

## **Quality attributes, chemical properties, and selected bioactive compounds of pomegranate juice (*Punica granatum* L.) processed from arils after freezing storage**

**Elfazazi Kaoutar <sup>(1)</sup>, Chafki Loubna <sup>(1,2)</sup>, Elkettabi Zahra <sup>(1,4)</sup>, Noutfia Younes <sup>(3)</sup>,  
Charafi Jamal <sup>(4)</sup> and Fakhour Samir <sup>(5)</sup>**

Kaoutar.elfazazi@inra.ma

1: Agro-food Technology and Quality Laboratory, Regional Center of Agricultural Research of Tadla, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco

2: Laboratory of Biotechnology and Valorization of Phyto-genetic Ressources, faculty of science and technology, Sultan Moulay Slimane University, Béni Mellal, Morocco. 23000

3: Department of Agri-Food and Quality, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco

4: Research Unit of Plant Breeding and Plant Genetic Resources Conservation, National Institute of Agricultural Research, Regional Agricultural Research Center of Meknes, PO. Box 578, Meknes 50000, Morocco.

5: Plant protection laboratory, Regional Center of Agricultural Research of Tadla, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principale, 10090 Rabat, Morocco

## Abstract

Pomegranate juice quality depends essentially on its chemical and nutritional components stability. The valuable nutritional components may be reduced during its processing or storage. This study examined the effect of pomegranate arils' frozen storage on juice quality, in terms of physicochemical properties and bioactive compounds stability. The physicochemical criteria (pH, TSS, TA, color attributes) and biochemical criteria (Total Phenolic content, Total anthocyanin content and antioxidant activity) were assessed on pomegranate juice extracted from frozen arils stored at (-18°C) for 6 months. Two cultivars 'Sefri' and 'Wonderful' were subject to this study. Results showed that the pH, TSS, and TA were generally stable in frozen arils juice. However, arils freezing had a significant effect on pomegranate juice color parameters. In fact, a significant decrease of  $a^*$  values were revealed in juice samples for both cultivars. Therefore, the color intensity (chroma) has decreased significantly and total color differences ( $\Delta E^*_{ab}$ ) also showed a significant difference ( $p < 0.05$ ) between fresh juice and frozen arils juice. Arils freezing conditions (-18 °C) for 6 months didn't affect pomegranate juice physicochemical criteria. However, bioactive compounds of pomegranate juice were reduced significantly. In fact, total phenolic content decreased by about 27% and 31% for 'Sefri' and 'Wonderful' cultivars, respectively. Total anthocyanins content decreased by 20% and 30% for 'Sefri' and 'Wonderful' cultivars, respectively. Antioxidant activity, measured based on the juice's radical scavenging properties using the 2,2-diphenyl-1-picrylhydrazyl method, decreased by about 50% for 'Sefri' cultivar and 60% for 'Wonderful' cultivar.

**Keywords:** Pomegranate (*Punica granatum* L); Frozen arils storage; Pomegranate juice; physicochemical properties; bioactive compounds, Morocco.

## Attributs de qualité, propriétés chimiques et composés bioactifs du jus de grenade (*Punica granatum* L.) transformé à partir d'arilles après congélation

### Résumé

La qualité du jus de grenade dépend essentiellement de la stabilité de ses propriétés chimiques et nutritionnelles. L'objectif de cette étude est d'examiner l'effet de la congélation des arilles de grenade sur la qualité du jus, en termes de propriétés physicochimiques et de stabilité des composés bioactifs. Les critères physicochimiques (pH, TSS, TA, paramètres de la couleur) et biochimiques (teneur en polyphénols totaux, teneur en anthocyanes totaux et l'activité antioxydante) ont été évalués sur le jus de grenade extrait d'arilles congelées stockées à (-18°C) pendant 6 mois. Deux cultivars 'Sefri' et 'Wonderful' ont fait l'objet de cette étude. Les résultats ont montré que le pH, le TSS et le TA étaient généralement stables dans le jus d'arilles congelé. Cependant, la congélation des arilles a eu un effet significatif sur les paramètres de couleur du jus de grenade. En fait, une diminution significative des valeurs  $a^*$  a été révélée dans les échantillons de jus pour les deux cultivars. Par conséquent, l'intensité de la couleur (chroma) a diminué de manière significative et les différences totales de couleur ( $\Delta E^*_{ab}$ ) ont également montré une différence significative ( $p < 0,05$ ) entre le jus frais et celui d'arilles congelées. La teneur des polyphénols totaux du jus d'arilles congelées a diminué d'environ 27% pour le cultivar 'Sefri' et de 31% pour le cultivar 'Wonderful'. De plus, la teneur totale en anthocyanes a diminué de 20% pour le cultivar 'Sefri' et de 30% pour le cultivar 'Wonderful'. L'activité antioxydante, mesurée sur la base des propriétés de piégeage des radicaux du jus par la méthode du 2,2-diphényl-1-picrylhydrazyl, a diminué d'environ 50% pour le cultivar 'Sefri' et de 60% pour le cultivar 'Wonderful'. Le stockage des arilles dans des conditions de congélation (-18 °C) pendant 6 mois n'a pas affecté les critères physico-chimiques du jus de grenade, cependant, les composés bioactifs du jus de grenade ont été réduits de manière significative.

**Mots clés :** Grenadier (*Punica granatum* L.) ; congélation; jus de grenade; propriétés physico-chimiques ; composés bioactifs, Maroc.

## صفات الجودة والخصائص الكيميائية والمركبات النشطة بيولوجياً لعصير الرمان المستخرج من حب الرمان بعد تخزينه بالتجميد

كوثر الفزازي، لبنى شفيق، زهرة كتابي، يونس نوظفيا، جمال شرفي، سمير فخور

### ملخص

تعتمد جودة عصير الرمان بشكل أساسي على استقرار مكوناته الكيميائية والغذائية. الهدف من هذه الدراسة هو فحص تأثير التخزين المبرد لحب الرمان على جودة العصير من حيث الخصائص الفيزيائية والكيميائية واستقرار المركبات النشطة بيولوجياً. تم تقييم المعايير الفيزيائية والكيميائية (الرقم الهيدروجيني، البريكس، معدل الحموضة واللون) والمعايير البيوكيميائية (إجمالي محتوى الفينول، إجمالي محتوى الأنثوسيانين والنشاط المضاد للأكسدة) على عصير الرمان المستخرج من حب الرمان المبرد لمدة 6 أشهر عند (-18 درجة مئوية). خضع الصنفان "Sefri" و "Wonderful" لهذه الدراسة. نتائج الأس الهيدروجيني، البريكس ومعدل الحموضة كانت مستقرة بشكل عام في عصير حب الرمان المبرد. لكن، عملية التجميد لها تأثير مهم على معاملات لون عصير الرمان. حيث تم الكشف عن انخفاض مهم في قيم  $a^*$  في عينات العصير لكلا الصنفين. لذلك، انخفضت كثافة اللون (الكروما) بشكل كبير وأظهرت الفروق اللونية الكلية ( $\Delta E^*_{ab}$ ) أيضاً فرقاً مهماً ( $p < 0.05$ ) بين العصير الطازج وعصير حب الرمان المبرد. لم تؤثر ظروف التجميد (-18 درجة مئوية) لمدة 6 أشهر بشكل عام على المعايير الفيزيوكيميائية لعصير الرمان. ومع ذلك، انخفضت المركبات النشطة بيولوجياً لعصير الرمان بشكل ملحوظ. في الواقع، انخفض إجمالي محتوى الفينول بحوالي 27% و 31% لأصناف "Sefri" و "Wonderful" على التوالي. انخفض إجمالي محتوى الأنثوسيانين بنسبة 20% و 30% لأصناف "Sefri" و "Wonderful" على التوالي. نشاط مضادات الأكسدة، الذي تم قياسه بناءً على خصائص الكسح الجذرية للعصير باستخدام طريقة 2،2-diphenyl-1-picrylhydrazyl، انخفض بحوالي 50% للصنف "Sefri" و 60% للصنف "Wonderful".

**الكلمات المفتاحية:** حب الرمان المبرد، عصير الرمان، المعايير الفيزيائية والكيميائية، مركبات بيولوجية، جودة غذائية، تخزين.

## Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits. It is widely grown in parts of Asia, North Africa, around the Mediterranean areas and in the Middle East (Sarkhosh et al., 2006). In Morocco, thanks to the ambitious strategies adopted by the Kingdom within the Green Morocco Plan (PMV) to improve pomegranate production, this sector now covers more than 12,000 ha and produces annually about 141,000 tons which allows the Kingdom to rank 6th internationally by its production. Beni Mellal-Khenifra region is considered to have the largest production with 48 900 tons, representing more than 33% of national production (MAPMDREF, 2019).

Pomegranate fruits have achieved great attention for its nutritional and health benefits in the last years (Fawole et al., 2013, Hmid, 2013; Vázquez-Araújo et al., 2014; Loukhmas et al., 2020; Catania et al., 2020, Chafki et al. 2021). In fact, pomegranate arils is a rich source of anthocyanins, ellagic acid, flavonoids, condensed and hydrolysable tannins and organic acids, some of which are important antioxidants (Mousavinejad et al. 2009; Tezcan et al. 2009, Benchagra et al., 2021). Several studies reported that pomegranate juice have an important antioxidant activities due to these major compounds that work against the damaging effects of free radicals (Sartippour et al., 2008; Basu and Penugonda, 2009; Viuda-Martos et al., 2010; Fawole et al., 2012). Epidemiological studies have associated the consumption of pomegranate fruit to a reduced risk of coronary heart disease, diseases that are not transmissible, such as cancer, and diabetes as a result of its high antioxidant capacity [Zhao et al., 2017, Benchagra et al., 2021]. These functional properties of pomegranate arils are unstable and depend highly on post-harvest storage conditions (Mirsaeedghazi et al., 2011, Mphahlele et al., 2014) ,and since the pomegranate is a seasonal fruit, it is important to select a processing technology that could extend the fruit availability during the year and preserve its nutritional quality. One of these important processing technologies is freezing.

Frozen storage is a common method generally used for preserving food materials such as some fruits. This preservation technology is widely used for highly perishable and seasonal fruits and vegetables, (Korus et al., 2011). In the food industry, a storage temperature of (-18 °C) effectively reduces chemical and microbial spoilage of foods. In fact, lowering the temperature below the product's freezing point allows metabolic activities inhibition, the kinetics of microbiological growth and qualitative degradation reactions are slowed (Neri et al., 2014, Paciulli et al., 2015; Bonat Celli et al., 2016). 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). In another study, freeze-drying process significantly decreased the antioxidant activity and phytochemicals contents of pomegranate seeds whereas freezing process had less negative effect (Al-Sanabani et al., 2016). Cortés et al. (2005) investigated the impact of freezing on carotenoids and ascorbic acid

concentrations in orange-carrot juice. After storing the juice at (-40 °C) for 132 days, they observed that vitamin A activity increased significantly.

However, according to several studies, freezing can damage cell membranes and break down their physical structure, resulting in quality degradation such as color changes, drip loss, softening, and nutritional and bioactive component loss (Rickman et al., 2007; Sacchetti et al., 2009; Chassagne-Berces et al., 2009). Mirsaeedghazi et al. (2011) examined the effect of frozen storage at -25 °C on some chemical characteristics of pomegranate juice. Total anthocyanin content of pomegranate juice decreased by 11% after 20 days of frozen storage. Phenolic components decreased by 29% after 20 days of frozen storage. Antioxidant activity, measured using the DPPH method decreased by 50% after 20 days of frozen storage.

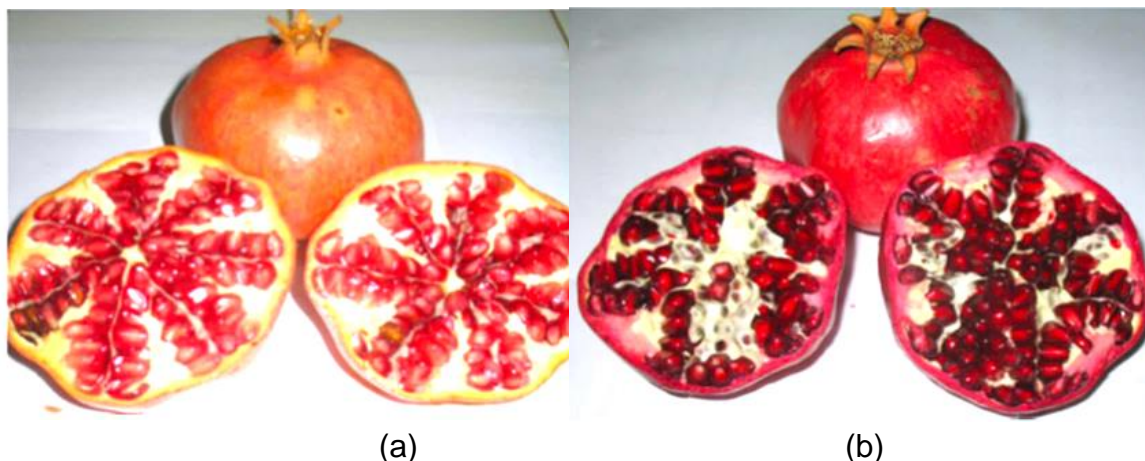
Pomegranate juice's nutritional value is an important factor that makes it preferable to other juices, thus its preservation is essential from the point of view of consumer health. However, to our knowledge, no previous study is published on Sefri Moroccan cultivar examining the pomegranate juice characteristics processed from frozen arils. In this work, the physicochemical properties (pH, Total Soluble Sugar, Titrable Acidity), the color attributes and the nutritional components of pomegranate juice (total anthocyanins content, phenolic components and antioxidant activity) were evaluated before freezing and after frozen storage for Two varieties cultivated in Morocco. The generated information will be useful in evaluating the suitable variety for pomegranate juice production from frozen arils.

## Materials and Methods

### Plant Material

Pomegranate fruits (*P. granatum* L., cv. Sefri) and (*P. granatum* L., cv. Wonderful) were harvested at commercial ripening (fully mature according to commercial practice) from the same orchard (with the same agricultural practices) located in Beni Mellal Region (Morocco) and immediately transported to laboratory (Figure 1). 50 fruits per cultivar were manually harvested. For each cultivar, 5 fruits were randomly collected from 10 sampled trees. Pomegranates with defects (sunburn, cracks, bruises and cuts in the husk) were discarded and only fruit with healthy outer skins and uniform in size and appearance were used.

'Sefri Ouled Abdellah' is a local dominant variety in Beni Mellal region labeled as protected geographical indication. This variety is mostly intended for fresh consumption and homemade juice; however, it is underexploited in terms of industrial processing. The 'Wonderful' is an American variety introduced recently in Beni Mellal region and mainly intended for exportation and pomegranate juice production.



**Figure 1:** Photo Pomegranate fruits: (a) 'Sefri Ouled Abdellah'; (b) 'Wonderful'.

### **Fruit processing, freezing and juice extraction**

Pomegranate fruits were washed with cold tap water, then carefully cutted with sharpened knives and arils manually extracted from the pith and capillary membranes. The arils were collected in a tray, washed in a solution containing 100  $\mu\text{L}$ / L chlorine ( $\text{NaOCl}$ ) for 5 min and further rinsed in tap water, drained and excess water removed from arils with paper towels, as reported by López-Rubira et al. (2005) and Al-Sanabani et al., (2016). Arils were packed and refrigerated in 4°C for 1hour, then stored in the freezer (Liebherr, Germany) at a temperature of -18°C for 6 months. The samples' temperature was controlled during storage using an infrared digital thermometer scanner.

The pomegranate juice (control, frozen arils) was extracted by a juice centrifuge (Easy Fruit Metal Juicer-JU610 Moulinex, France). The extracted juice was pureed (with passing from mesh filter No. 10 to remove the large extra particles) and homogenized and immediately analyzed.

Fresh juice (control) physicochemical and biochemical properties for each cultivar were assessed before storage. After 6 months of storage, the chemical characteristics of frozen arils juice were analyzed after complete defrosting.

### **Physicochemical analysis**

#### ***pH, total titratable acidity and soluble solid content***

The pH was measured using a pH meter (Thermo Orion 3 Star, France). Total titratable acidity (TA) was determined potentiometrically using 0.1 M NaOH to the end point of pH 8.1 and expressed as grams of citric acid per milliliter (g/mL) (AOAC, 1984). Total soluble solids, or Brix (expressed in °Bx), was determined at room temperature (25 °C) using a digital refractometer (Mettler-Toledo GmbH, Germany) (IFU, 1991).

## Color Measurement

Juice color change for all trials was measured as change in color before and after arils freezing. Measurements were performed using a Chromameter (CR-410, Konika Minolta Sensing Inc., Osaka, Japan) which gives the Colorimetric variables  $L^*$ ,  $a^*$  and  $b^*$ .

Colorimetric variables ( $L^*$ ,  $a^*$ ,  $b^*$ ) were measured and color change ( $\Delta E^*_{ab}$ ), chroma ( $C^*$ ) and hue ( $H^*$ ) were calculated from:

$$\Delta^*_{ab} = \sqrt{(\Delta L^*{}^2 + \Delta a^*{}^2 + \Delta b^*{}^2)} \quad (1)$$

$$C^* = \sqrt{(a^*{}^2 + b^*{}^2)} \quad (2)$$

$$H^* = \tan^{-1} \left( \frac{a^*}{b^*} \right) \quad (3)$$

Color measurement was performed on 30 mL samples in Petri dishes against a background of white tiles (Yawadio and Morita, 2007).

## Biochemical analysis

**Total Polyphenols Content (TP):** TP was determined by using the Folin–Ciocalteu method (Singleton et al., 1965). 300  $\mu$ L of diluted pomegranate juice in the ratio of 1:100 with methanol: water (6:4) was mixed with 1.5 mL of 10-fold-diluted Folin–Ciocalteu reagent and 1.2 mL of 7.5% sodium carbonate. The mixture was allowed to stand for 90 min at room temperature before the absorbance was measured by UV–Visible spectrophotometer at 760 nm. Gallic acid was used as a standard. The results were expressed as mg gallic acid equivalent in a liter of fruit juice (mg GAE/L of juice).

**Total Anthocyanins Content (TA):** TA was determined by pH differential method according to Giusti and Wrolstad (2005) using two buffers' systems: sodium acetate buffer pH 4.5 (0.4 M) and potassium chloride buffer pH 1.0 (0.025 M). The total anthocyanins of the samples, expressed as mg of cyanidin-3-glucoside/100ml of juice, are calculated according to the following equation:

$$\text{Total Anthocyanins composition} = (\text{Abs} \cdot \text{PM} \cdot \text{FD} \cdot 100 / \text{CAM}). \quad (4)$$

With Abs: absorbance; MW: Molecular weight (449.2); DF: Dilution factor; CAM: Molar absorption coefficient of Cyanidin-3- glucoside (26.9) (Giusti and Wrolstad, 2005)).

**Antioxidant activity:** The antioxidant activity of juice samples was measured in terms of their Radical-Scavenging Activity (RSA), by using the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method (Brand-Williams et al., 1995; Sorrenti et al., 2006). Briefly, 100  $\mu$ l of pomegranate juice diluted 1:100 with methanol: water (6:4) then, mixed with 2 ml of 0.1 mM DPPH prepared in methanol. The Absorbance of the resulting solution is measured at 517 nm by a UV-Visible spectrophotometer after



incubation in the dark for 30 min. The reaction mixture without DPPH is used for background correction.

Results are expressed as percent DPPH inhibition, as follows:

$$\text{Antioxidant capacity (\%)} = [1 - (\text{Abs}_{517\text{ nm sample}} / \text{Abs}_{517\text{ nm control}})] \times 100. \quad (4)$$

## Statistical Analysis

Each experiment was conducted with three repetition and measurements were done in triplicates (n = 3), unless elsewhere specified. The results were expressed as mean values  $\pm$  standard error of mean (SD) and were analyzed by (Version 22.0 SPSS Inc). One way analysis of variance was performed using ANOVA Method. 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

### Physicochemical analysis

#### *pH, total titratable acidity and soluble solid content*

The physicochemical criteria (pH, Total soluble solids (TSS), Titratable acidity (TA)) of pomegranate juice samples of 'Sefri' and 'Wonderful' cultivars obtained from frozen arils are presented in Table 1.

**Table 1.** pH, Total Soluble Solids (TSS), Titratable Acidity (TA) values of pomegranate juice samples obtained from frozen arils.

Cultivars	Pomegranate juice	pH	TSS(°Bx)	TA (g/100mL)
<b>Sefri</b>	fresh juice	3.86 $\pm$ 0.17 <sup>a</sup>	17.02 $\pm$ 0.22 <sup>a</sup>	0.24 $\pm$ 0.02 <sup>a</sup>
	frozen arils juice	3.92 $\pm$ 0.23 <sup>a</sup>	17.45 $\pm$ 0.45 <sup>a</sup>	0.31 $\pm$ 0.09 <sup>a</sup>
<b>Wonderful</b>	fresh juice	3.02 $\pm$ 0.14 <sup>b</sup>	15.35 $\pm$ 0.15 <sup>b</sup>	0.50 $\pm$ 0.03 <sup>b</sup>
	frozen arils juice	3.15 $\pm$ 0.35 <sup>b</sup>	15.89 $\pm$ 0.17 <sup>b</sup>	0.58 $\pm$ 0.51 <sup>b</sup>

*Note: Data are expressed as mean values  $\pm$  standard deviation. Values in each column having different letters are significantly different from one another at  $p < 0.05$ .*

According to results shown in Table 1, a significant difference was revealed between pomegranate cultivars for all physicochemical parameters before freezing. In fact, pH, TSS, and TA values of 'Sefri' cultivar were 3.86, 17.02 °Brix, and 0.24 g/100 mL, respectively. For 'Wonderful' cultivar, pH, TSS, and TA values were 3.02, 15.35 °Brix, and 0.50 g/100 mL, respectively. These results were similar with those previously

reported on pomegranate fresh juice (hmid et al., 2013; Poyrazoglu et al., 2002; Tehranifar et al., 2010).

Regarding pomegranate juice samples of frozen arils, the obtained results showed that there is no significant effect on the pH, TSS, and TA characteristics for both cultivars. We can conclude that the freezing storage of arils at (-18°C) for 6 months couldn't affect significantly the pH, TSS, and TA criteria of the extracted pomegranate juice.

### Color parameters

Color attributes ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma ( $C^*$ ), hue ( $H^*$ ), and color change ( $\Delta E^*_{ab}$ )) of pomegranate juice samples of 'Sefri' and 'Wonderful' cultivars obtained from frozen arils are presented in Table 2.

**Table 2.** Color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma ( $C^*$ ), hue ( $H^*$ ), and  $\Delta E^*_{ab}$ ) values of pomegranate juice samples obtained from frozen arils.

Cultivar	Pomegranate juice	$L^*$	$a^*$	$b^*$	$C^*$	$H^*$	$\Delta E^*_{ab}$
Sefri	Fresh juice	21.70 <sup>a</sup> ±0.31	43.66 <sup>a</sup> ± 0.09	31.45 <sup>a</sup> ±0.0 3	53.23 <sup>a</sup> ±0.09	34.90 <sup>a</sup> ±0.23	0.00±0.00
	Frozen arils juice	22.15 <sup>a</sup> ±0.15	40.08 <sup>b</sup> ± 0.18	32.49 <sup>a</sup> ±0.0 2	52.11 <sup>b</sup> ±0.20	38.00 <sup>b</sup> ±0.17	3.42 <sup>a</sup> ±0.27
Wonderful	Fresh juice	23.89 <sup>b</sup> ±0.42	45.97 <sup>c</sup> ± 0.14	33.06 <sup>b</sup> ±0.1 2	55.81 <sup>c</sup> ±0.04	37.40 <sup>c</sup> ±0.03	0.00±0.00
	Frozen arils juice	24.17 <sup>b</sup> ±0.10	44.10 <sup>d</sup> ± 0.20	33.25 <sup>b</sup> ±0.2 1	52.23 <sup>bc</sup> ±0.16	37.00 <sup>c</sup> ±0.02	2.31 <sup>b</sup> ±0.18

Note: Data are expressed as mean values ± standard deviation. Values in each column having different letters are significantly different from one another at  $p < 0.05$ .

To objectively measure color variation, color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma ( $C^*$ ), hue ( $H^*$ ), and color change ( $\Delta E^*_{ab}$ )) should be investigated (Wrolstad et al., 2005). Therefore, we have evaluated these key color parameters in order to measure color changes in pomegranate juice samples obtained from frozen arils (Table 2).

According to results, no significant variation of  $L^*$  and  $b^*$  values were observed in pomegranate juice samples extracted from frozen arils for both cultivars. However, significant decrease of  $a^*$  value was observed in frozen arils juice samples for both cultivars. The decrease of  $a^*$  value of 'Sefri' cultivar was higher compared to 'Wonderful' cultivar. These results are in accordance with Alighourchi et al. (2009) who reported a significant decrease of  $a^*$  values on pomegranate juice stored at 4°C for 210 days. These decrease of  $a^*$  values can be attributed to the degradation or polymerization of anthocyanins and indicate a fading of the typical red color of pomegranate juice. Consequently, the color of juices became browner (Kalt et al., 2000, Martí et al., 2002; Pérez-Vicente et al., 2004, Yuksel and Koka, 2008).

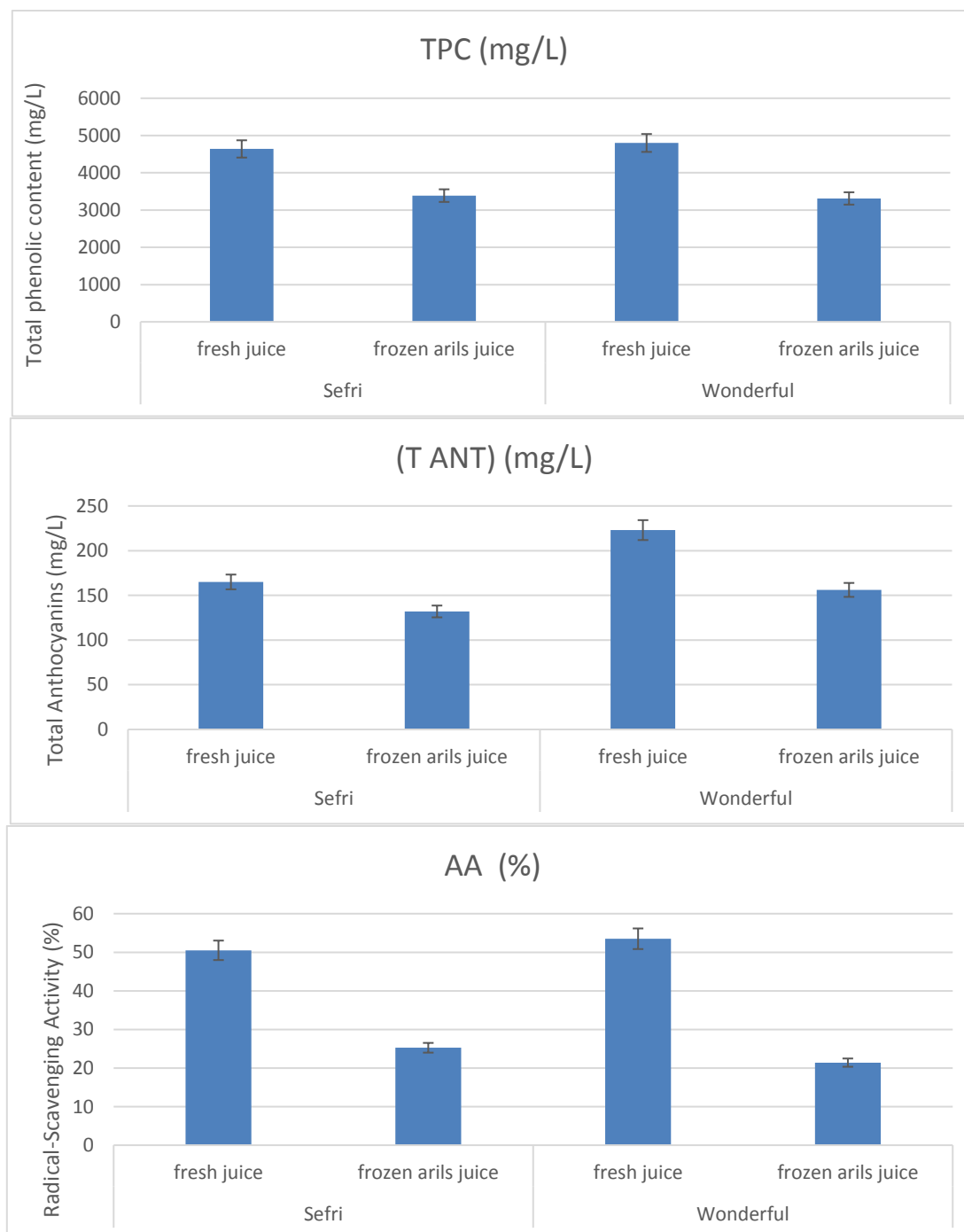
In general, hue, chroma, and  $\Delta E^*_{ab}$  values have significantly been different in frozen arils juices for both cultivars ( $p < 0.05$ ). The chroma value, which represents color

intensity, have decreased significantly. Similar decreasing trend were found by Alighourchi et al. (2009) on reconstituted pomegranate juices stored at 4°C for 210 days.

Total color differences ( $\Delta E^*ab$ ), which indicate the magnitude of the color difference between fresh and frozen arils pomegranate juices, showed significant difference ( $p < 0.05$ ). This effect could be related to the degradation of anthocyanins content as suggested by Pérez-Vicente et al. (2004) who reported that total anthocyanin concentration of pomegranate juices highly correlated with CIE  $a^*$  value and  $\Delta E$  value. These results suggests that arils freezing had a significant influence on color attributes of the pomegranate juices.

### ***Changes in Total phenolic content, total anthocyanins and antioxidant activity***

Total phenolic content, total anthocyanins and antioxidant activity of pomegranate juice samples of 'Sefri' and 'Wonderful' cultivars obtained from frozen arils are presented in figure 2.



**Figure 2:** Changes in total phenolic content (TPC), total anthocyanins content (T ANT) and antioxidant activity (AA) of pomegranate juice samples of ‘Sefri’ and ‘Wonderful’ cultivars obtained from frozen arils.

Phytochemical's 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. Pomegranate (*Punica granatum* L) is a rich source of anthocyanins, ellagic acid and other phenolic compounds with proven antioxidant activity (Poyrazoğlu et al. 2002; Mousavinejad et al. 2009; Tezcan et al. 2009; Mena et al., 2013).

According to results in figure 2, fresh pomegranate juice of 'Sefri' and 'wonderful' cultivars have an important total phenolic (4640 mg/L; 4800 mg/L) and total anthocyanins content (165mg/L; 223 mg/L), respectively. These results are similar to those reported by Aviram et al. (2000), Hmid (2013), and Benchagra et al. 2020)

The evaluation of the frozen arils' juice showed that total phenolic contents decreased by about 27% for 'Sefri' cultivar and 31% for 'Wonderful' cultivar. This reduction could be related to ellagic acid degradation, as a major phenolic component, during arils' frozen storage (Mousavinejad et al. 2009). Al-Sanabani et al., (2016) reported a significant decrease in total phenolics of pomegranate arils after freezing at (-18°C). In similar studies on other fruits, Oszmianski et al, (2009) reported that freezing storage for 6 months at -20°C results in a loss of 5 to 35% of the total polyphenols initially contained in the strawberry. Also, De Ancos et al. (2000) found that the freezing process decreased the total phenolics content by 4-20% in four cultivars of raspberries.

Regarding Anthocyanins (figure 2), the results revealed a decrease of 20% for 'Sefri' cultivar and 30% for 'Wonderful' cultivar. These results were in contrast with those of Mirsaeedghazi et al. (2014) reported on frozen storage of pomegranate juice who concluded that total anthocyanins and phenolic contents in pomegranate juice stored at -25 °C highly decreased after 20 days' frozen storage. Also, Al-Sanabani et al., (2016) reported a significant decrease in total anthocyanins of pomegranate arils after freezing at (-18°C). This reduction in these valuable compounds may be attributed to the presence and action of oxidative enzymes as reported by Lester *et al.*, (2004). In fact, anthocyanins are highly unstable and easily susceptible to degradation. Their stability is strongly affected by pH, temperature, anthocyanin concentration, oxygen, light, enzymes (Wrolstad et al., 2005; Howard et al., 2010; Jaiswal et al., 2010).). Several studies have reported the effect of processing and storage on the stability of fruit juice with high anthocyanin content (Garzon and Wrolstad 2002; Wang and Xu 2007; Jiménez et al. 2010). However, limited Data are reported on the degradation of anthocyanin in pomegranate juice produced from frozen arils and concentrate during frozen storage (Al-Sanabani et al., (2016), Mirsaeedghazi et al (2014)). In fact, Mirsaeedghazi et al (2014) evaluated individual anthocyanin degradation during storage at -25°C for 20 days using the LC–MS method and reported that pelargonidin 3,5- diglucoside had the most reduction between all anthocyanins analyzed.

Regarding antioxidant activity of frozen arils' juices highly decreased by about 50% for 'Sefri' cultivar and 60% for 'Wonderful' cultivar (figure 2). In general, the phenolics, anthocyanins components and antioxidant activity losses were higher in 'Wonderful' cultivar compared to 'Sefri' cultivar.

Antioxidant activity is considered one of the most important nutritional parameters of pomegranate juice, which is attributed to the high content and composition in Punicalagins, anthocyanins, phenolic acids and ascorbic acid, either alone or in combination (Hmid et al., 2013; Scalzo et al., 2004; Tezcan et al. 2009; Borochov-Neoriet al., 2009). Several studies confirmed that The antioxidant capacity is most significantly correlated with the contents of total phenolics and anthocyanins (Proteggente et al., 2002 ; Alighourchi et al., 2009 ; Al-Sanabani et al., 2016). This could explain the decrease of antioxidant capacity of our pomegranate juice produced with frozen arils due to total phenolics and anthocyanins decreases. These results are similar to those found by Al-Sanabani et al., (2016) who reported a significant decrease in antioxidant activity in frozen pomegranate arils at (-18°C). in other research, Poiana et al. (2010) concluded that freezing strawberry, sweet cherry and sour cherry juices at -18 °C decreased bioactive components and consequently, their antioxidant activity.

Freezing can influence the content of polyphenols that are easily oxidized, oxidation reactions lead to the formation of more or less polymerized substances, which lead to changes in food quality, especially color and organoleptic characteristics (Manach et al., 2004). In fact, fruits can be damaged during freezing and frozen storage, and thus, the final quality of the products and its functional properties may be lower than the corresponding fresh product. Cell breakages can lead to the decompartmentalization of antioxidants, such as anthocyanins and other phenolic compounds, and to their degradation due to the interaction with oxidative enzymes (Khattab et al., 2015).

Moreover, soluble antioxidants can undergo possible exposure to oxygen and separation in the unfrozen phase in which oxidation reactions could proceed faster due to the increased concentration of the reactants (Manzocco et al., 2006a; Manzocco et al., 2006b). However, some authors have also shown that freezing operations may also exert positive effects on the quality and functional properties of plant foods, since after freezing, a release of bioactive compounds as bound phenolic acids and anthocyanins can occur, resulting in an increase of antioxidant activity (Mullen et al., 2002; Sacchetti et al., 2008).

## **Conclusion**

Evaluation of physiochemical properties, color attributes and some key phytochemical compounds of pomegranate juice obtained from frozen arils of 'Sefri' and 'Wonderful' cultivars showed no significant effect on the pH, TSS, and TA characteristics. Regarding color attributes, arils freezing had a significant influence on pomegranate juice color. In fact, a significant decrease of  $a^*$  values and color intensity (chroma) were revealed on juice samples for both cultivars. Storage of pomegranate arils at  $-18^{\circ}\text{C}$  caused a significant decrease in the concentration of total phenolics (27-31%) and total anthocyanins (20-30%) for 'Sefri' and 'Wonderful' cultivars, respectively. The reduction in these major antioxidant components has led to a high decrease in antioxidant activity by about 50% to 60%.

his study concludes that frozen storage of pomegranate arils at  $-18^{\circ}\text{C}$  for 6 months cannot preserve the nutritional value and color attributes of pomegranate juice. More research is being conducted by authors to determine the maximum storage duration at which bioactive component and organoleptic degradation becomes highly important.

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