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The Effect of Technological Treatments of the Pasteurized Juice Processing on the Antioxidant Compounds Content and the Antioxidant Activity of Pomegranate *(Punica granatum L.)* Fruits Juice

Abeer Hassan A. Janbi and Stota Ahmed Al-Said

Nutrition and Food Science Dept., Faculty of Education for Home Economy and Art Education, King Abdul Aziz University, Jeddah, Saudi Arabia

ABSTRACT

This research was carried out to throw the light on the effect of technological treatments of pasteurized pomegranate juice processing; juice extraction methods, clarification process procedures and pasteurization process on its antioxidant capacity and the naturally occurrence antioxidant compounds content. The obtained results showed that fresh whole pomegranate fruits juice extracted by pressing procedure contained 81.90 % moisture and 18.10 % total solids. It also contained 0.45, 13.30, 12.80, 0.50, and 0.45 % from crude protein, total sugars, reducing sugars, non- reducing sugars and pectin contents, on wet weight basis; respectively. Therefore, total sugars are being the major solids constituent of fresh pomegranate whole fruits juice (73.48% of total solids) and the reducing sugars content represented 70.72 % of the total solids of fresh pomegranate juice. In addition that the fresh pomegranate juice extracted from whole fruits by pressing method was rich in the determined antioxidant compounds; polyphenols, tannins, anthocyanin pigments and L-ascorbic acid, which were naturally occurred at concentration of 2609.4 mg gallic acid equivalents (GAE), 3.65 mg tannic acid equivalents (TAE), 66.01 mg cyanidin-3-glucoside equivalents (CGE) per liter of juice; respectively, as well as it had a good antioxidant activity (719.2 mg trolox equivalents / liter of juice). The present results also showed that pressing extraction juice method exhibited a higher efficiency in extracting the all tested antioxidant compounds with pomegranate juice than the blending method and therefore the pomegranate juice batches extracted from whole fruits by using the pressing extraction method had the highest level from the determined antioxidant compounds; total polyphenols, total tannins, anthocyanin pigments and L-ascorbic acid, as well as it had the highest antioxidant activity, when compared with the other extracted juice batches. Clarification process of pomegranate juice caused a noticeable reduction in its content of the total polyphenols (16.40- 24.93), total tannins (12.79 – 31.51), anthocyanin pigments (10.71 – 31.51) and L-ascorbic acid (0.86 – 31.83 %) at different rates depending upon the antioxidant compound itself and the clarification method. Furthermore, the multi-technological treatment of clarification followed by pasteurization process for pomegranate juice batches caused a high loss in the determined antioxidant compounds content ranged between 25.41 and 31.96 % for total polyphenols , 26.58 and 41.92 % for total tannins, 25.82 and 41.17 % for the anthocyanin pigments, 56.76 and 65.38 % for L-ascorbic acid, depending upon the clarification method and the antioxidant compound itself, as well as it resulted in the reduction of the antioxidant activity of pomegranate juice batches by 20.80 – 32.97 %. Therefore, the most sensitive antioxidant compounds for the elimination effect of pasteurization process were found to be L-ascorbic acid. Regardless of the reduction effect of the tested technological treatments for processing of the pasteurized pomegranate fruit juice batches on their content of the determined antioxidant compounds and their antioxidant activities, these juice batches characterized with a high antioxidant compounds level and a good antioxidant activity. Therefore, the present results are recommended with increasing the utilization of pomegranate juice in food processing and future researching for novel technological treatments which can extend the shelf-life with less adverse effects on product quality, properties and the antioxidant compounds in pomegranate juice and in the other food products containing it.

Keywords: Pomegranate juice, Technological treatments, Blending juice extraction, Pressing juice extraction, Clarification treatments, Pasteurization process, Chemical composition, Physico-chemical quality characteristics.

Introduction

Pomegranate (*Punica granatum L.*) belongs to the punicaceae family. It is native to southwest Asia and has been cultivated and naturalized over the whole Mediterranean region of Asia, Africa and Europe, and the caucus since ancient times. In particular, its successful adaptation to the Mediterranean climate has produced a wide diffusion in various countries thus originating several local genotypes along the centuries. Pomegranate is widely cultivated throughout Afghanistan, Algeria, Armenia, Azerbaijan, Iran, Iraq, India, Pakistan, Syria, Turkey, Egypt, Italy, the dried parts of Southeast Asia, Peninsular Malaysia, the East Indies, and tropical Africa.

Corresponding Author: Abeer Hassan Ali Janbi, Nutrition and Food Science Dept., Faculty of Education for Home Economy and Art Education, King Abdul Aziz University, Jeddah, Saudi Arabia

In addition, it is now cultivated in parts of California and Arizona for juice production (Hobani and Elansari, 2004 Ahmed *et al*., 2005; Martinez *et al*., 2006; Ozgen *et al*., 2008 and Al-Said *et al*., 2009). The pomegranate is grown in many areas of Saudi Arabia particularly the Taif city and its neighboring villages and is one of the important commercial fruits in Saudi Arabia (Al-Maiman and Ahmed, 2002 and Hobani and Elansari, 2004). Pomegranate tree is the famous tree in Al-Taif city, because it has an economic important and consider one of the most natural resources in this area rather than of others in kingdom of Saudi Arabia. The pomegranate tree is mentioned in a holy Quran for its beneficial to human health. Recently, the products of pomegranate tree (including, peels, juice, leaves, seeds, flower. etc.) have medicinally and industry importance. (Negi *et al*., 2003 and Malik *et al*., 2005). Pomegranate is an ancient mystical fruit used in folkloric medicine as a treatment for many diseases such as diarrhea, parasitic worm infections, urinary tract infections and kidney stones (Navarro *et al*., 1996 and Sudheesh & Vijayalakshmi, 2005). It has been reported that *Punic granatum* can slow bacterial growth and inhibit bacterium-induced toxins (Braga *et al*., 2005 and Ghosh *et al*., 2008). Rabbits that received oral doses of aqueous extract of *Punica granatum* peel (100 mg /kg body weight) for 10 consecutive days had stimulated immune system and enhanced the cellular immunity (Gracious *et al*., 2001). Several additional studies have demonstrated the therapeutic effects of *P. granatum* fruit, peel and juice as powerful antioxidants and anti-inflammatory substances that include polyphenols and tannins (Aviram and Dornfeld, 2001; Aviram *et al*., 2002; Kim *et al*., 2002; Afaq *et al*., 2005 and Gasemian *et al*., 2006). Pomegranate also plays a great role in protecting against certain cancers such as breast, prostate and colon cancers among other degenerative diseases (Malik *et al*., 2005; Mertens-Talcott *et al*., 2006; Syed *et al*., 2007 and Hong *et al*., 2008) and its juice is effective in protecting neuron cells from Alzheimer's disease (Hong *et al*., 2008).

The market of pomegranate products has steadily grown, which is presumably due to the increasing consumer awareness of the potential health benefits attributed to pomegranate and phytochemical thereof (Martinez *et al*., 2006 and Lansky & Newman, 2007). A recent increasing demand for pomegranate products by consumers all over the world is especially supported for medicinal and nutritional properties (Lansky and Newman, 2007), due to the antioxidant properties of this fruit (Gil *et al*., 2000 and Seeram *et al*., 2008) that contains anticarcinogenic (Kim *et al*., 2002; Afaq *et al*., 2005; Malik *et al*., 2005 and Hong *et al*., 2008), antimicrobial (Reddy *et al*., 2007), antiviral (Kotwal, 2007) and antiatherosclerotic compounds even able to reduce blood pressure and LDL oxidation (Aviram *et al*., 2002). These activities are mainly attributed to the pomegranate's high levels of antioxidant activity and its high total polyphenols content (Gil *et al*., 2000 and Tzulker *et al*., 2007). Pomegranate juice was shown to possess a 3-fold higher antioxidant activity than that of red wine or green tea (Gil *et al*., 2000), and 2-, 6- and 8-fold higher levels than those detected in grape/cranberry, grapefruit, and orange juice; respectively (Rosenblat and Aviram, 2006).

Arils are the edible part of pomegranate fruit and contain around 80% of Juice and 20% of seed (Al-Maiman and Ahmed, 2002 and Mousavinejad *et al*., 2009). The pomegranate juice is rich in sugar, organic acids, vitamins, polysaccharides, essential minerals and the antioxidant compounds; polyphenols, tannins, anthocyanin pigments and L-ascorbic acid (Al-Maiman and Ahmed, 2002; Hobani and Elansari, 2004; Kulkarni and Aradhya, 2005 and Al-Said *et al*., 2009). Pomegranate fruit is consumed directly as fresh arils as well as fresh juice. It is also used extensively in the food and beverages industry for flavouring and colouring agents, as well as for making juice, jellies and grenadine or wine (Gill *et al*., 2000; Maesstre *et al*., 2000; Al-Maiman and Ahmed, 2002 and Al- Said *et al*., 2009). In many parts of the world, pomegranate kernels are also used as a garnish for salads and desserts (Al-Maiman and Ahmed, 2002 and Al- Said *et al*., 2009). In addition to its use in the food industry pomegranate juice is now increasingly used widely as dye in healthcare and cosmetic products such as shampoos and high-value carpets (Al- Said *et al*., 2009). In Saudi Arabia, the edible part of the pomegranate fruits is mainly consumed fresh, but it is also used in the preparation of fresh juice; which is a very popular beverage. The pomegranate juice sacs may be frozen intact or the extracted juice may be concentrated and frozen for further use. Pomegranate juice is widely regarded as a delicacy and is made into excellent sherbet and it is consumed with the addition of water and sugar. It is also used in preparing syrups, jellies, marmalades, and pomegranate molasses (called dibs rumman in Arabic). Pomegranate molasses is heavy pomegranate syrup used in some parts of the world in cooking and also to marinate meat, due to its content of proteolytic enzymes, which acts as a meat tenderizer (Hobani and Elansari, 2004).

Consumer demand for freshly squeezed fruit juices in increasing but such products are susceptible to spoilage and thus have a limited shelf-life (Buzrul *et al*., 2008). Thermal processing; pasteurization process, is the most commonly used preservation technique to extend the shelf-life of juices. However, this process may have adverse effects on sensory and nutritional values of juices. Therefore, color quality of anthocyanins containing pomegranate juice is undesirably lost during thermal process (Plaza *et al*., 2006). It has been reported that the higher antioxidant activity of commercial pomegranate juice is due to the punicalagins and ellagic acid derivatives, which are mostly, located in the fruit rind more than other hydolizable tannins from the arils. Therefore it is recommended that the juice be extracted from whole fruits for a higher antioxidant capacity, although this results in the increase of bitterness and astringency in the juice (Gil *et al*., 2000). Another technological treatment carries out sometimes during the processing of pomegranate juice is clarification. The

aim of that step is to reduce the amount of phenolic substances and improve some sensory properties, such as color, turbidity, overall appearance and bitterness. However, this practice leads to a reduction in the juice's health benefits (Vardin and Fenercioglu, 2003).

Although the high production of pomegranate juice and its extensive use in the food and beverages industry in Saudi Arabia, the little efforts have been done and no sufficient published data were found about the stability of the antioxidant compounds naturally present in pomegranate juice throughout its processing. Therefore, this research was performed to evaluate the effect of the technological treatments of pasteurized pomegranate juice processing, juice extraction methods, clarification process procedures and pasteurization process on the antioxidant compounds and the antioxidant activity of pomegranate juice.

Materials and Methods

1. Materials:

(a). Pomegranate fruits:

Fresh fully ripe and reddish yellow pomegranate (*Punica granatum L.*) fruits, grown at fruiting season of 1428 A.H in Al-Taif city, were obtained from the local market in Jeddah city, Saudi Arabia. The obtained pomegranate fruits were packed in plastic boxes and immediately transported to the laboratory research for post-graduate students, Fac. of Education for Home Economics and Art Education, King Abdel Aziz University, Jeddah, Saudi Arabia.

(b)- Chemicals and Reagents:

All chemicals and reagents used in this investigation were of analytical grade. Pure standard tannic acid, gallic acid, trolox [(+1-)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2- carboxylic acid], ABTS [2,2-azinobis-(3-ethylbenzthiazoline-6- sulfonic acid) diammonium salt], Folin-Ciocalteu regent, cyanidine-3-glucoside and Folin-Denis reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the other chemicals, solvants and reagents used in our investigation were purchased from Beijing Chemical Co., Beijing, China.

2. Technological Methods:

2. 1.Preparation of Pasteurized pomegranate Juice Batches:

Processing of the pasteurized pomegranate juice included the 3 main technological treatments; juice extraction, clarification process and pasteurization process, as the following:-

(a)- Extraction of pomegranate juice batches:

Tested pomegranate juice batches were extracted from either whole pomegranate fruits or pomegranate fruit arils. To obtain the whole pomegranate fruits juice batches, fully ripe pomegranate fruits were cleaned , washed with tap water ,steam for 5 minutes for enzyme inactivation, cut into halves with a stainless steel sharp knife and then divided into the two batches; the first batch was used for extraction the juice by the manual pressing using a stainless steel hand operated juice extractor and the second batch was used for extraction the juice by blending using an electric juice blender (Model ju 2000 vitae, Moulinex, Barcelona, Spain). To obtain the pomegranate arils juice batches, the pomegranate fruits were cleaned, washed with tap water, let to drain for 5 minutes and peeled manually by stainless steel sharp knife and the arils were .separated. Then, the separated arils were divided into 2 batches, which were steamed individually for 5 minutes for enzyme inactivation. The first batch of steamed arils was rolled with press cloth to use for the extraction of the arils juice batch by the manual pressing using a stainless steel hand operated juice extractor, while the second batch of steamed arils was used for the extraction of the arils juice batch with blending treatment by using an electric juice blender (Model ju 2000 vitae, Moulinex, Barcelona, Spain). The extracted pomegranate juice batches were filtrated through a 3 layers of cheese cloth.

(b)- Clarification process of the extracted pomegranate juice batches:

The extracted pomegranate juice batches were clarified by either (1) the addition of 0.2% gelatin with the gently mixed, (2) centrifugation treatment at 4000 rpm for 15 minutes or by (3) thermal treatment at 80 ± 2°C for 1 minute. The clarified pomegranate juice batches were filtrated through a 3 layers of cheese cloth.

(c)- Pasteurization process:

The clarified pomegranate juice batches were pasteurized individually at 90 ± 5°C for10 minutes and packed in closed tetra pack and then cooled suddenly into the ambient temperature (25 ± 5°C) by using tap water. After that, juice packs were stored under frozen storage conditions (at - 18± 2°C) until used and analysis.

3. Analytical Methods:

The recorded results in this research for all tested parameters represented the mean of the determination result for tested parameter in triplicate samples of juice ± standard error.

3.1. Determination of chemical composition:

The chemical components; moisture, total soluble solids (TSS), crude protein, Total sugar, reducing and non-reducing sugars and pectin contents of fresh whole pomegranate fruit juice extracted by pressing process were determined according to the standard methods of the AOAC (2000).

3.2. Determination of the pH *value and total acidity:*

The pH value and total titrable acidity (TA) of whole pomegranate fruit juice which extracted by pressing process were determined according to the standard methods of the AOAC (2000). The pH value of pomegranate juice was measured using a digital pH meter (Beckman model 3550, USA) .Titrable acidity, expressed as % citric acid. Whereas, the juice sample (10 ml) was filtrated with a filter paper in a vacuum flask was determined by titration definite quantity of sample 0.1 N Na OH and then added to 190 ml distilled water and homogenized for 2 min. Aliquot of 50 ml of the former mixture was titrated with 0.1 M Na OH solution to the end point of pH 8.2 as indicated by phenol phthalein indicator.

3.3. Determination of total polyphenols (TPPs) content:

Total polyphenols (TPPs) content of pomegranate juice was determined according to the Folin-Ciocalteau method with some modifications (Singleton *et al*., 1999 and Chen *et al*., 2008). Briefly, Stock was prepared by dissolving 100 µl of pomegranate juice in 1ml of deionized water. Then, 300 µl of stock solution and the blank solution were pipted into separate vortex and 300 µl of Folin-Ciocalteau reagent were added to each of them. The mixture was mixed well and allowed to equilibrate. After 2 min, 2.4 ml of a 5% (W/ V) sodium carbonate (Na_2CO_3) solution was added and the mixture was swirled After 15 minutes of incubation at ambient temperature, 10 ml of deionized water were added and the formed precipitate was removed by centrifugation at 4000 xg for 5 minutes. Finally, the absorbance was measured at 740 nm using the UV-VIS spectrophotometer (Model: Spectroscan 80 DV, Biotech Eng., Management Company Ltd., UK) and compared to a gallic acid equivalents (GAE) calibration curve the obtained results were expressed as mg GAE per liter of juice.

3.4. Determination of total tannins content:

Total tannins content of pomegranate juice was determined colorimetrically using the Folin-Denis method as described in the standard methods of the AOAC (2000). In this method, 5-10 ml of pomegranate juice sample was boiled under a reflux condenser for 30 min with 75 ml of distilled water. The mixture was cooled, diluted to 100 ml with distilled water and filtrated. An aliquot of the filtrate (5 ml) transferred into the 100 - volumetric flask containing 75 ml of distilled water. Then, 5 ml of Folin-Denis reagent and 10 ml of saturated sodium carbonate (Na_2CO_3) solution were added to the volumetric flask and diluted up to the mark with distilled water. The solution was mixed well, incubated at ambient temperature for 30 min and the absorbance (A) of the formed color was measured at 760 nm, against the distilled water experimental blank instead of the tested juice, by using the UV-VIS spectrophotometer (Model: Spectroscan 80 DV, Biotech Eng., Management Company Ltd., UK). The total tannins content was calculated as mg tannic acid equivalents (TAE) per liter of juice by using the standard curve of gradual concentrations of pure tannic acid from the following equation:

Total tannins content (mg TAE/L) = mg of tannic acid against the measured A on the calibration curve x Dilution x 100 / ml of sample taken x weight of sample taken x 1000.

3.5. Determination of anthocyanin pigments content:

Total anthocyanins content of pomegranate fruits juice batches was determined by the pH-differential method, using 2 buffer systems; namely potassium chloride solution (pH 1, 0.025 M) and sodium acetate

solution (pH 4.5, 0.4 M) according to the procedure of Serrano *et al*. (2005). In this procedure 1 ml of tested pomegranate juice was mixed well with 9 ml of buffer solution. Then, the absorbance was measured against the distilled water as a blank at 510 and 700 nm using the UV-VIS spectrophotometer (Model: Spectroscan 80 DV, Biotech Eng., Management Company Ltd., UK). Total anthocyanins content was calculated as mg of cyaniding-3-glucoside equivalents (CGE) per liter of juice by using the following equation:

Total anthocyanins content (mg CGE/L) = A × MW × DF × 100 / ϵ × 1

Where:

A= [(A520 – A700) pH 1.0 – (A510 – A700) pH 4.5]; Mw (molecular weight for cyaniding-3 glucoside) = 449.2 g mol^{-1}; DF= dilution factor; 1=path length in cm; ϵ (molar extinction coefficient) = 26,900. All analysis were done as 3 replicates (n=3) and the obtained results were expressed as mg of cyaniding-3-glucoside equivalents (CGE) per liter of juice.

3.6. Determination of L-ascorbic acid:

L-ascorbic acid in pomegranate fruits juice batches was extracted in 2% oxalic acid solution and determined quantitatively according to the standard method described in the AOAC. (2000) based on the reduction of 2.6 di-chlorophenol indophenol by L-ascorbic acid.

3.7. Determination of antioxidant activity:

Antioxidant activity values of tested pomegranate fruits juice batches were measured using an improved ABTS method as described by Re *et al*. (1991); Cai *et al*. (2004) and Biglari *et al*. (2008). The $ABTS^{\cdot+}$ (2.2-azinobis [3-ethylbenzothiazoline -6- sulphonic acid] diammonium salt) solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at 23±2°C in the dark for 16 h. The $ABTS^{\cdot+}$ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. $ABTS^{\cdot+}$ solution (3.9 ml; absorbance of 0.700 ± 0.005) was added to 0.1 ml of the test sample and mixed vigorously. The reaction mixture was allowed to stand at 23±2ºC for 6 min and the absorbance at 734 nm was immediately measured by using the UV-VIS spectrophotometer (Model: Spectroscan 80 DV, Biotech Eng., Management Company Ltd., UK). A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0 to 15 μM) in 80% ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard and the results were expressed as mg Trolox equivalents (TE) per liter of juice.

4. Statistical analysis:

Statistical analysis for the obtained data was carried out according to the procedure of Snedecor and Cochran (1980) and that of Helwing (1983) using the analysis of variance (ANOVA) and Duncan's Multiple Range Test. The obtained data were recorded as the mean of the determination result for tested parameter in triplicate pomegranate juice samples ± standard error and the differences between the means at $P < 0.05$ were considered statically significant.

Results and Discussion

1- Chemical Composition and the Most Important Physico-Chemical Quality Characteristics Value (on wet weight basis) of Tested Fresh Whole Pomegranate Fruits Juice Extracted by Pressing Process:

The present results of Table (1) evident the chemical Components and the most important quality criteria (on wet weight basis) of tested fresh pomegranate juice extracted from whole fruits by pressing process.

As shown in Table (1), fresh whole pomegranate fruits juice extracted by pressing procedure contained 81.90 % moisture and 18.10 % total solids. It also contained 0.45, 13.30, 12.80, 0.50, and 0.45 % from crude protein, total sugars, reducing sugars, non- reducing sugars and pectin contents, on wet weight basis; respectively. Therefore, total sugars are being the major solids constituent of fresh pomegranate whole fruits juice (73.48% of total solids) and the reducing sugars content represented 70.72 % of the total solids of fresh pomegranate juice. These results are in nearly agreement with those found by El-Nemr *et al*. (1992); Melgarejo *et al*. (2000); Al-Maiman & Ahmed (2002) and El-Said *et al*. (2009); with some slight variations as the result of the difference of the pomegranate variety, stage of ripening, the location and climatic conditions of growing area, and the analytical procedure used for these determinations.

From the obtained results (Table 1), it could be also showed that the pH value of tested pomegranate whole fruits juice was 3.56 and its titrable acidity value was found to be 1.10 % (as citric acid). In addition, as illustrated in Table (1), the fresh whole pomegranate fruits juice was rich in the determined antioxidant

compounds; polyphenols, tannins, anthocyanin pigments and L-ascorbic acid, which were naturally occurred at concentration of 2609.4 mg gallic acid equivalents (GAE), 3.65 mg tannic acid equivalents (TAE), 291.3 mg cyanidine-3-gulcoside equivalents (CGE) and 66.01 mg L-ascorbic acid (L-AA) per liter of juice; respectively. Whereas, the antioxidant activity of fresh whole pomegranate fruits juice was found to be 719.2 mg trolox equivalents (TE) per liter of juice. Therefore, whole pomegranate fruits juice exhibited a good antioxidant capacity and it was an effective scavenger of several reactive oxygen species, primarily due to its high levels of polyphenolic compounds, anthocyanins and L-ascorbic acid. The current results are in nearly accordance with those reported by El-Nemr *et al*. (1992); Gil *et al*. (2000), Al-Maiman & Ahmed (2002); Kulkarni and Aradhya (2005); El-Said *et al*. (2009) and Tezcan *et al*. (2009).

Table 1: Chemical composition and the most important physico-chemical quality characteristics of fresh whole pomegranate fruit juice extracted by pressing process.

Tested Parameter	Parameter value (M±SE)*
1. Chemical Components (%):	
Moisture content	81.90 ± 6.47
Total solids (TS)	18.10 ± 1.29
Crude protein content	0.45 ± 0.003
Total sugars content	13.30 ± 1.67
Reducing sugars content	12.80 ± 0.89
Non-Reducing sugars content	0.50 ± 0.005
Pectin content	0.45 ± 0.003
2. Physico-Chemical Quality Characteristics:	
The pH value	3.56 ± 0.013
Titrable acidity value (% as citric acid)	1.10 ± 0.007
Total polyphenols content (mg GAE/L)[1]	2609.4 ± 104.8
Total tannins content (mg GAE/L)[2]	3.65 ± 0.13
Anthocyanins content (mg CGE /L)[3]	291.3 ± 17.6
L-Ascorbic acid content (mg L-AA /L)	66.01 ± 0.39
Antioxidant activity (mg TE/L)[4]	7.19.2 ± 28.30

M±SE*: Mean of the determination result for tested parameter in triplicate pomegranate juice samples ± standard error; 1: Data are expressed as mg gallic acid equivalents (GAE) per liter of juice; 2: Data are expressed as mg tannic acid equivalents (TAE) per liter of juice; 3: Data are expressed as mg cyaniding-3-glucoside equivalents (CGE) per liter of juice 4: Data are expressed as mg trolox equivalents (TE) per liter of juice.

2- The Effect of some Technological Treatments of the Pasteurized Pomegranate Fruits Juice Processing on Its Antioxidant Compounds Content and the Antioxidant Activity:

The effect of some technological treatments used in the commercial production of pasteurized pomegranate juice; including the juice extraction, clarification and pasteurization processes, on the naturally present antioxidant compounds (polyphenols, tannins, anthocyanin pigments and L-ascorbic acid) content and the antioxidant activity in pomegranate fruits juice was studied as the following :

(a) – Effect of Juice extraction process on the antioxidant activity of pomegranate fruits juice:

The effect of extraction procedure of pomegranate juice from either whole fruits or arils by using either pressing extraction method or blending extraction method, on the antioxidant compounds (polyphenols, tannins, anthocyanin pigments and L-ascorbic acid) content and the antioxidant activity in pomegranate juice was investigated , and the obtained results were recorded as in Table (2).

Table 2: Effect of juice extraction method on the antioxidant compounds level and the antioxidant activity of pomegranate juice batches.

Tested parameter	Tested parameter value (M±SE)*			
	Juice Extraction Method			
	Fruit		Arils	
	Pressing	Blending	Pressing	Blending
Total polyphenols (TP_s)	2609.4^{d}	2296.8^{c}	1841.2^{b}	1629.6^{a}
content[1]	± 49.18	± 31.02	± 42.30	± 46.09
Total tannins (TT_s)	3.65^{c}	2.15^{b}	1.37^{a}	1.33^{a}
content[2]	± 0.29	± 0.36	± 0.19	± 0.16
Anthocyanins ($Anth_s$)	291.3^{b}	282^{ab}	267.1^{a}	266.5^{a}
content[3]	± 7.37	± 6.81	± 7.67	± 8.28
L-Ascorbic acid (L-A.A)	66.01^{c}	59.3^{b}	63.9^{c}	57.1^{a}
content	± 4.45	± 3.07	± 4.86	± 3.13
Antioxidant activity (A.A)	719.2^{d}	636.9^{c}	553.2^{b}	497.7^{a}
Conten[4]	± 51.6	± 57.3	± 49.7	± 43.2

M±SE*: Mean of determination result for tested parameter in triplicate samples of pomegranate juice ± standard error; the means, within the same row, having different superscripts are varied significantly (at $p \leq 0.05$); 1: Data are expressed as mg gallic acid equivalents (GAE) per liter of juice; 2: Data are expressed as mg tannic acid equivalents (TAE) per liter of juice; 3: Data are expressed as mg cyaniding-3-glucoside equivalents (CGE) per liter of juice; 4: Data are expressed as mg trolox equivalents (TE) per liter of juice.

As given in Table (2), whole pomegranate fruits juice extracted by pressing method contained the determined antioxidant compounds; total polyphenols, total tannins, anthocyanin pigments and L-ascorbic acid, at concentration of 2609.4 mg gallic acid equivalents (GAE), 3.65 mg tannic acid equivalents(TAE), 291.3 mg cyanidin-3-glucoside equivalents (CGE) and 66 mg L-ascorbic acid (L-AA) per liter of juice, versus 2139.8 mg GAE,2.15 mg TAE, 282 mg CGE and 59.3 mg L-AA per liter of juice extracted from pomegranate whole fruits by using the blending method; respectively. From the obtained results (Table 2) it could be also observed that pomegranate juice extracted from arils by pressing extraction method contained the former antioxidant compounds at level of 1841.2 mg GAE, 1.37 mg TAE, 267.1 mg CGE and 63.9 mg L-AA per liter of juice, against 1629.6 mg GAE, 1.33 mg TAE, 266.5 mg CGE and 57.1 mg L-AA per liter of juice extracted from pomegranate arils by using the blending extraction method; respectively. In addition, the antioxidant activity of pomegranate juice batches extracted from whole fruits using either pressing or blending extraction method was found to be 719.2 mg and 636.9 mg trolox equivalents (TE) per liter of juice, versus 553.2 and 497.7 mg TE per liter of juice extracted from pomegranate arils by using the former individual extraction methods; respectively. Therefrom, pressing extraction juice method exhibited a higher efficiency in extracting the all tested antioxidant compounds with pomegranate juice than the blending method and therefore the pomegranate juice batches extracted from whole fruits by using the pressing extraction method had the highest level from the determined antioxidant compounds; total polyphenols, total tannins, anthocyanin pigments and L-ascorbic acid, as well as it had the highest antioxidant activity, when compared with the other extracted juice batches. The present results are harmony with those found by Gil *et al*. (2000). Therefore, the pomegranate whole fruits juice extracted by using the pressing extraction method was selected for investigation the following technological treatments; clarification and pasteurization processes.

(b) Effect of clarification process on the antioxidant compounds content and the antioxidant activity of pomegranate fruits juice:

Clarification process is carried out sometimes during the processing of vegetables and fruits juices. The aim of this step is to reduce the amount of phenolic substances and improving some sensory properties such as color turbidity, overall appearance, and bitterness. However, this practice leads to a reduction in the juices health benefits (Vardin and Fenercioglu, 2003). Therefore, the effect of clarification process on the naturally occurrence antioxidant compounds content and the antioxidant activity of pomegranate whole fruits juice extracted by pressing method was studied and the obtained data were recorded as in Table (3).

Table 3: Effect of clarification method on the antioxidant compounds level and the antioxidant activity of pomegranate juice.

Tested parameter	Tested parameter value (M±SE)*			
	Clarification Method Treatments			
	Control (Unclarified)	Gelatin addition (0.2 %)	Centrifuge 4000 rpm for 15 min	Thermal treatment at (80 ± 2°C for 1 min)
Total polyphenols (TP_s) content[1]	2609.4^{d}	1958.9^{a}	2181.5^{c}	2042.2^{b}
	± 49.18	± 33.05	± 45.81	± 37.57
Reduction in TP_s content (%)**	–	24.93	16.4	21.74
Total tannins (TT_s) content[2]	3.65^{d}	2.50^{a}	3.18^{c}	2.91^{b}
	± 0.29	± 0.36	± 0.27	± 0.23
Reduction in TT_s content (%)**	–	31.51	12.79	20.27
Anthocyanins ($Anth_s$) content[3]	291.3^{d}	211.0^{a}	260.1^{c}	239.8^{b}
	± 7.37	± 6.81	± 9.39	± 7.82
Reduction in $Anth_s$ content (%)**	–	27.57	10.71	17.68
L-Ascorbic acid (L-A.A) content[4]	66.01^{c}	65.44^{c}	57.89^{b}	45.7^{a}
	± 4.45	± 2.29	± 1.66	± 1.59
Reduction in L-A.A content (%)**	–	0.86	12.3	31.83
Antioxidant activity (A.A) value[5]	719.2^{c}	561.8^{a}	606.3^{b}	552.9^{a}
	± 51.6	± 39.3	± 43.9	± 36.1
Reduction in AA value (%)**	–	21.89	15.7	23.12

M±SE*: Mean of the determination result for tested parameter in triplicate samples of juice ± standard error; the means, within the same row, having different superscripts are varied significantly (at $p \leq 0.05$); 1: Data are expressed as mg gallic acid equivalents (GAE) per liter of juice; 2: Data are expressed as mg tannic acid equivalents (TAE) per liter of juice; 3: Data are expressed as mg cyaniding-3-glucoside equivalents (CGE) per liter of juice; 4: Data are expressed as mg trolox equivalents (TE) per liter of juice; Reduction (%)**: Related to the parameter value for fresh unclarified juice sample (control).

From the obtained results (Table 3), it could be illustrated that the unclarified pomegranate juice (control batch) contained the total polyphenols, total tannins, anthocyanin pigments and L-ascorbic acid at concentration of 2609.4 mg gallic acid equivalents (GAE), 3.65 mg tannic acid equivalents (TAE), 291.3 mg cyanidin-3-glucoside equivalents (CGE) and 66.01 mg L-ascorbic (L-AA), per liter of juice; respectively, as well as it had a

high antioxidant activity of 719.2 mg trolox equivalents (TE) per liter of juice. The obtained results (Table 3) also showed that clarification process of pomegranate juice caused a noticeable reduction in its content of the total polyphenols (16.40- 24.93), total tannins (12.79 – 31.51), anthocyanin pigments (10.71 – 31.51) and L-ascorbic acid (0.86 – 31.83 %) at different rates depending upon the antioxidant compound and the clarification method, as well as it caused a high loss in its antioxidant activity (15.70 – 23.12 %). Whereas, clarification process with addition 0.2 % gelatin exhibited the highest effective method in the reduction of all tested antioxidant compound of pomegranate juice followed by thermal clarification treatment (at 80±2°C for 1 min) was the least effective, with the exception of the thermal clarification method which showed the highest effective treatment in the loss of L-ascorbic content of pomegranate juice. On the other hand, the highest antioxidant activity value (606.3 mg TE L^{-1}) in clarified pomegranate juice batches was observed for that treated with centrifugation clarification treatment followed by that (561.8 mg TE L^{-1}) for the juice batch clarified with addition of 0.2 % gelatin treatment. The reduction effect of clarification process with gelatin addition treatment on the antioxidant compounds in pomegranate juice could be attributed mainly to bound some phenolic compounds and the other antioxidant compounds in pomegranate juice which having negative charged with the positive charged molecules of gelatin resulting in the precipitation of some antioxidant compounds. While, the elimination effect of thermal clarification for pomegranate juice batches may be attributed to the thermal coagulation and denaturation of some suspended antioxidant compounds resulting in their precipitation, as well as to the thermal degradation of some antioxidant compounds, especially L-ascorbic acid. The present results are in nearly conformity with those reported by El-Nemr *et al*. (1992); Vardin and Fenercioglu (2003) and Mirdehghan *et al*. (2006).

Table 4: Effect of pasteurization process on the antioxidant compounds level and the antioxidant activity of pomegranate juice.

Tested parameter	Tested parameter value (M±SE)*							
	Unpasteurized Juice batches				Pasteurized Juice batches			
	A	B	C	D	A	B	C	D
Total polyphenols (TP_s) content	2609.4^{e}	1958.9^{c}	2181.5^{d}	2042.2^{cd}	2150.4^{d}	1804.1^{b}	1946.4^{c}	1618.9^{a}
(mg GAE / L)	± 49.18	± 33.05	± 45.81	± 37.57	± 41.03	± 29.79	± 33.07	± 27.13
Reduction in TP_s content (%)**	–	24.93	16.40	21.74	17.59	30.86	25.41	37.96
Total tannins (TT_s) content	3.65^{e}	2.50^{b}	3.18^{d}	2.91^{c}	3.26^{d}	2.12^{a}	2.68^{bc}	2.40^{b}
(mg TAE / L)	± 0.29	± 0.36	± 0.27	± 0.23	± 0.31	± 0.27	± 0.23	± 0.27
Reduction in TT_s content (%)**	–	31.51	12.79	20.27	10.68	41.92	26.58	34.25
Anthocyanins ($Anth_s$) content	291.2^{f}	211.0^{bc}	260.1^{e}	239.8^{d}	235.0^{d}	201.7^{b}	216.0^{c}	171.3^{a}
(mg CGE / L)	± 7.37	± 6.81	± 9.39	± 7.82	± 6.27	± 5.91	± 6.27	± 5.91
Reduction in $Anth_s$ content (%)**	–	27.57	10.71	17.68	19.30	30.73	25.82	41.17
L-Ascorbic acid (L-A.A) content[4]	66.01^{f}	65.44^{f}	57.89^{e}	45.70^{d}	30.21^{c}	28.54^{bc}	27.40^{b}	22.85^{a}
(mg / L)	± 2.45	± 2.29	± 1.66	± 1.59	± 1.77	± 2.01	± 2.13	± 1.33
Reduction in L-A.A content (%)**	–	0.86	12.30	31.83	54.23	56.76	58.49	65.38
Antioxidant activity (A.A) value[5]	$7.19.2^{e}$	561.8^{c}	606.3^{d}	559.9^{c}	611.7^{d}	507.3^{b}	569.6^{c}	482.1^{a}
(mg TE / L)	± 51.6	± 39.3	± 43.9	± 36.1	± 41.3	± 33.9	± 27.3	± 23.9
Reduction in A.A value (%)**	–	21.89	15.70	23.12	14.95	29.46	20.80	32.97

M±SE*: Mean of the determination result for tested parameter in triplicate samples of juice ± standard error; the means, with the same row, having different superscripts are varied significantly (at $p \leq 0.05$) 1: Data are expressed as mg gallic acid equivalents (GAE) per liter of juice; 2: Data are expressed as mg tannic acid equivalents (TAE) per liter of juice; 3: Data are expressed as mg cyaniding-3-glucoside equivalents (CGE) per liter of juice; 4: Data are expressed as mg trolox equivalents (TE) per liter of juice; Reduction (%)**: Related to the parameter value for fresh unclarified and unpasteurized juice sample; A: Control Juice sample without any clarification treatment; B: clarified juice sample with addition of 0.2 % gelatin treatment; C: clarified juice sample with centrifuge process at 4000 rpm for 15 minutes; D: clarified juice sample with thermal treatment (at 80 ± 2°C for 1 min).

(C) Effect of pasteurization process (at 90 ± 5° C for 10 min) on the antioxidant compounds level and the antioxidant activity of pomegranate fruits juice:

Consumer demand for freshly squeezed fruit juices is increasing, but such products are susceptible to spoilage and thus have a limited shelf-life (Buzrul *et al*., 2008). Thermal processing (pasteurization) is the most commonly used preservation technique to extend the shelf-life of juices. However, this process may have adverse effects on sensory quality attributes, nutritive value and bioactive phytochemicals (Plaza *et al*., 2006). Therefore, color quality of anthocyanin containing juices is undesirably lost during thermal process. Thereupon, the effect of pasteurization step (at 90 ± 5°C for 10 min) on the antioxidant compounds content and the

antioxidant activity of unclarified and clarified pomegranate whole fruits juice batches extracted by pressing method was investigated, and the obtained results were recorded as in Table (4) and Figure (1).

As shown in Table (4) and Figure (1), the control fresh unclarified pomegranate whole fruits juice batches extracted by pressing method contained the total polyphenols, total tannins, anthocyanin pigments and L-ascorbic acid at concentration of 2609.4 mg GAE per liter, 3.65 mg TAE per liter, 291.2 mg CGE per liter and 66.01 mg L-ascorbic acid per liter; respectively, as well as it had a high antioxidant activity of 719.2 mg trolox equivalents (TE) per liter of juice. From the obtained results (Table 4), it could be also noticed that the pasteurization process (at 90 ± 5°C for 10 min) of fresh unclarified pomegranate fruits juice caused a considerable reduction in its contents of total polyphenols, total tannins, anthocyanin pigments and L-ascorbic acid by 17.59, 10.68, 19.30 and 54.25 %; respectively, as well as it caused a marked loss in its antioxidant activity by 14.95 %.

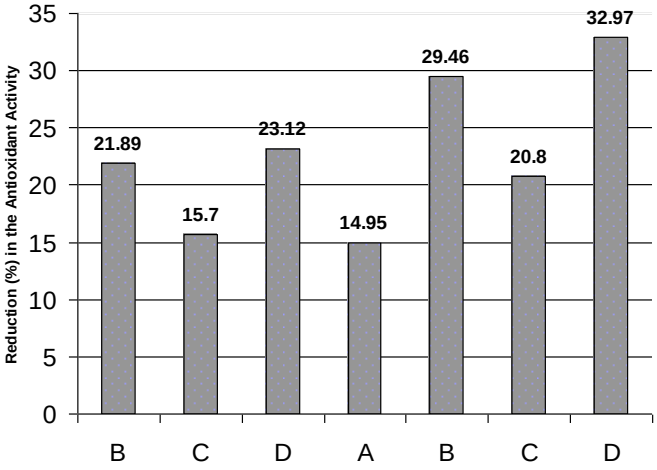

Fig. 1: The effect of clarification and pasteurization processes on the antioxidant activity of pomegranate whole fruits juice.

A: *Control Juice sample without any clarification treatment;* ***B:*** *clarified juice sample with addition of 0.2 % gelatin treatment;* ***C:*** *clarified juice sample with centrifuge process at 4000 rpm for 15 minutes;* ***D:*** *clarified juice sample with thermal treatment (at 80 ± 2°C for 1 min).*

On the other hand , as recorded in Table (4) and Figure (1), the multi-technological treatment of clarification followed by pasteurization process for pomegranate juice batches caused a high loss in the determined antioxidant compounds content ranged between 25.41 and 31.96 % for total polyphenols , 26.58 and 41.92 % for total tannins, 25.82 and 41.17 % for the anthocyanin pigments, 56.76 and 65.38 % for L-ascorbic acid, depending upon the clarification method and the antioxidant compound itself, as well as it resulted in the reduction of the antioxidant activity of pomegranate juice batches by 20.80 – 32.97 %. Therefore, the most sensitive antioxidant compounds for the elimination effect of pasteurization process were found to be L-ascorbic acid. Regardless of the reduction effect of the tested technological treatments for processing of the pasteurized pomegranate fruit juice batches on their content of the determined antioxidant compounds and their antioxidant activities, these juice batches characterized with a high antioxidant compounds level and a good antioxidant activity. The elimination effect of pasteurization process could be attributed to the thermal degradation of antioxidant compounds and the coagulation and denaturation of some suspended colloidal materials bounded with the antioxidant compounds in the juice resulting in the precipitation of these compounds. The current results are in nearly conformity with those reported by Gil *et al*. (2000); Maskan (2006) and Tezcan *et al*. (2009).

Conclusion and Recommendation:

From the present results, it could be concluded that of pomegranate juice extracted from whole fruits by using pressing extraction method exhibited a higher content of the determined antioxidant compounds and antioxidant activity. In addition, regardless the loss of the antioxidant compounds in pasteurized pomegranate whole fruits juice batches throughout the technological treatments of its processing, it characterized with a high content of the antioxidant compounds and a good antioxidant activity. Therefore, the present results are recommended with increasing the utilization of pomegranate juice in food processing and future researching for novel technological treatments which can extend the shelf-life with less adverse effects on product quality, as well as retaining the antioxidant compounds in pomegranate juice and in the other food products containing it.

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