Asian Journal of Scientific Research

ISSN 1992-1454

OPEN ACCESS Asian Journal of Scientific Research

ISSN 1992-1454
DOI: 10.3923/ajsr.2017.290.298

Research Article
Effect of Different Concentration Techniques on Some Properties of Fresh and Stored Pomegranate Juice

[1]Marwa Hanafy Mahmoud, [2]Faten Lotfi Seleet and [2]Mervat Ibrahim Foda

[1]Department of Food Technology, National Research Centre, 33 Bohouth St. P.O. Box 12622, Dokki, Giza, Egypt
[2]Dairy Department, National Research Centre, 33 Bohouth St. P.O. Box 12622, Dokki, Giza, Egypt

Abstract
Background and Objective: Pomegranate is a well-known fruit consumed as fresh or beverages and has chemo-prevention activities of various types of cancers. Pomegranate juice concentration was very important to keep its quality and the stability of sensory properties. The aim of this study was to evaluate different concentration techniques such as rotary evaporator, conventional direct heat and microwave on some properties of concentrated pomegranate juice and color stability. **Materials and Methods:** Total Soluble Solids (TSS), total phenols, anthocyanin contents and antioxidant activity of the fresh concentrated samples either fresh and during cold storage at (7±2°C) for 30 days were determined and statically analyzed using one way (ANOVA). Hunter instrument was used to measure colors changes in the juice and all concentrated samples. **Results:** The TSS of concentrated samples were 49.4, 49.5 and 51.6° Brix by using direct heat, microwave and rotary evaporator, respectively. Sample was concentrated by rotary evaporator had the highest value of the redness (a*) followed by microwave and direct heat compared with the juice sample. The rotary evaporator caused higher anthocyanin content (761.47 mg/100 mL) compared with other techniques. Prolong the cold storage increased moral content of phenols, anthocyanin and the antioxidant activity of all concentrated samples compared with the juice. **Conclusion:** It can be concluded that concentrated pomegranate juice by either microwave or rotary evaporator resulted in less degradation of total phenol and anthocyanin contents. Concentration of pomegranate juice by different techniques could be used to fortified several food products including dairy products.

Key words: Pomegranate, anthocyanin content, fruit concentration, microwave, rotary evaporator, pomegranate color

Received: May 18, 2017 Accepted: August 09, 2017 Published: September 15, 2017

Citation: Marwa Hanefy Mahmoud, Faten Lotfy Seleet and Mervat Ibrahim Foda, 2017. Effect of different concentration techniques on some properties of fresh and stored pomegranate juice. Asian J. Sci. Res., 10: 290-298.

Corresponding Author: Mervat Ibrahim Foda, Dairy Department, National Research Centre, 33 Bohouth St. P.O. Box 12622, Dokki, Giza, Egyp
Tel: +201010469901

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fruits and vegetables contain bioactive compounds capable of neutralizing the free radicals in human organism that cause oxidative damage to the lipids, proteins and nucleic acids which cause many diseases. So, fruits and vegetables could prevent the human from certain diseases[1]. Pomegranate is one of the most well-known fruits consumed as fresh fruit, beverages and other food products such as jams and jellies. Pomegranate juice may provide protection against cardiovascular and stroke diseases, by acting as a potent antioxidant against Low Density Lipoprotein (LDL) oxidation and inhibition of atherosclerosis development[2,3]. Pomegranate phytochemicals show potential in chemoprevention of various types of cancers, by exerting anti-proliferative effects on tumor cells[4]. Some clinical studies suggested that pomegranate juice increased the Prostate Specific Antigen (PSA), improvement of sperm quality and erectile dysfunction in male patients[5] and may help against Alzheimer's disease[6], cancer[7] and can protect rats against the HFD-induced obesity[8]. On the other hand, concentrated juice process is very important to increase the shelf life of the extracted juice and for easier transportation. Fruit processing industries are so concerned about concentrate fruit juice for keeping the quality and stable sensory properties (flavor, color, aroma, appearance and mouth feel) of the concentrate[9] and removing the water partly without changes in fruit total solids composition including minerals and vitamins. Concentrated fruit juice can be used as ingredients in many food products as fruit syrups, jellies and fruit juices beverages[10] in addition to ice creams and different dairy products. Concentration of fruit juices by conventional evaporation methods can cause loss in the volatile compounds and increase the color degradation which is an important attribute because it can be easily observed by the consumer, measured instantaneously for online quality control during the thermal processing[9].

However, the properties change in concentrated pomegranate juice need more investigations. So, the objective of the present study was to evaluate different concentration techniques such as direct heat, microwave and vacuum evaporation on some juice properties as the antioxidant activity, total phenols and anthocyanin contents and color changes of the concentrate pomegranate juice either fresh or cold stored for 30 days.

MATERIALS AND METHODS

Fresh pomegranates (*Punica granatum* L.) were purchased from Cairo local market. All reagents for chemical analysis were analytical grade.

Preparation of fresh and concentrated pomegranate juice: This study was carried out at National Research Centre, Cairo, Egypt on October 2015. Pomegranates were washed in cold tap water, while damaged pomegranates were discarded. The arils were separated by hand, blended, placed in a muslin cloth and pressed with a laboratory hand press and then clear filtrate was concentrated. The juice (15°Brix) was concentrated to a final 50°Brix, using three different techniques as follows:

Microwave treatment: About 1000 mL of the juice sample was poured into 2000 mL beaker and replaced in the center of turn table of the programmable domestic microwave oven (Samsung, Model MF245, Korea) with maximum output of 945 W. Rotary vacuum evaporator: 1000 mL of the juice sample was concentrated by a laboratory rotary vacuum evaporator (Heidolph, VV2000, Germany) at 40°C using 66 rpm. Direct heat: 1000 mL of the juice sample was poured into 2000 mL beaker replaced on the electric laboratory heater and stirred during the process. All concentrated samples were stored at 7±2°C for 30 days and samples were taken periodically every 5 days for different analyses.

Determination of soluble solids content: Total soluble contents (°Brix) of the juice during concentration processes were determined by an automatic digital refractometer (Atago Rx-7000a, Tokyo, Japan) at 20°C.

Determination of total phenolic content: Total phenolic contents of the juice and different concentrates were determined using Folin–Ciocalteu method and UV-Visible spectrophotometer (T80-UV/Vis PG instruments Ltd. (England). The final results were expressed as gallic acid equivalents[11].

Determination of total anthocyanin content: The total anthocyanin contents were estimated by pH differential method using two buffer systems: potassium chloride buffer, pH 1.0 (25mM) and sodium acetate buffer, pH 4.5 (0.4M)[12].

The absorbance was calculated using the following equation:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$$

Results were expressed as mg of cyanidin-3-glucoside per 1 L of juice, using a molar absorptive coefficient (e) of 26,900 and a molecular weight of 449.2.

Determination of antioxidant activity: Antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) as free radical reagent[13].

Color measurement: The color of juice and different concentrated samples were carried out using a Hunter colorimeter model D2s A-2 (Hunter Assoc. Lab Inc., USA), according to Maskan[14] . The L* variable represents lightness (0 for black, 100 for white), the a* scale represents the red-green dimension (- a for greenness, + a for redness) and the b* scale represents the yellow-blue dimension (- b for blueness, + b for yellowness). Color difference (ΔE*) was calculated from a, b and L parameters, using equation according to Hunter[15] as follows:

$$\Delta E^* = (\Delta a^2+\Delta b^2+\Delta L^2)^{1/2}$$

Where:

$$a = a\text{-}a^o,\ b = b\text{-}b^o \text{ and } L = L\text{-}L^o$$

Statistical analysis: Data were analyzed by Statistical Analysis System (SAS) software Version 9.1 using analysis of variance one-way (ANOVA) and differences among means were determined for significance at p<0.05 using Tukey's test according to Waller and Duncan[16].

RESULTS AND DISCUSSION

Chemical properties: The effect of concentration techniques and storage period on Total Soluble Solids (TSS) of pomegranate juice is shown in (Fig. 1). The (TSS) content of the juice was (15°Brix), this result was in agreement with the range of (10-16.5°Brix) which reported by Fadavi *et al.*[17] and the value of (15.07°Brix) which obtained by Tehranifar *et al.*[18], Hmid *et al.*[19] and lower than (16.28-16.46°Brix) which reported by Turfan *et al.*[20]. Concentrated Juices were reached 49.4, 49.5 and 51.6°Brix for direct heat, microwave and rotary evaporation vacuum, respectively. Prolong the cold storage (7±2°C) to 30 days slightly increased the (TSS) of all the concentrates, this result was in agreement with those obtained by Alighourchi *et al.*[21] and O'Grady *et al.*[22].

Total phenolic (TP) content: Phenols considered the most important ingredients characterized in the pomegranate, they are mostly in the form of the red pigment anthocyanin. The effect of different concentration techniques on the total phenols is presented in Table 1. It could be noticed that the TP in fresh juice was (796.67 mg GAE 100 g^{-1}), this value among the results of 20 varieties of Iranian pomegranate studied by Tehranifar *et al.*[18] who found the TP values ranged from 295.79-985.32 (mg GAE 100 g^{-1}) and lower than the range of 6 Turkish varieties (1245-2076 mg gallic acid)[23,24].

Prolonging the cold storage to 10 days decreased the total phenols content (not significant) to (726.67 mg GAE 100 g^{-1}), while after 10 days the juice was rejected for microbiological point of view. Concentrated pomegranate juice increased the TP significantly (p<0.05) compared to the fresh juice and between the 3 concentration techniques. Also, prolong the cold storage affected the total phenols content of the pomegranate concentrates significantly during the cold period (30 days). However, the pomegranate juice concentrate was more stable by using the microwave compared to other concentration techniques.

Total anthocyanin content: Anthocyanin pigments are considered the most important elements of the quality of the pomegranate where it is responsible for the red color of the pomegranate fruits. Different concentration techniques and storage period which were affected the total anthocyanin contents (expressed as cyanidin 3-glucoside) of pomegranate juice are shown in Fig. 2. It could be noticed the total anthocyanin in fresh juice was 105 mg/100 mL, this result was higher than that obtained by Tehranifar *et al.*[18] who found the

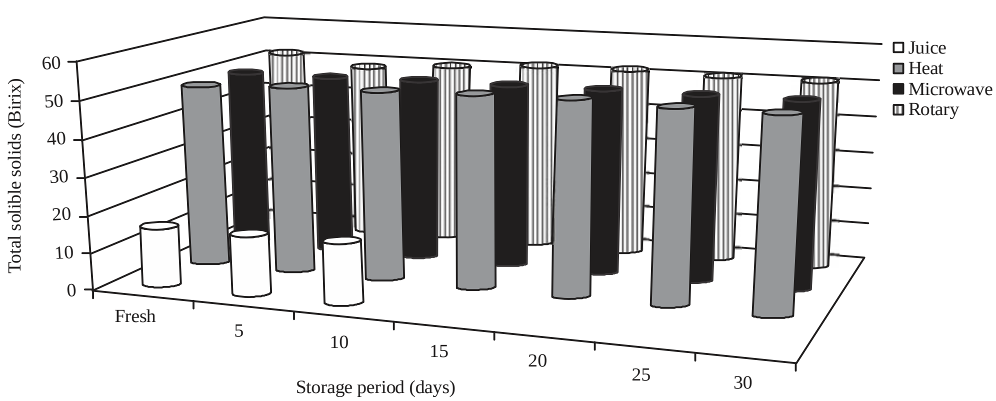


Fig. 1: Effect of different concentration techniques and storage period on total soluble solids of pomegranate juice

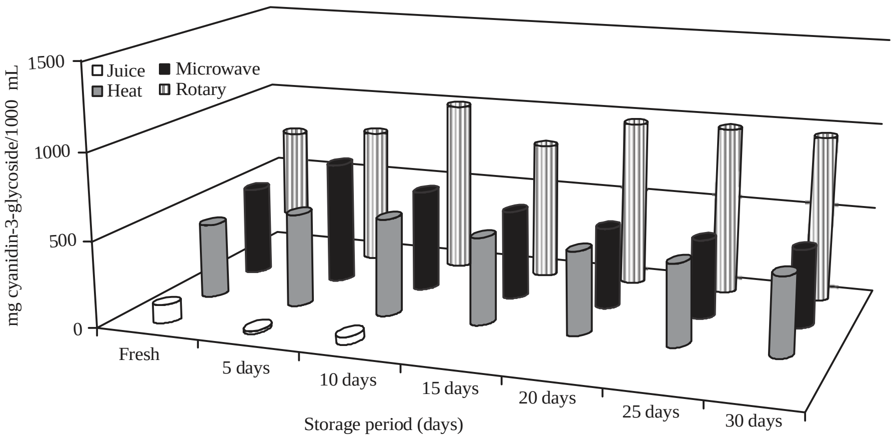


Fig. 2: Effect of different concentration techniques and storage period on total anthocyanin of pomegranate juice

Table 1: Effect of different concentration techniques and storage period on total phenols (mg GA /100 mL) of pomegranate juice

	Storage period (Days)						
Concentration techniques	Fresh	5	10	15	20	25	30
Juice	796.67 ± 1.6^{ca}	788.55 ± 1.2^{cb}	796.67 ± 1.6^{Da}				
Heat	3463.33 ± 1.9^{Bd}	3546.67 ± 0.7^{Ad}	5150 ± 0.9^{Cb}	5210.00 ± 0.6^{Cb}	6203.30 ± 0.7^{Ba}	5536.67 ± 0.6^{Bb}	4830.00 ± 1.0^{Bc}
Microwave	3483.33 ± 1.1^{Bd}	3366.67 ± 1.4^{Bd}	6700.0 ± 1.1^{Aa}	5913.32 ± 0.8^{Bc}	6283.32 ± 0.9^{Bb}	6003.30 ± 0.9^{Ac}	6360.00 ± 1.7^{Ab}
Rotary evaporator	3763.33 ± 1.2^{Ad}	3683.33 ± 1.0^{Ad}	5780 ± 1.2^{Bb}	6706.67 ± 1.4^{Aa}	6420.000 ± 0.40^{Aa}	5420.0 ± 0.4^{Bb}	4843.33 ± 0.8^{Bc}

Data are mean values±Standard Deviation, data obtained from 3 independent measurements. Different capital letters within the same column and small letters within the same row are significantly different ($p\leq0.05$)

highest amount of total anthocyanin (30.11 mg cy-3-glu 100 g^{-1}) and Cam *et al.*[25] who found the range between (8.1 and 36.9 mg 100 g^{-1}) of juice with different cultivars grown in Turkey and lower than Spain cultivars (217 mg cy-3-glu 100 g^{-1})[26].

Prolonging the cold storage (10 days) decreased juice total anthocyanin to 52.32 mg/100 mL. This reduction could due to the degradation of anthocyanin pigments by the cold storage. Ochoa *et al.*[27] reported that total anthocyanin pigment decreased $p<0.05$ significantly through storage, strongly dependent on storage temperature and most of the anthocyanin was polymerized rather than being lost during storage. Choi *et al.*[28] reported that many factors affect the stability of ACs including temperature, pH, oxygen, enzymes, ascorbic acid etc and the loss of anthocyanin pigments is probably due to oxidation as well as due to condensation of anthocyanin pigments with ascorbic acid and degradation of ascorbic acid and/or monosaccharides, may accelerated ACs degradation during storage. Previous study by Aarabi *et al.*[29] showed that the pomegranate juices stored at 4°C had lost 100% of their initial ascorbic acid content after 15 days. Concentrated juice by rotary evaporator under vacuum obtained the highest anthocyanin content (761.47 mg/100 mL) followed by the microwave (522.68 mg/100 mL) then the direct heat (435.84 mg/100 mL) as shown in Fig. 2. These results could be due to the low temperature and fast mass transfer obtained by the vacuum[30]. While, microwave heating transfers rapid energy and generates low temperature drying process[31].

Prolonging the cold storage increased the total anthocyanin pigments in the concentrated pomegranate with the three techniques could be as a result of water removal during storage.

Antioxidant activity: The effect of different concentration techniques and storage period on the antioxidant activity of pomegranate juice is shown in Table 2. Data show that the antioxidant activity of fresh juice was (1.5%) increased significantly (8.55%) by prolong the cold storage to 10 days. These results were in agreement with 7 Turkish commercial pomegranate juices (0.37-67.46%)[32,33] and lower than 8 Iranian pomegranate juices (18.6-42.8%)[34]. These differences could be due to the different pomegranate cultivars in the 3 countries and sample extraction method used in the experiments.

Fresh concentrated juice by Microwave technique Table 2 obtained highest antioxidant activity followed by Rotary evaporated method with significant ($p<0.05$) increase compared to direct heat method. The antioxidant activity of stored concentrated juice was affected strongly by the concentration methods as found that direct heat caused significant $p<0.05$ increased to 20 days of storage then the antioxidant activity decreased dramatically till the end of storage period (30 days). Microwave method had increased the antioxidant activity of the stored concentrated juice gradually to 10 days then decreased $p<0.05$ significantly to the end of storage period. Rotary evaporation method increased the antioxidant activity to reach the maximum value after 15 days then decreased significantly.

Correlation: High correlations between Total Phenols (TP) and Total Anthocyanin (TA) of pomegranate juice ($r = 0.94$) similar results were reported[35,36]. On the other hand, there were high significant correlation ($p>0.01$) between the antioxidant activity and total phenol content in concentrated juice (0.98, 0.96 and 0.94) using rotary evaporator, direct heat and microwave, respectively. While, there was no significant correlation between anthocyanin content and the antioxidant activity of concentrated pomegranate juice, which indicated that the anthocyanin pigments did not contribute to the pomegranate antioxidant properties. These findings were in agreement with those obtained by Fawole *et al.*[37].

Changes of pomegranate juice color: The red color of pomegranate is one of the most important parameter affecting its quality. The Hunter color parameters L, a and b have been widely used to describe the color changes during thermal processing of fruit and vegetable products.

The variation of L*, a*, b* and DE values of fresh pomegranate juice which were affected by different concentration techniques as shown in Fig. 3.

Results showed that concentration techniques under test decreased all the Hunter color parameter compared to the juice. Rotary evaporator sample recorded the highest value of the redness (a*) followed by microwave sample compared to the direct heat. While, the direct heat recorded the highest value of whiteness (L*), followed by samples concentrated by rotary evaporator compared to the microwave. For the color difference (ΔE*) rotary evaporator recorded the highest value, followed by the microwave compared to direct heat and the juice. Previous study by Maskan[14] showed that all concentration processes decreased the color parameters (L, a and b values) of pomegranate juice significantly and the product colors turned to reddish brown. The extent of color degradation increased with soluble solids content and during

Table 2: Effect of different concentration techniques and storage period on the antioxidant activity of pomegranate juice

	Strong sample (Days)						
Samples	Fresh	5	10	15	20	25	30
Juice	1.50±0.65[c]	4.75±1.33[b]	8.55±1.3[a]				
Heat	13.16±1.78[Ce]	21.88±0.64[d]	23.77±0.61[Bc]	25.67±1.2[Bb]	28.67±0.93[Aa]	23.93±1.28[Bc]	21.73±1.8[Bd]
Microwave	15.02±0.55[Ae]	21.26±1.95[d]	26.53±1.4[Aa]	25.27±1.55[Bb]	25.33±0.8[Bb]	24.70±0.36[Ac]	22.93±0.94[Ad]
Rotary	14.09±0.38[Be]	21.57±0.5[d]	23.87±1.8[Bc]	28.47±1.1[Aa]	26.03±0.8[Bb]	23.30±0.98[Bc]	20.73±0.64[Bd]

Data are mean values±Standard Deviation, data obtained from three independent measurements. Different capital letters within the same column and small letters within the same row are significantly different ($p\leq0.05$)

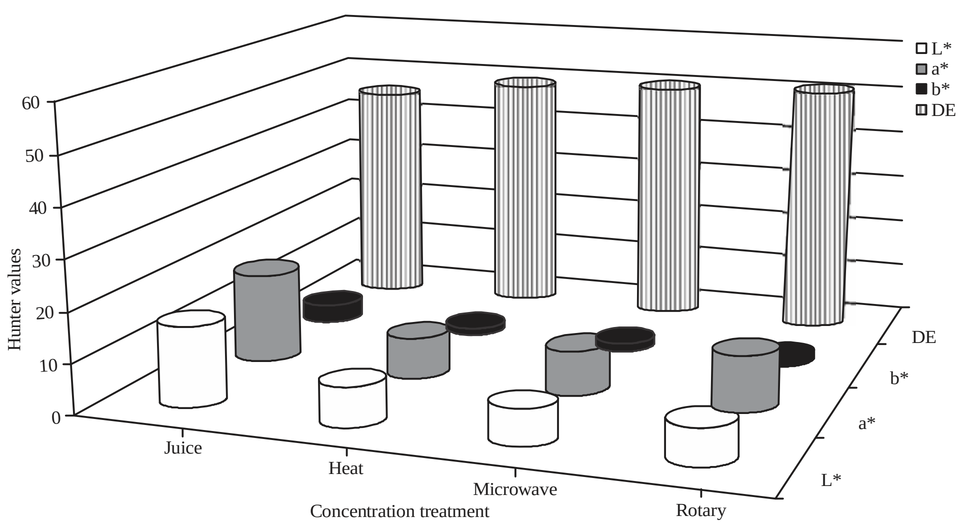
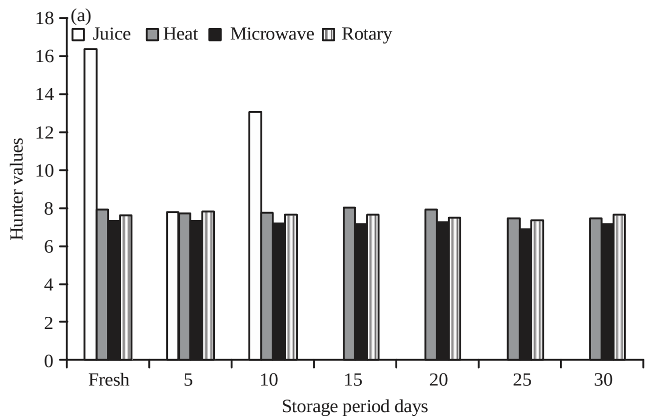
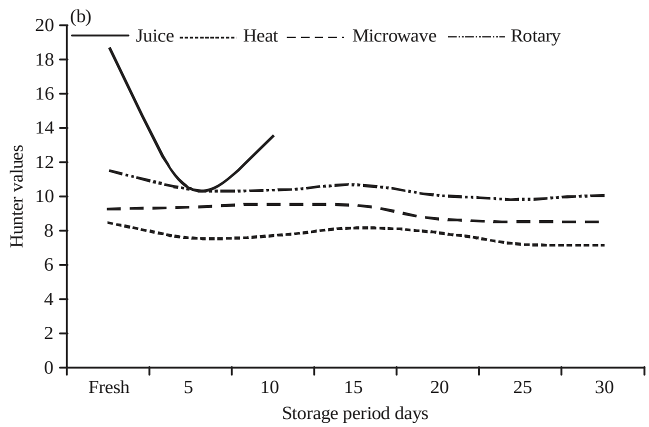


Fig. 3: Hunter color parameters of fresh and concentrated pomegranate juice





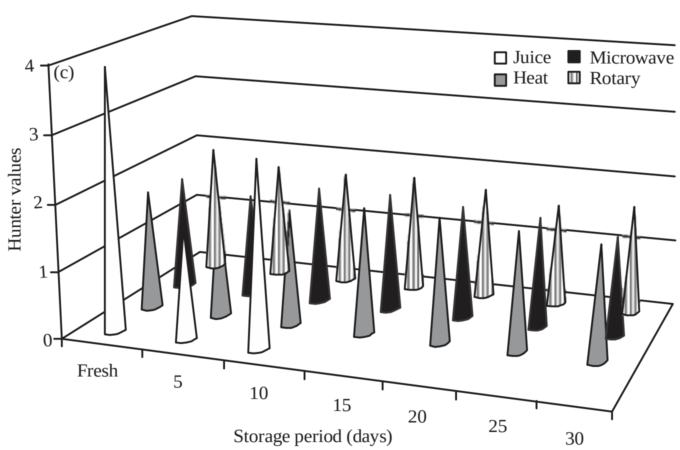


Fig. 4(a-c): (a) Effect of different concentration techniques and storage period at (7±2°C) for 30 days on lightness (L*) of pomegranate juice, (b) Redness (a*) of fresh concentrated pomegranate juice and (c) Brightness (b*) of concentrated pomegranate juice

thermal processing sugar and sugar degradation products have been found to be effective on accelerating anthocyanin pigment breakdown and enhance non-enzymatic browning[38]. The L* variable which represents the lightness of fresh and stored juice concentrated by different techniques are shown in (Fig. 4a). It could be noticed that juice recorded higher values either fresh or during the cold storage for 10 days. Concentrated juice by microwave recorded lower lightness values during the cold storage (30 days), while other 2 concentrated methods got higher values with no significant differences. The extent of loss of L value is higher in the samples treated with rotary vacuum with no statistically significant between other heating processes[14].

The redness (a*) of pomegranate juice which are affected by different concentration techniques and storage period are shown in Fig. 4b. Samples concentrated by rotary evaporator caused the highest redness (a*) value followed by microwave and direct heat caused the lowest value (11.51, 9.28 and 8.4), respectively compared to the juice (18.75).

Both a* and L* values were decreased during the cold storage period, probably due fading of the red color as heat destroyed anthocyanin pigments which are unstable in fruit juices[39] and polymerization of anthocyanin with other phenols[40]. Consequently, the color of concentrated juices became browner during storage.

Figure 4c shows the effect of different concentration techniques and cold storage on brightness (b*) of pomegranate juice. It could be noticed that there are no significant changes on the brightness (b*) of concentrated pomegranate juice by different concentrations methods. The parameters L* and b* values of all samples had increased while a* value had decreased[41]. From all data above could recommended that using rotary evaporator to concentrate pomegranate juice can be good applicable method for different fruits which used as main ingredient in food and dairy products[42].

CONCLUSION

It could be concluded that both rotary evaporation and microwave heating processes are considered good concentration techniques for causing less deterioration on antioxidant capacity, total phenol and total anthocyanin of the concentrated juice. Prolonging storage period (7$\pm$2°C) for 30 days increased the total phenols, total anthocyanin and the antioxidant activity of concentrated pomegranate juice. Concentration techniques under test decreased all the Hunter color parameter compared to the juice. Concentrated juice by microwave recorded lower lightness values during the cold storage (30 days) and the other two concentrated methods recorded higher values with no significant differences and both a* and L* values were decreased during the cold storage period.

SIGNIFICANCE STATEMENTS

This study discovers the best method to concentrate pomegranate juice, which keep the stability of the favorable sensory properties of the concentrate and remove the water partly without changing its total solids composition, minerals and vitamins. This study will help in future by recommended to juice companies to concentrate different types of fruits juice. This study pointed to a new area in Food Science that many researchers could not able to explore.

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