses were found to be four times greater than those found in the juice. Molasses has the strongest antioxidant properties *in vitro* compared to juice. This could indicate that, high temperature

helps polyphenols to be released from pomegranate fruit cells as there is no extraction with a solvent in the preparation of pomegranate molasses (Porrini and Riso, 2008 and Visioli *et al.*, 2011).

In conclusion, Pomegranate molasses produced by rotary methods were found to achieve the highest quality characteristics compared with other pomegranate products of the present study. Pomegranate molasses produced by rotary without sugar recorded the highest increase in total phenol content, total flavonoids and antioxidant activity. Pomegranate molasses produced by heating method showed the lowest values for color indices and antioxidant activity as a result of severe heat treatment. Pomegranate jam achieved the highest scores for different sensory. Administration of pomegranate juice and pomegranate molasses caused an improvement in liver marker enzymes, activity as well as antioxidant status and immune functions. Therefore, pomegranate juice, molasses and jam products should be served in the human diet to obtain their nutritional benefits.

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### دراسات كيميائية وحيوية على بعض منتجات الرمان

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#### ملخص

تستخدم ثمرة الرمان بكثرة في الغذاء والصناعات الغذائية ويرجع ذلك لفوائدها الغذائية الممتازة وقيمتها الصحية العالية لذلك فان الهدف من هذه الدراسة هو: انتاج عصير الرمان و مولاس الرمان (عصير الرمان المركز او الدبس) بعدة طرق مختلفة ومربى الرمان، وقياس خواص الجودة الاساسية لهذة المنتجات ودراسة تأثير هذة المنتجات على الحالة العامة لمضادات الاكسدة وبعض عوامل المناعة في الفئران السليمة صغيرة السن لوحظ ان مولاس الرمان المنتج بجهاز الروتارى مع السكر ومربى الرمان حصلت على اعلى الدرجات للقابلية الكلية لدى المستهلك ، وكانت المواد الصلبة الكلية لمولاس الرمان المنتج بالروتارى مع السكر اعلى بالمقارنة مع باقى عينات مولاس الرمان و ذلك نتيجة اضافة السكر، بينما اظهر مولاس الرمان المنتج بطريقة التسخين التقليدية اقل المعدلات لعوامل اللون بالمقارنة مع باقى المنتجات وذلك للمعاملة الحرارية العالية. يحتوى مولاس الرمان على نسب عالية من الحديد، الزنك، الكالسيوم بالمقارنة مع العصير. احتوى مولاس الرمان المنتج بالروتارى بدون سكر على اعلى قيمة من عديد الفينولات ( $18.93 \pm 0.63$  ملجم/100جم) ومحتوى الفلافونيات الكلية ( $44.71 \pm 3.54$  ملجم/100جم) مع اعلى نشاط كمضادات الاكسدة.

ادت تغذية الفئران السليمة صغيرة السن على مختلف منتجات الرمان السابقة ادت الى انخفاض معنى فى نشاط انزيمات الكبد بالمقارنة مع فئران الكنترول وزيادة معنى فى نشاط انزيم الجلوتاثيون بيروكسيداز فى سيرم الفئران، وكانت اعلى زيادة فى نشاط الجلوتاثيون بيروكسيداز بالتغذية على مولاس الرمان المنتج بالروتارى بدون سكر (11.75%) يليه مولاس الرمان المنتج بالروتارى مع السكر (7.81%). كما لوحظ ان تغذية الفئران على مختلف منتجات الرمان سببت زيادة معنى فى مستويات الحديد فى السيرم والجلوبيولينات المناعية.

يمكن القول من النتائج المتحصل عليها من هذه الدراسة بأن مولاس الرمان المنتج بطرق الروتارى اعطى اعلى خواص الجودة بالمقارنة مع باقى منتجات الرمان. يمكن استخدام منتجات الرمان فى العديد من الاغذية والمنتجات لتحسين الحالة العامة لمضادات الاكسدة والوظائف المناعية ويرجع ذلك لاحتوائها على العديد من المركبات الحيوية.

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (64) العدد الرابع (أكتوبر 2013): 396-408.