Impact of Freezing and Freeze-drying Processes on Color, Phytochemical Contents and Antioxidant Capacity of Pomegranate Seeds

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Abstract: The effects of freezing and freeze-drying processes on the antioxidant capacity and some phytochemical contents as well as the color of pomegranate seeds were evaluated. The fresh pomegranate seeds exhibited high antioxidant activity (79.50% and 3894.73 µmol trolox/100 g dry weight, measured by DPPH and ABTS assays, respectively). The pomegranate seeds had high contents of total anthocyanins (22.80 mg/100 g), total phenolics (633.55 mg GAE/100 g) and total flavonoids (593.23 mg quercetin equivalent/100 g). Processing did not significantly affect the color attributes of the pomegranate seeds except the hue angle values. Freeze-drying process significantly decreased the antioxidant activity and phytochemicals contents of pomegranate seeds whereas freezing process had less negative effect. The total anthocyanins, total phenolic and total flavonoid contents of pomegranate seeds well correlated with their antioxidant capacity. The best correlations were between the total anthocyanins content with DPPH radical scavenging activity (R^2= 0.9652) and $ABTS^{\bullet+}$ scavenging capacity (0.9752) values.

***Keywords*:** Pomegranate seeds, freezing, freeze-drying, antioxidant capacity, phenolics, flavonoids, anthocyanins.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits. It is cultivated extensively in Iran, Afghanistan, India, Mediterranean countries (Egypt, Syria, Tunisia, Turkey, Jordan, Lebanon, Greece, Italy, Spain and Morocco) and to some extent in USA, China, Pakistan, Japan and Russia (Sepúlveda *et al.,* 2010). The edible part of the fruit is called arils which are eaten fresh and can be preserved as frozen or dried arils, juices, syrup or used for making jam, jellies and others (Al-Maiman and Ahmad, 2002). Over recent years there has been a great increase in pomegranate commercial farming, due to the high quality attributes of pomegranate arils and their potential health benefits, such as anti-mutagenic, anti-hypertension, antioxidant activities, prevention of cardiovascular diseases and some cancer types (Sartippour *et al.,* 2008; Basu and Penugonda, 2009; Viuda-Martos *et al.,* 2010; Fawole *et al.,* 2012).

Phytochemicals profile of pomegranate seeds has been associated with the broad array of biological properties of pomegranate products (Mena *et al.,* 2011), turning this fruit into a product of growing interest for consumers and industry. The total phenolic content in pomegranate juice ranges from 1808 to 2566 mg/L (Gil *et al.,* 2000). Cyanidin and delphinidinare linked to pigmentation and antioxidant activity of pomegranate arils and juice (Drogoudi *et al.,* 2005; Tzulker *et al.,* 2007). Pelargonidin, cyanidin and delphinidin are the most prominent anthocyanidins found in pomegranate fruit juice and cause the red, blue and an intermediate color, respectively (Noda *et al.,* 2002). Other phenolic compounds such as catechin, gallocatechin, epicatechin, gallic acid and caffeic acid have also been detected in pomegranate. Anthocyanins stability is influenced by various factors such as temperature, pH, light and oxygen (Jaiswal *et al.,* 2010). Anthocyanins also may be susceptible to degradation by oxidizing enzymes (Ju and Howard, 2003). The β-carotene content of pomegranate arils declined during storage (O'Grady *et al.,* 2014). Therefore, there is a need for alternative methods of processing which can preserve nutritional and bioactive characteristics (Patras *et al.*, 2009).

Freezing causes minimal destruction to phenolic compounds in fruits, with retention levels dependent on cultivar. As an example, raspberries have been shown to lose up to 12% of phenolics in an early harvest cultivar, but a 12% gain of phenolics in a late harvest cultivar (Gonzalez *et al*., 2003). Late harvest raspberries have also been shown to contain higher levels of antioxidants in particular, total anthocyanins after freezing (Ancose *et al.,* 2000). Freezing fruits and vegetables does not cause any substantial loss of vitamin A and β-carotene (Shofian *et al.*, 2011). Also, freezing and even more drastic freeze-drying processes have little effect on some antioxidants in fresh fruits but could markedly affect others. It was established that the ascorbic acid content of freeze-dried tropical fruits and melons was not affected greatly, but beta-carotene levels decreased in some samples (Shofian *et al.*, 2011). Drying processes lead to flavonoids degradation. The proportion lost depends on the drying method. Freeze-drying is the less aggressive method whereas hot air drying leads to major losses (Zainol *et al.,* 2009; Zhang *et al.,* 2009; Dong *et al.,* 2011).

Accordingly, the aim of the present study was to evaluate the effect of freezing and freeze-drying processes on color attributes, phytochemicals and antioxidant capacity as well as the correlations between them in pomegranate seeds.

MATERIALS AND METHODS

Materials:

Pomegranate

Pomegranate fruits (*P. granatum* L., cv. Assiuty) were harvested at commercial ripening (fully mature according to commercial practice) from different locations of Egypt and immediately transported to the frozen plant (Whiz Company for Modern Food Industries), Ismailia, Egypt during the 2014 season. Pomegranates with defects (sunburn, cracks, bruises and cuts in the husk) were discarded and only fruit with

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healthy outer skins and uniform in size and appearance were used. Husks (about 300) were carefully cut with sharpened knives and seeds manually extracted. The seeds were collected in a tray, washed in a solution containing 100 µl L^{-1} chlorine (NaOCl) for 5 min and further rinsed in tap water, drained and excess water removed from seeds with paper towels, as reported by López-Rubira *et al.* (2005) and sampled. Seeds were then subjected to freezing process using Instant Quick Freezer (IQF) at –35ºC for 12-13 min and then packed before storage at -18ºC.

For studying the effect of freeze-drying process, fresh seeds from the same batches were collected and transported to the laboratory and then were freeze-dried by a vertical freeze-drier (CPERON, FDU-7006, Gyeonggi, Korea) for 36 hours at -70°C. The freeze-dried seeds were ground and maintained at freezing temperature until analyses.

Chemicals and reagents

Folin-Ciocalteu's phenol reagent, anhydrous sodium carbonate, gallic acid, aluminum chloride and sodium hydroxide were purchased from Fluka (Fluka Chemie GmbH, Switzerland). Sodium nitrite, quercetin, 2.2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), potassium persulfate and 2,2´-azino-bis (3-ethylbenzothiazoline–6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Methanol, ethanol and hydrochloric acid (analytical grade) were from Scharlab (Scharlab Chemies s. a., Barcelona, Spain).

Methods:

Moisture content

The moisture content of all studied samples was determined by oven at 70°C under vacuum until constant weight according to the method described by AOAC (2002).

Color measurements

Color attributes; lightness (L^*), redness (a^*) and yellowness (b^*) were performed using a Minolta Color Reader CR-10 (Minolta Co. Ltd., Osaka, Japan). The color intensity (C^*) was calculated as $C^* = (a^{*2} + b^{*2})^{0.5}$. Furthermore, the hue angle (h_{ab}) was calculated as $h_{ab} = \tan^{-1}(b^*/a^*)$, where h_{ab}= 0º for a red hue and h_{ab}= 90º for a yellow hue (RØrå and Einen, 2003). To determine the overall color changes, a numerical total color difference (ΔE) was calculated according to Park *et al.* (2002) using the following equation: $\Delta E = [(L^*-L_0^*)^2 + (a^*-a_0^*)^2 + (b^*-b_0^*)^2]^{0.5}$, where L_0, a_0 and b_0 are the control values for the fresh sample. All attributes were measured or calculated before and after freezing and freeze-drying steps.

Determination of total anthocyanins

The total anthocyanins content of pomegranate seeds was determined on the fresh and the corresponding processed samples using the method proposed by Alarcao-E-Silva *et al.* (2001) with some modifications. One gram of the sample was blended in 10 ml of extraction solvent (0.01% v/v HCl in methanol) and extracted for 15 min. The sample was then filtered through filter paper No. 102 and the volume was adjusted to 10 ml by the extraction solvent. Absorbance at 530 nm was measured on the supernatant using a spectrophotometer (6505 UV/ VIS, Jenway LTD, Felsted, Dunmow, UK). The total anthocyanins content was calculated on the basis of molar extinction coefficient (30000) proposed by Deman (1999) taking the molecular weight (322.7) for cyanidin chloride as the following equation:

Total anthocyanins= (A*322.7*extract volume*1000)/ (30000*1000*sample weight)

Where A is the absorbance at 530 nm and the content was expressed as mg per 100 g dry weight.

Preparation of total phenolics, total flavonoids and antioxidants extracts

The antioxidants and bioactive compounds extract was prepared according to the method described by Barros *et al.* (2011) with some modifications as follows: one half gram of the sample was stirred with 25 ml of methanol at 100 rpm on Orbital Shaker (LAB-LINE Instruments, Inc., USA) for one hour at room temperature (32 ± 1°C) and then filtered through filter paper No. 102. The residue was re-extracted with 25 ml of methanol. The methanolic extracts were combined and stored at 4°C until further analyses.

Determination of total phenolics content

Total phenolics content was determined according to the Folin-Ciocalteu assay with slight modifications (Barros *et al.*, 2011). A half ml aliquot of the extract was mixed with 5 ml of Folin–Ciocalteu phenol reagent (diluted with water 1:10 v/v) and 4 ml of sodium carbonate (75 g/ L). The tubes were vortexed for 30 s and allowed to stand for 60 min at room temperature (32 ± 1°C) for color development. The absorbance was measured at 765 nm by spectrophotometer. A calibration curve (R^2= 0.9986) of gallic acid (0 – 0.10 mg/ml) was prepared and treated in similar conditions. The results were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW).

Determination of total flavonoids content

Total flavonoids content was determined by technique reported by Barros *et al.* (2011). Briefly, a half ml aliquot of the extract was mixed with 2 ml of distilled water followed by addition of 0.15 ml of $NaNO_2$ (5%) solution. After 6 min, 0.15 ml of $AlCl_3$ solution (10%) was added and allowed to stand for another 6 min before 2 ml of NaOH solution (4%) was added. The mixture was brought to 5 ml with distilled water. Then the mixture was mixed well and allowed to stand for 15 min. The absorbance was measured at 510 nm. A calibration curve of quercetin was prepared and total flavonoids content was determined from the linear regression equation (R^2= 0.9976) of the calibration curve. The results were expressed as mg quercetin equivalents per 100 g of dry sample.

Determination of DPPH radical–scavenging activity

The antioxidant activity of the methanolic extracts was determined by DPPH method modified by Ravichandran *et al.* (2013). Shortly, 0.1 ml of the extract was mixed for 30 s with 3.9 ml of DPPH

solution (6 x 10^{-5} M), and left to react for 30 min, after which the absorbance of the mixture was measured at 515 nm. The DPPH solution without extract was analyzed as blank. The antioxidant activity was calculated as follows, on dry weight basis:

DPPH radical–scavenging activity (%)= [(A_{blank} - A_{sample})/A_{blank}] x 100

Where A is the absorbance at 515 nm.

$ABTS^{\bullet+}$ assay (trolox equivalent antioxidant capacity, TEAC)

The ability of the samples extract to scavenge the $ABTS^{\bullet+}$ radical was determined using the trolox equivalent antioxidant capacity (TEAC) assay. The method modified by Rufino *et al.* (2010) was used. Briefly, $ABTS^{\bullet+}$ radical cations were produced by reacting 7 mM ABTS stock solution with 145 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.800 ± 0.020 at 734 nm. After addition of 30 µl of the sample extract or trolox standard to 4 ml of diluted $ABTS^{\bullet+}$ solution, absorbance was recorded after 6 min of mixing. Ethanolic solutions of known trolox concentrations (0-10 µg per ml) were used for calibration (R^2= 0.9988) and results were expressed as µmol trolox per 100 g dry sample.

Statistical analysis:

Each experiment was done in six replicates. The results were expressed as mean ± standard deviation and were analyzed by SPSS (version 17.0 SPSS Inc). One–way analysis of variance was performed using ANOVA procedures. Significant differences between the means were determined by Duncan's Multiple Range test. $P \leq 0.05$ was considered as a level of significance.

RESULTS AND DISCUSSION

Impact of freezing and freeze-drying processes on the color attributes of pomegranate seeds

The effects of freezing and freeze-drying techniques on the color attributes of pomegranate seeds are presented in Table (1). The measured initial values of the lightness (L^*), redness (a^*) and yellowness (b^*) of the fresh seeds were 43.50, 14.45 and 2.80, respectively which indicated that fresh seeds had low intensity (Chroma, 14.72) red color (hue angle, 10.97). From Table (1), freezing or freeze-drying techniques did not significantly affect the color attributes values of pomegranate seeds, except the hue angle values. The a^* values of both techniques (16.10 for frozen and 13.97 for freeze-dried seeds) were not significantly different. The frozen seeds denoted a redder Chroma (16.16). The hue angle values of frozen (4.90) and freeze-dried (3.40) pomegranate seeds were significantly different between each technique and the fresh ones (10.97) (Table 1).

The total color difference (ΔE) values did not significantly varied between frozen and freeze-dried pomegranate seeds. Therefore, it could be confirmed that freezing or freeze-drying techniques did not affect on the color attributes of the resultant pomegranate seeds.

Table (1): Impact of freezing and freeze-drying processes on the color attributes of pomegranate seeds

Treatment	L^*	a^*	b^*	C^*	h_{ab}	ΔE
Fresh pomegranate seeds	43.50^a ±7.50	14.45^a ±4.32	2.80^a ±2.67	14.72^a ±3.10	10.97^a	-
Frozen pomegranate seeds	42.95^a ±2.45	16.10^a ±2.56	1.38^a ±0.60	16.16^a ±1.55	4.90^b	2.25^a
Freeze-dried pomegranate seeds	46.50^a ±4.40	13.97^a ±1.23	0.83^a ±0.27	13.99^a ±1.08	3.40^b	3.62^a

Data are expressed as means ± standard deviation

Means within the same column having the same superscript letter are not significantly different ($p \leq 0.05$)

Impact of freezing and freeze-drying processes on some phytochemicals content and antioxidant capacity of pomegranate seeds

Phytochemical components reported in pomegranate seeds in variable proportions are anthocyanins, ascorbic acid and β-carotene. Additionally, pomegranate fruit is rich in vitamin C, fatty acids, organic acids and mineral elements (Al-Said *et al.,* 2009; Opara *et al.,* 2009; Mena *et al.,* 2012). Punicalagins, anthocyanins, phenolic acids and ascorbic acid, either alone or in combination, are responsible for the antioxidant activity of pomegranates (Scalzo *et al.,* 2004).

The total anthocyanins (as cyanidin chloride) content in fresh pomegranate seeds (22.80 mg $100g^{-1}$) was 17.54% higher than that of frozen seeds (18.80 mg $100g^{-1}$) and 61.40% higher than that of freeze-dried seeds (8.80 mg $100g^{-1}$) as shown in Table (2). Anthocyanins content was not stable by the processing techniques. The reduction in total anthocyanins content may be attributed to the presence of oxidative enzymes (Lester *et al.,* 2004). During processing and storage, anthocyanin degradation and polymerization usually lead to its discoloration (Kalt *et al.,* 2000; Yuksel and Koka, 2008). Jaiswal *et al.* (2010) reported that anthocyanins were relatively heat stable without oxygen, but reduced considerably (65%) in the combined presence of heat and oxygen. They suggested that the enzyme polyphenol oxidase (PPO) might facilitate the destruction of total anthocyanins over time and that boiling and oven-drying arils could destroy PPO and preserve these anthocyanins. Anthocyanins

may be released from the cellular matrix by freezing, although this may appear as an increase in values due to efficient extraction, it is possible that analysis of these released anthocyanins may render the nutrient more instrumentally detectable although there is no indication that these increased levels will be available biologically (Lohachoompol *et al.,* 2004).

From Table (2), the results showed that total phenolics and flavonoids contents in freeze-dried treated seeds were significantly lower than those of the frozen or fresh seeds. The calculated decrement percentages for total phenolics and flavonoids content were 4.01 and 3.82, respectively of the frozen seeds while, in freeze-dried seeds they were 22.37 and 17.09. Ancose *et al.* (2000) found that the freezing process decreased the total phenolics content by 4-20% in four cultivars of raspberries. Marques *et al.* (2006) reported that during freeze-drying treatment, there may be a chance of decline in the content of antioxidants due to degradation of certain compounds. Also, Chang *et al.* (2006) reported that the composition of some antioxidants of the fruits is affected by freeze-drying. Temperature (degree and duration), oxygen, pH, light illumination, water activity, presence of saccharides and their degradation products and activities of various enzymes are considered to be important factors influencing anthocyanins stability and bioactive compounds content (Skrede *et al.,* 2004; Wrolstad *et al.,* 2005; Howard *et al.,* 2010).

The results in Table (2) showed also, the DPPH radical-scavenging activity (%, DW) and the $ABTS^{\bullet+}$ scavenging capacity (µmol trolox 100 g^{-1}, DW) of the processed pomegranate seeds. The $ABTS^{\bullet+}$ scavenging capacities of the processed samples were significantly ($p \leq 0.05$) different as compared to the fresh sample. Freeze-drying process caused a significant decrease in antioxidant capacity measured by DPPH assay of pomegranate seeds as compared to that for fresh and frozen seeds. Ancose *et al.* (2000) reported that the freezing process decreased the free radical scavenging capacity of four cultivars of raspberries. The DPPH and $ABTS^{\bullet+}$radical scavenging activities of the samples extracts indicated that some of the compounds present in the extracts were electron donors and could react with free radicals to terminate radical chain reactions and therefore, were able to boost the natural antioxidant defense mechanism (Xu and Chang, 2008).

Table (2): Impact of freezing and freeze-drying processes on some phytochemicals content (mg 100 g^{-1} DW) and antioxidant capacity of pomegranate seeds

Treatment	Total anthocyanins	Total phenolics	Total flavonoids	DPPH radical-scavenging activity (%, DW)	$ABTS^{\bullet+}$ scavenging capacity (µmol trolox 100 g^{-1}, DW)
Fresh pomegranate seeds	22.80^{a} ±1.50	633.55^{a} ±97.45	593.23^{a} ±88.44	79.54^{a} ±1.90	3894.73^{a} ±175.48
Frozen pomegranate seeds	18.80^{b} ±2.80	608.09^{a} ±41.26	570.58^{a} ±69.27	77.39^{a} ±3.01	3295.29^{b} ±190.25
Freeze-dried pomegranate seeds	8.80^{c} ±0.90	491.84^{b} ±63.89	496.04^{b} ±61.50	68.91^{b} ±0.72	2956.59^{c} ±120.69

DW= dry weight basis
Data are expressed as means ± standard deviation
Means within the same column having the same superscript letter are not significantly different ($p \leq 0.05$).

Data represented in this study showed a positive correlation between the total anthocyanins content with the color attributes of pomegranate seeds (Figure 1). The best polynomial regressions (3rd degree) were with the Chroma (C^{*}, 0.9449), b^{*} (0.9303) and h_{ab} (0.9253) values.

This result confirmed that, the anthocyanins are responsible for the attractive color of pomegranate seeds and some of the fruit's anti-oxidant activity (Borochov-Neori *et al.,* 2009).

From Table (2), the decrement of total anthocyanins, total phenolics and total flavonoids contents of pomegranate seeds well correlated with their antioxidant capacity measured by DPPH radical-scavenging activity and the $ABTS^{\bullet+}$ scavenging capacity methodologies. The antioxidant capacity is most significantly correlated with the contents of total phenolics and anthocyanins (Prior *et al.,* 1998; Proteggente *et al.,* 2002). Figure (2) showed a high correlation between the total anthocyanins content with DPPH radical scavenging activity values (R^2= 0.9652). Also, showed a high positive correlation between total phenolics (R^2= 0.9152), total flavonoids (R^2= 0.9242) content and DPPH radical scavenging activity of pomegranate seeds.

The same well correlations (polynomial regression, 3rd degree) were observed with the $ABTS^{\bullet+}$ scavenging capacity (Figure 3) of pomegranate seeds. The best correlation was with the total anthocyanins content (0.9752) followed by the total phenolics content (0.8672). Phenolics can act as efficient free radical scavengers by donating their alcoholic hydrogen or one of their delocalized electrons to radicals for their stabilization (Brown, 1995).

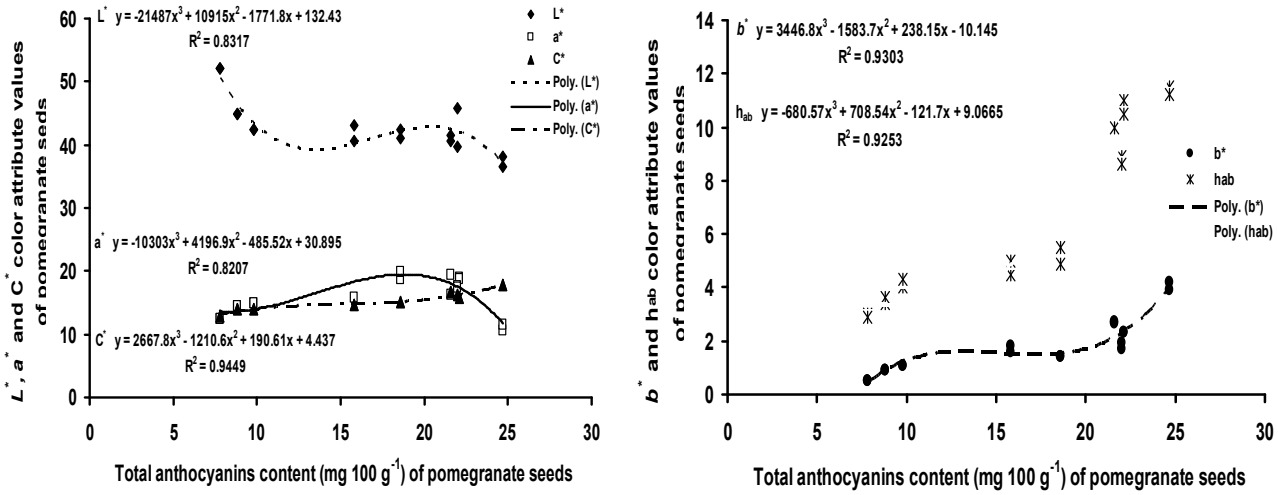

Figure (1): The polynomial regressions of the total anthocyanins content with Hunter color coordinates as affected by freezing and freeze-drying of pomegranate seeds

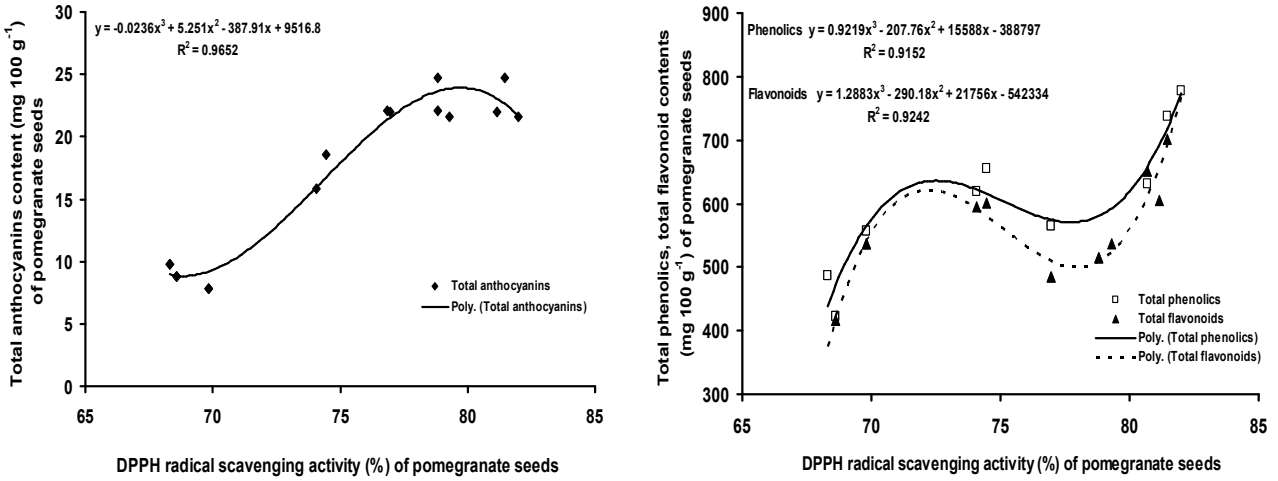

Figure (2): The polynomial regressions of the total anthocyanins, total phenolics, total flavonoid contents with the DPPH radical-scavenging activity as affected by freezing and freeze-drying of pomegranate seeds

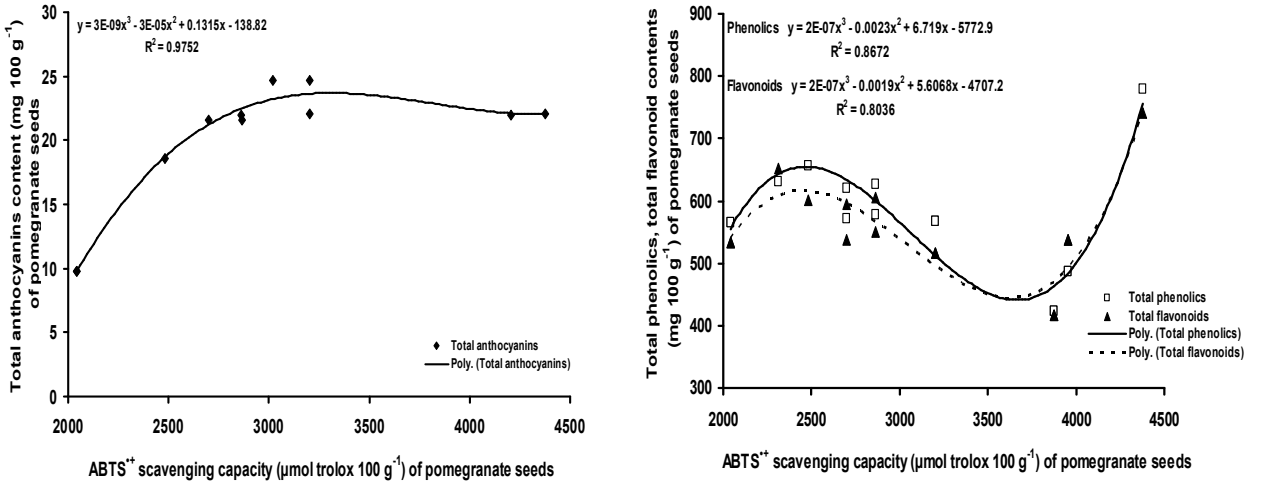

Figure (3): The polynomial regressions of the total anthocyanins, total phenolics, total flavonoid contents with the $ABTS^{\bullet+}$ scavenging capacity as affected by freezing and freeze-drying of pomegranate seeds

CONCLUSION

The results of the present study showed the importance of pomegranate seeds as a good source of natural antioxidants. Freezing and freeze-drying processes had significant effects on the antioxidant activity and phytochemical compounds of pomegranate seeds. Optimizing these processes is important to maintaining pomegranate seeds desirable properties.

REFERENSES

Alarcao-E-Silva, M., A. Leitao, H. Azinheira and M. Leitao (2001). The arbutus berry: studies on its colour and chemical characteristics at two mature stages. J. Food Compo. Anal., 14:27–35.

Al-Maiman, S. A. and D. Ahmad (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. Food Chem., 76: 437–441.

Al-Said, F. A., L. U. Opara and R. A. Al-Yahyai (2009). Physico-chemical and textural quality attributes of pomegranate cultivars (*Punica granatum* L.) grown in the Sultanate of Oman. J. Food Eng., 90:129–134.

Ancose, B., E. M. Gonzalez and P. Cano (2000). Ellagic acid, Vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. J. Agric. Food Chem., 48:4565-4570.

AOAC (2002). Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Inc., Washington DC, USA.

Barros, L., L. Cabrita, M. V. Boas, A. M. Carvalho and I. C. F. R. Ferreira (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. Food Chem., 127:1600–1608.

Basu, A. and K. Penugonda (2009). Pomegranate juice: a heart-healthy fruit juice. Nutr. Rev., 67:9-56.

Borochov-Neori, H., S. Judeinstein, E. Tripler, M. Harari, A. Greenberg, I. Shomer and D. Holland (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. J. Food Comp. Anal., 22:189–195.

Brown, H. W. (1995). Organic Chemistry. Saunders College Publishing, New York, USA, 521.

Chang, C. H., H. Y. Lin, C. Y. Chang and Y. C. Liu (2006). Comparison on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. J. Food Eng., 77: 478–485.

Deman, J. M. (1999). Principles of food chemistry (3rd Edition). Aspen Publishers, Inc., Gaithersburg, Maryland.

Dong, J., X. Ma, Z. Fu and Y. Guo (2011). Effects of microwave drying on the contents of functional constituents of *Eucommia ulmoides* flower tea. Ind. Crops Prod., 34:1102–1110.

Drogoudi, P. D., C. Tsipouiridis and Z. Michailidis (2005). Physical and chemical characteristics of pomegranates. HortSci., 40:1200–1203.

Fawole, O. A., U. L. Opara and K. I. Theron (2012). Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. Food Bioproc. Technol., 5:2934–2940.

Gil, M. I., F. A. Tomás-Barberán, B. Hess-Pierce, D. M. Holcrof and A. A. Kader (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem., 48:4581–4589.

Gonzalez, E. M., B. de Ancos and M. P. Cano (2003). Relation between bioactive compounds and free radical-scavenging capacity in berry fruits during frozen storage. J. Sci. Food Agric., 83:722-726.

Howard, L. R., C. Castrodles, C. Brownmiller and A. Mauromoustakos (2010). Jam processing and storage effects on blueberry polyphenolics and antioxidant capacity. J. Agric. Food Chem., 58:4022–4029.

Jaiswal, V., A. Der Marderosian and R. J. Porter (2010). Anthocyanins and polyphenol oxidase from dried arils of pomegranate (*Punica granatum* L.). Food Chem., 118:11–16.

Ju, Z. Y. and L. R. Howard (2003). Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. J. Agric. Food Chem., 51:5207–5213.

Kalt, W., J. E. McDonald and H. Donner (2000). Anthocyanins, phenolics, and antioxidant capacity of processed lowbush blueberry products. J. Food Sci., 65:390–393.

Lester, G. E., D. M. Hodges, R. D. Meyer and K. D. Munro (2004). Pre-extraction preparation (fresh, frozen, freeze-dried, or acetone powdered) and long-term storage of fruit and vegetable tissues: effects on antioxidant enzyme activity. J. Agric. Food Chem., 52:2167–2173.

Lohachoompol, V., G. Srzednicki and J. Craske (2004). The change of total anthocyanins in blueberries and their antioxidant effect after drying and freezing. J. Biomed. Biotechnol., 5:248–252

López-Rubira, V., A. Conesa, A. Allende and F. Artés (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. Postharvest Biol. Technol., 37:174–185.

Marques, L. G., A. M. Silveira and J. T. Freire (2006). Freeze-drying characteristics of tropical fruits. Drying Technol., 24:457–463.

Mena, P., L. Calani, C. Dall'Asta, G. Galaverna, C. García-Viguera and R. Bruni (2012). Rapid and comprehensive evaluation of (Poly) phenolic compounds in pomegranate (*Punica granatum* L.) Juice by UHPLC–MSn. Molecules, 17:14821–14840.

Mena, P., C. García-Viguera, J. Navarro-Rico, D. A. Moreno, J. Bartual, D. Saura and N. Martí (2011). Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. J. Sci. Food Agric., 91:1893–1906.

Noda, Y., T. Kaneyuka, A. Mori and L. Packer (2002). Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin. J. Agric. Food Chem., 50:166–171.

O'Grady, L., G. Sigge, O. J. Caleb and L. U. Opara (2014). Effects of storage temperature and duration on chemical properties, proximate composition and selected bioactive components of pomegranate (*Punica granatum* L.) arils. LWT - Food Sci. Technol., 57:508-515.

Opara, L. U., M. R. Al-Ani and Y. S. Al-Shuaibi (2009). Physico-chemical properties, vitamin C content, and antimicrobial proper-ties of pomegranate fruit (*Punica granatum* L.). Food Biopro. Technol., 2:315–321.

Park, S. J., J. L. Lee and J. Park (2002). Effects of a combined process of high-pressure carbon dioxide and high hydrostatic pressure on the quality of carrot juice. J. Food Sci., 67:1827-1834.

Patras, A., N. P. Brunton, S. Da Pieve and F. Butler (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. Inn. Food Sci. Emer. Technol., 10:308–313.

Prior, R. L., G. H. Cao, A. Martin, E. Sofic, J. McEwen, C. O'Brien, N. Lischner, M. Ehlenfeldt, W. Kalt, G. Krewer and C. M. Mainland (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J. Agric. Food Chem., 46:2686–2693.

Proteggente, A. R., A. S. Pannala, G. Paganga, L. van Buren, E. Wagner and S. Wiseman (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Rad. Res., 36:217–233.

Ravichandran, K., N. M. M. T. Saw, A. A. A. Mohdaly, A. M. M. Gabr, A. Kastell, H. Riedel, Z. Cai, D. Knorr and L. Smetanska (2013). Impact of processing of red beet on betalain content and antioxidant activity. Food Res. Int., 50:670–675.

RØrå, A. M. B. and O. Einen (2003). Effects of freezing on quality of cold smoked Salmon based the measurements of physicochemical characteristics. J. Food Sci., 68:2123-2128.

Rufino, M. S. M., R. E. Alves, E. S. de Brito, J. Pérez-Jiménez and F. Saura-Calixto (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem., 121:996-1002.

Sartippour, M., N. Seeram, D. Heber and A. Pantuck (2008). Ellagitannin-rich pomegranate extract inhibits angiogenesis in prostate cancer *in vitro* and *in vivo*. Int. J. Oncol., 32:475–480.

Scalzo, J., A. Politi, N. Pellegrini, B. Mezzetti and M. Battino (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutr., 21:207-213.

Shofian, N. M., A. A. Hamid, A. Osman, N. Saari, F. Anwar, M. S. P. Dek and M. R. Hairuddin (2011). Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. Int. J. Mol. Sci., 12:4678–4692.

Skrede, G., V. Bryhn Larsen, K. Aaby, A. Skivik Jørgensen and S. E. Birkeland (2004). Antioxidative properties of commercial fruit preparations and stability of bilberry and black currant extracts in milk products. J. Food Sci., 69:S351-S356.

Sepúlveda, E., C. Sáenz, A. Peña, P. Robert, B. Bartolomé and C. Gómez-Cordovés (2010). Influence of the genotype on the anthocyanin composition, antioxidant capacity and color of Chilean pomegranate (*Punica granatum* L.) juices. Chil. J. Agric. Res., 70:50–57.

Tzulker, R., I. Glazer, I. Bar-Ilan, D. Holland, M. Aviram and R. Amir (2007). Antioxidants activity, polyphenol content and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. J. Agric. Food Chem., 55:9559–9570.

Viuda-Martos, M., M. C. López-Marcos, J. Fernández-López, E. Sendra, J. H. López-Vargas and J. A. Pérez-Álvarez, J. A. (2010). Role of fiber in cardiovascular diseases: A review. Comp. Rev. Food Sci. Food Safe, 9:240–58.

Wrolstad, R. E., R. W. Durst and J. Lee (2005). Tracking color and pigment changes in anthocyanin products. Trends Food Sci. Technol., 16: 423-428.

Xu, B. and S. K. C. Chang (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chem., 110:1-13.

Yuksel, S. and I. Koka (2008). Color stability of blackberry nectars during storage. J. Food Technol., 6:166-169.

Zainol, M. M., A. Abdul-Hamid, F. A. Bakar and S. P. Dek (2009). Effect of different drying methods on the degradation of selected flavonoids in *Centella asiatica*. Int. Fd. Res. J., 16:531-537.

Zhang, M., N. S. Hettiarachchy, R. Horax, P. Chen and K. F. Over (2009). Effect of maturity stages and drying methods on the retention of selected nutrients and phytochemicals in bitter melon (*Momordica charantia*) leaf. J. Food Sci., 74: C441-C446.

تأثير عمليتي التجميد والتجفيد على خصائص اللون، المحتوى من المركبات الفعالة والنشاط المضاد للأكسدة في بذور الرمان

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تم تقدير تأثير عمليتي التجميد والتجفيد علي النشاط المضاد للأكسدة ومحتوى بعض المركبات الفعالة وكذلك خصائص اللون في بذور الرمان. وأوضحت النتائج أن لبذور الرمان الطازجة نشاط مضاد للأكسدة مرتفع (٧٩.٥٠٪ و ٣٨٩٤.٧٤ميكرومول ترولوكس لكل ١٠٠جرام مادة جافة مقدرة بطريقتي DPPH و ABTS، علي الترتيب). كما احتوت البذور الطازجة علي محتوى مرتفع من الأنثوسيانينات الكلية (٢٢.٨٠ مجم/١٠٠جم) والفينولات الكلية (٦٣٣.٥٥ مجم/١٠٠جم) والفلافونيدات الكلية (٥٩٣.٢٣ مجم/١٠٠جم). لم تؤثر عمليتي التجميد والتجفيد معنويا على خصائص لون بذور الرمان. أدت عملية التجفيد إلي انخفاض معنوي في النشاط المضاد للأكسدة ومحتوى بذور الرمان من المركبات الفعالة، بينما كان لعملية التجميد تأثير سلبي أقل. أوضحت النتائج أيضا وجود علاقة جيدة ما بين محتوى بذور الرمان من الأنثوسانين، الفينولات والفلافونيدات الكلية والنشاط المضاد للأكسدة لها. وكانت أفضل العلاقات تلك ما بين الأنثوسيانينات الكلية والنشاط المضاد للأكسدة مقدرا بطريقتي DPPH (معامل ارتباط ٠.٩٦٥٢) و ABTS (معامل ارتباط ٠.٩٧٥٢).