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Assessment of antioxidant properties and *in-vitro* bioaccessibility of some pomegranate products

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Abstract

In this research, pomegranate molasses, pomegranate sour sauces and pomegranate jams were evaluated in terms of some physicochemical properties including hydroxymethylfurfural, total phenolic content, antioxidant capacity and bioaccessibility. While ascorbic acid of these products ranged between 0.89-19.78 mg 100g^{-1}, total phenolic contents changed between 31.40-2061.10 mg gallic acid equivalent 100g^{-1}. Antioxidant capacities of the products were determined as 34.01-2377.52 mg trolox equivalent 100g^{-1} with DPPH assay and 18.9-6439.0 mg trolox equivalent 100g^{-1} with CUPRAC assay. The bioaccessibilities regarding phenolic substance and antioxidant capacity after simulated gastrointestinal digestion ranged between 74-247% and 53-213%, respectively. High HMF levels, which were reflected on color and sensory features, have indicated the necessity of improving the production and storage conditions. On the other hand, the highest ascorbic acid, total phenolic content and antioxidant capacity of pomegranate molasses and the bioaccessibility of pomegranate jams in terms of antioxidants showed the importance of consumption of these products in the daily diet. So, this study can be regarded as a case surveillance study that can be used by producers, nutritionals and authorities to make assessments on manufacturing conditions, consumer health and nutrition.

Keywords: *Pomegranate, antioxidant capacity, polyphenols, HMF, bioaccessibility.*

Bazı nar ürünlerinin antioksidan özellikler ve *in-vitro* biyoerişilebilirlik açısından değerlendirilmesi

Öz

Bu çalışmada, nar ekşisi, nar ekşili sos ve nar reçelleri hidroksimetilfurfural, toplam fenolik madde, antioksidan kapasite ve biyoerişilebilirliği de kapsayacak şekilde bazı

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fizikokimyasal özellikler yönünden değerlendirilmiştir. Ürünlerin askorbik asit içeriği 0.89-19.78 mg 100g^{-1} arasında değişirken, toplam fenolik madde miktarları 31.40-2061.10 mg gallik asit eşdeğeri 100g^{-1}arasında saptanmıştır. Ürünlerin antioksidan kapasiteleri DPPH yöntemi ile 34.01-2377.52 mg troloks eşdeğeri 100g^{-1}; CUPRAC metodu ile 18.9-6439.0 mg troloks eşdeğeri 100g^{-1} arasında belirlenmiştir. Simüle edilmiş gastrointestinal sindirim sonrası fenolik madde ve antioksidan kapasiteye ilişkin biyoerişilebilirlik değerleri sırasıyla %74-247 ve %53-213 arasında değişim göstermiştir. Renk sonuçları ve duyusal özelliklere yansıyan yüksek HMF seviyeleri, üretim ve depolama koşullarının iyileştirilmesi gerektiğini ortaya koymuştur. Diğer taraftan, nar ekşilerinin yüksek askorbik asit, toplam fenolik madde içeriği ile antioksidan kapasitesi; nar reçellerinin ise antioksidanlar açısından yüksek biyoerişilebilirliğe sahip olması, bu ürünlerin günlük diyette tüketiminin önemini göstermiştir. Sonuç olarak, bu çalışma üreticiler, beslenme uzmanları ve diğer otoriteler tarafından üretim koşulları, tüketici sağlığı ve beslenme ile ilgili değerlendirmelerde kullanılabilecek bir durum tespit çalışması olarak değerlendirilebilir.

Anahtar kelimeler: *Nar, antioksidan kapasite, polifenoller, HMF, biyoerişilebilirlik.*

1. Introduction

The pomegranate belongs to the genus *Punica* of the *Punicaceae* family and the most important species is *Punica granatum* L. [1]. The bioactive compounds of this fruit (punicalagin, ellagic acid, gallic acid, ellagitannins, and gallotannins) exhibit functional and therapeutic properties such as antioxidant, antiviral, anticancer, antibacterial, antidiabetic, antineoplastic, antihyperlipidemic and play a role in the prevention of many diseases [2, 3].

Recent studies showed a higher antioxidant activity of pomegranate juice than red wine and grape, apple, blackberry, cornelian cherry, blueberry juices regarding its high phenolic content [4, 5]. So the edible portion of this favorite fruit can be consumed fresh or processed into different products with a long shelf life such as jam, juice, molasses, sour sauces, leather or wine. It is also used as a sweetener or colorant in food formulations [6].

Sour pomegranate varieties (with 2-3 pH), which cannot be consumed as tableware, are pressed, clarified, then concentrated to minimum 68 brix for pomegranate molasses production [7]. This is a product with high nutritional value and contains at a significant level of minerals (K, P, Mg, Ca) and phenolics [8]. Pomegranate sour sauce dissimilarly contains pomegranate syrup, water, pomegranate aroma, acidity regulator (citric acid), colorant and preservative [9]. Pomegranate jam is a kind of another product obtained by adding some sugar, pectin, citric acid on the arils and concentrating to a certain level of brix. Pomegranate jam, pomegranate molasses and pomegranate sour sauce are concentrated products that are subjected to long term heat treatment during their production. As well as commercial production, adulterations can be made to these products with inappropriate production and storage conditions. In this sense, it is important to carry out studies on checking the composition of pomegranate products.

Recent studies have mainly focused on the composition of pomegranate juice, pomegranate molasses, clarification of pomegranate juice, pomegranate juice powder

production, bioactive components of pomegranate arils, peel and seed oil [10-12]. There is a limited number of studies on pomegranate products and their bioactive potentials [13, 14]. In addition, there is not enough research in the literature about the changes in the functional properties of pomegranate products in gastro-intestinal track. There are some studies on the bioavailability of only ellagic acid found in the pomegranate [15]. In this study, it is aimed to assess some physicochemical and biochemical properties of pomegranate products belonging to different commercial brands. The ascorbic acid, hydroxymethylfurfural, antioxidant capacity, total phenolic content of these products were analyzed within this scope. In order to evaluate the functional aspects, the bioaccessibilities of phenolic content and antioxidants after *in-vitro* gastrointestinal (GI) digestion were also determined. It is thought that this research will give an information about the reliability of these products and provide up-to date data for further studies.

2. Materials and method

In this study, commercially available pomegranate molasses (PM), pomegranate sauces (PS) and pomegranate jams (PJ) with two different brands (encoded as 1 and 2) were used with three replications. Attention was paid to the purchase products with closer production dates.

2.1. Chemicals

All reagents were in analytical grade. TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) and bile salts were purchased from Fluka (Switzerland). Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), neocuproine (2,9-dimethyl-1,10-phenanthroline), DPPH (2,2-diphenyl-2-picrylhydrazyl), methanol, sodium carbonate, gallic acid, oxalic acid, sodium bicarbonate and sodium hydroxide were obtained from Sigma Aldrich (MO, Germany). Pepsin, pancreatin, Folin-Ciocalteu reagent, 2,6 dichlorophenol indophenol, copper (II) chloride, ammonium acetate and hydrochloric acid were supplied from Merck (Darmstadt, Germany).

2.2. Physicochemical analysis

The pH values of pomegranate products were measured with Sartorius Basic PB-11 model pH meter [16] and the amount of water-soluble dry matter (WSDM) content (brix) were determined with RA-500 KEM model refractometer [17]. Total acidity was analyzed by potentiometric method in diluted samples [18]. The color of the samples (*L, a, b, Chroma, hue angle*) were measured with CR Konica Minolta CR-5 model device [19]. Ascorbic acid content was determined by Shimadzu UV 1208 spectrophotometer by using 2-6, dichlorophenolindophenol dye solution [20]. In HMF analysis, the absorbance of the red color formed by the reaction of clarified samples with p-toluidine and barbituric acid was determined at the wavelength of 550 nm [21].

2.3. Total phenolic content

Total phenolic content was determined using Folin-Ciocalteau reagent. The products were diluted with pure water at a certain concentration to obtain absorbance in the range of the calibration curve. Then, 1 mL of Folin-Ciocalteu reagent (1:3) was added to 1 mL of diluted sample, and after 5 minutes, 2 mL of 35% saturated sodium carbonate (Na_2CO_3) was added to the mixture. In the final stage, 2 mL of distilled water was added and the test tubes were vortexed (Vortex Mixer Classic, Velp Scientifica, Italia). The mixture was allowed to stand for 30 min in the dark and blue color formed is measured at 700 nm

against the control sample. Results were expressed as milligram gallic acid equivalents (GAE) $100g^{-1}$ WSDM [22].

2.4. Antioxidant Capacity

The antioxidant capacity (AC) of pomegranate products was measured by DPPH assay (2,2-diphenyl-1-picrylhydrazyl) [23] and CUPRAC (cupric ion reducing antioxidant capacity) method [24]. According to the literature, both of these methods were used to determine the AC of pomegranate, so these methods were chosen.

In DPPH method, diluted pomegranate products were mixed with DPPH radical (6×10^5 M) and the reaction mixture was allowed to stand for 30 min at 25ºC in the dark. Absorbance of the reduced radical and control sample was measured at 515 nm using methanol as a blank. Antioxidant capacity was expressed as the percentage inhibition of DPPH radical and determined by the following equation [25];

$$\text{AC (\%)} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \text{ x } 100 \quad (1)$$

A trolox calibration curve (R^2=0.9951) was obtained by measuring the reduction in the absorbance of DPPH solution in the presence of different concentrations of trolox (10-100 μmol L^{-1}). The results as mg trolox equivalent (TE) $100g^{-1}$ of WSDM were calculated using this curve.

Estimation of cupric ion reducing antioxidant capacity was achieved based on the method of Apak et al. [26]. 3 mL of CUPRAC solution [1 mL of 1 x 10^{-2} M copper (II) chloride + 1 mL of 7.5 x 10^{-3} M neocuproine + 1 mL of 1 M ammonium acetate] was added to 1 mL diluted sample. Then final absorbance of green color was measured at 450 nm after waiting 30 min in the dark. Calculation of antioxidant capacity was done as mg trolox equivalents (TE) $100g^{-1}$ of WSDM by using calibration curve (R^2=0.9978).

2.5. In-vitro digestion procedure

An *in-vitro* digestion enzymatic extraction method, slightly modified version of the one described by Vitali et al. [27], that mimics the conditions in the GI tract was used to measure the bioaccessibility of antioxidants and total phenolics. The simulation of GI conditions using commercial digestive enzymes (pepsin and pancreatin) is a widely used method for specifying the potential availability of bioactives. Briefly, 10 mL of distilled water and 0.5 mL of pepsin (20 g L^{-1} in 0.1 mol L^{-1} HCl) were added to 0.5 g of sample, pH was adjusted to 2 using 5 mol L^{-1} HCl and sample was incubated at 37 °C in a shaking water bath (Memmert WNB 22 model) for 1 h. Simulation of gastric digestion was stopped by the addition of 1 M $NaHCO_3$ (to adjust pH to 7.2). 2.5 mL of bile/pancreatin solution (2 g L^{-1} of pancreatin and 12 g L^{-1} of bile salt in 0.1 M $NaHCO_3$) and 2.5 mL of NaCl/KCl (120 mmol L^{-1} NaCl and 5 mmol L^{-1} KCl) were added to the samples and simulation of intestinal digestion was conducted for the following 2 h at 37 °C. Samples were centrifuged (Sigma 3K30 model) at 3500 rpm for 10 min and the supernatant was used for the analysis. After gastric and intestinal digestion, digested samples were used to determine the bioaccessibility of antioxidants and phenolics as described above. *In-vitro* bioaccessibility was calculated as the percentage of antioxidant capacity and total phenolics of digested and undigested concentrations.

2.6. Sensory analysis

The products were evaluated based on color, odor, appearance, consistency, taste and flavor by a panel comprising 10 panelists. The evaluation was done using 5-point structured scales, 5 being the best and 1 the worst quality [28]. The results were interpreted on average scores.

2.7. Statistical analysis

The experiment was conducted in a completely randomized design with three replications. The results were statistically evaluated by one-way analysis of variance (ANOVA) using the JMP software package version 7.0.1 (SAS Institute Inc. NC, 27513). When significant differences were found ($p<0.05$), the Least Significant Difference (LSD) test was used to determine the differences among means.

3. Results and discussion

The highest pH values and so the lowest total acidity levels were seen in jams as seen in Table 1. According to Turkish Standardization Institute (TS 4953) PM standard, the total acidity and brix values of PM is at least 7.5% and 68%, respectively [29]. While the brix values of the samples were in accordance with the standard, the total acidity of the PM 2 sample was determined below the standard. Karabiyikli et al. [30] found the pH values and total acidity of commercial PM between 2.51-2.64 and 12.6-18%, respectively. In the same study, the pH values and total acidity of PS were determined as 2.33-2.68 and 8.6-9.3%. Metin [9] found the pH values of PM and PS between 2.7-3.0 and 1.74-2.62, respectively. The pH, brix and total acidity (in citric acid) values were reported as 1.71-2.96; 62.40-75.00 g $100g^{-1}$ and 4.70-9.73 g $100g^{-1}$ in commercial PM by Akpinar-Bayizit et al. [31]. In another study, total acidity of homemade and two different brands of commercial PM were determined as 1.92 g $100g^{-1}$, 3.2 g $100g^{-1}$ and 3.52 g $100g^{-1}$ [10]. The results obtained from this study were substantially consistent with the literature data and it was thought that the possible differences were mainly due to the raw material and process conditions.

Abid et al. [14] reported the pH of the PJ prepared with different concentrations of sugar and low methoxyl pectin between 2.70 and 3.11. Üstün and Tosun [32] suggested that the required pH range is 3.0-3.5 in order to form a good gel and provide flavor balance in the jams. According to the Turkish Food Codex (2006/55), it was stated that the pH of traditional jam and extra traditional jam should be between 2.8-3.5. In this study, pH values of PJ were determined at these intervals. As known pectin gel is formed in the presence of at least 68% soluble dry matter in jams [33]. In relation to this, Abid et al. [14] determined dry matter of PJ between 59.8% and 66.5% manufactured by using pomegranate peel powder instead of pectin. A limited number of studies are available in the literature on PJ and it is thought that the differences between the results can be based on the composition of the fruit, recipe and process conditions.

While the highest amount of ascorbic acid (vitamin C) was determined in PM 2, the lowest value was found in PJ 1 (Table 1). The antioxidant capacity of PM 2 sample was found to be higher in accordance with high ascorbic acid content (Figure 2). The high HMF content of PJ 1 refers to inappropriate process conditions and this could cause a loss in ascorbic acid content. Eyigün [34] and Kamal et al. [35] determined the ascorbic acid contents of PM samples between 0.02-0.19 g L^{-1} and 0.154-0.250 g $100g^{-1}$ respectively.

The results of Eyigün [34] were consistent with our results, while the results of Kamal et al. [35] were found to be higher. This may be due to differences in raw material, production parameters and analysis methods.

PJ 1 and following PS 2 had the highest HMF level in concentrated pomegranate products. The contribution of sugar, added during the production process, to HMF formation has been revealed in PJ and PS samples. Incedayi et al. [36] and Metin [9] reported the amounts of HMF in PM between 18.56-1542.98 mg kg^{-1} and 91.10-11485.70 mg kg^{-1}. This value was found to be 41-151.9 mg kg^{-1} in PS [9]. Eyigün [34] produced PM under vacuum and atmospheric pressure using Hicaznar variety pomegranate and determined the HMF values of both products between 7.70-190.99 mg L^{-1} and 184.39-1380.64 mg L^{-1}, respectively. In the same study, HMF values of homemade PM were consumedly increased and found between 506.74 and 3266.35 mg L^{-1}. The amount of HMF in concentrated pomegranate products mainly varies according to the concentration technique (under atmospheric pressure or vacuum) and composition (pH, dry matter, reducing sugar and so on) [37]. Additionally, improper conditions and long times of storage may increase post-production HMF levels in these products [9]. Sabanci et al. [38] reported 2.70-5.4 mg L^{-1} HMF in vacuum evaporated and ohmic heating assisted vacuum evaporated pomegranate juice concentrates (40 brix). Also, Karaca [39] found that HMF level of concentrated pomegranate juice in 55-60 brix increased by about 92% compared to pomegranate juice.

The upper amount of HMF for PM is limited to 50 mg kg^{-1} according to Turkish Standards Institute (TS 4953). While PM 1 was complied with the standard, PM 2 had an undesirable HMF level. In previous studies on PM, it was remarkable that HMF contents differ and were mostly above the standard [9, 34, 36]. There is no arrangement with respect to PS and so any evaluation was made for HMF values of this product. Metin [9] determined the HMF content of commercially produced PM in the range of 41-151.90 mg kg^{-1} similar to this study. PS and PJ contain glucose syrup, water, pomegranate aroma, acidity regulator (citric acid), coloring and preservative, unlike PM. The differences in the HMF levels of PM and PS may be due to differences in production methods and inputs.

3.1.Color

While the *L, a, b* and chroma values were highest in PM 1, the lowest color values were determined in PM 2 (Table 2). This indicates that PM 1 has a lighter red color and higher color intensity than the other commercial products. The low HMF value of this product was also showed the lowest undesirable change in the original color. The color tone (h^o) was higher in PM 2. The degradation of anthocyanins and the formation of polymeric oxidation products cause the product color to change to yellowy-brown color over time, resulting in a complete variation in color values. The h^o value of PM 2 was thought to be high due to the brown colored pigments formed during the heat process [40].

Yilmaz et al. [41] found the *L, a* and *b* values of commercial PM in order of 1.88, 2.30, 2.39, whereas Kaya and Sözer [42] found the same values of the pomegranate juice concentrate (71º Brix) as 5.54, 0.57, -0.31, respectively. It was thought that the difference between the results varied depending on the raw material and process conditions. Abid et al. [14] stated the *L, a* and *b* values of PJ samples prepared with different concentrations of fruit, pectin and sugar between 31.82-51.61, 8.15-14.57 and -0.55-4.97, respectively. In parallel with our results, it was detected by Garrido et al. [43] that the longer concentration time of the jam samples with higher fruit content was caused to darkening

of the samples with non-enzymatic browning reactions. In addition, Kopjar et al. [44] presented that different pectin sources and concentrations directly affect the color of the products. Based on this information, factors as pomegranate ratio, pectin concentration and degree of esterification, concentration parameters (temperature and time), storage temperatures *etc*. could be the causes of differences in color values of PJ samples.

3.2. Total phenolic content and antioxidant capacity

The total phenolic content of the PM 2 was remarkably about 7 times higher than that of the PM 1 (Figure 1). This difference may be due to the phenolic level of the raw material and the process parameters. The higher brix value of PM 1 could have a greater loss of phenolic content due to prolonged heat treatment. The jams may also have a lower phenolic content than PM in as much as their high concentration and reaction of the Maillard reaction by-products by Folin Ciocalteu reagent [45].

In previous studies, the results of phenolic substances of PM were found as 2.74 mg GAE / g [46], 118.28-828.15 mg GAE g^{-1} [31], 52.6 mg GAE g^{-1} [41], 551.61-9695.17 mg GAE kg^{-1} [36]. In this study, total phenolic content of PM samples varied between 0.23-15.21 mg GAE g^{-1} on fresh weight and so accorded with the results of Incedayi et al. [36] and Öztan [46]. The difference with others may be the result of extraction methods and dissimilarity of the composition of the raw material.

The antioxidant capacity results of CUPRAC method were higher than DPPH assay (Figure 2). In parallel, Koçak [47] found higher antioxidant capacity values of strawberry jam samples in CUPRAC method. The antioxidant capacity of PS 1 could not be detected in DPPH method at the common dilution rate. It was found to be higher in PM 2 than the other products with both methods. The antioxidant ascorbic acid level and total phenolic content of the same sample were also observed to be similarly high (Table 1 and Figure 1).

Öztan [46] and Akpinar-Bayizit et al. [31] found the antioxidant capacity of the PM by DPPH method as 54.8 μmol TE g^{-1} and 560.23-1885.23 μmol TE g^{-1}, respectively. In this study, antioxidant capacity values of PM and PS (in DPPH method) varied between 1.25-70.10 μmol TE g^{-1} on fresh weight. Among these results, only PM 2 sample (70.10 μmol TE g^{-1} sample) with the highest antioxidant capacity was found closer to the result obtained by Öztan [46]. Differences between the results of the other study and this study could be explained by the extraction method or type and concentration of solvent as well as the properties of the raw material and so the product.

In general, the antioxidant capacity of jams with low phenolic and ascorbic acid contents was determined lower. Poiana et al. [48] showed a significant loss of antioxidant capacity with thermal treatment in jam production. Mena et al. [49] stated an increment in punicalagine with a high antioxidant effect and a decrement in the other bioactive components such as ellagic acids (free and glycoside forms) with heat treatment. The high antioxidant capacity of PM 2 could be related with the increase in bioactive components during the heat treatment or the antioxidant content of the raw material.

The differences in the composition, heat treatment parameters (temperature and time), production methods, storage conditions etc. could be affected the total phenolic content and antioxidant capacity of these concentrated products [39].

3.3. In-vitro bioaccessibility

As known, the biological action and health effects of phenolic compounds rely on the consumed amount and their bioaccessibility which is defined as the quantity of an ingested food constituent that is available for absorption in the gut after digestion [50, 51]. At this point, it is essential to comprehend the bioaccessibility of antioxidants because phytochemicals must be previously available to exert their biological activities [52].

Antioxidant capacity values of all samples could not be determined after simulated GI digestion by DPPH assay in this study. DPPH radical is a reagent with a limited effect on biological mediums due to its structure [53]. Therefore, it was thought that the reaction of the pomegranate samples with the DPPH radical could not be sufficient because of the changes in the structure of the bioactive components after GI digestion.

Biological properties of bioactive components may vary during *in-vitro* GI digestion. It is known that the antioxidant capacity of the phenolic compounds is significantly dependent on the pH level as far as their chemical structures. Aglycone forms have a higher antioxidant capacity than glycosides. Besides the presence, solubility and antioxidant capacity of polyphenols are influenced by the interaction of other compounds (dietary fiber, proteins, *etc*.) released during GI digestion with polyphenols. While antioxidants show higher capacity in gastric phase due to acidic pH conditions, these effects may be reduced in the gut phase. Bioaccessibilities of the constituents might be changed according to physical properties and chemical composition of the food, its release from the food matrix, possible interactions with other food components, the presence of suppressors or cofactors and individual digestive capacity in brief [54, 55].

The total phenolic content of the concentrated pomegranate products after simulated GI digestion was changed between 57.93-2723.49 mg GAE 100 g^{-1} WSDM. Contrary to others, PM 1 and PS 2 samples had a reduction of the total phenolic content after *in-vitro* digestion. The *in-vitro* bioaccessibility of total phenolic contents of the concentrated pomegranate samples was found to be 74-247% (Figure 3). The increase in the amount of bioaccessible phenolics of PJ 1 was also reflected in the antioxidant capacity after digestion (Figure 3). PS 2 had the lowest bioaccessibility in terms of total phenolic content and antioxidant capacity. The processing conditions effect the absorption kinetics of the food in the digestive system [56]. Kamiloğlu et al. [57] notified that the high sugar content of black carrot jam and marmalade was effective on the propagation of polyphenols during *in-vitro* GI digestion. The bioaccessibility of polyphenols depends on a variety of factors, including its digestive stability, its release from the solid food matrix during GI, cellular uptake, metabolism and further transport in the circulatory system. The structural changes after GI digestion affect both further polyphenol uptake and result in a significant loss of the antioxidant capacity.

In this study, after the *in-vitro* GI digestion, the total phenolic content of PM 2, PS 1, PJ 1 and PJ 2 and the antioxidant capacity of PS 1, PJ 1 and PJ 2 samples (after CUPRAC assay) were increased compared to undigested samples. This can be explained by the fact that the cell walls become more permeable as a result of the deterioration of their structure by the heat treatment applied during the production of foods. In this way, access to the compounds within the cell is facilitated. Heat treatment has a positive impact on making bioactive substances in food more accessible and increasing their extractability. Degirmencioglu et al. [58] identified a similar increase in total phenolic content of

vegetable juices and explained it by the metabolism of phenolics, such as hydrolysis via deglycosylation or cleavage by esterases during GI digestion. These structural changes could affect both their further uptake and bioactivity. As reported by Fang et al. [59], the increase of the antioxidant capacity of the products (PS 1, PJ 1 and PJ 2) might be explained by the increase of the total phenols. Generally, PM, PS and PJ are the products with differences between their composition and production methods. So, it was expected to see some differences for polyphenols present in these products during *in-vitro* digestion.

3.4. Sensorial evaluation

Each pomegranate product group (PM, PS and PJ) was evaluated separately in itself in terms of color, appearance, consistency, odor, taste and flavor as seen in Table 3. PS contains glucose syrup, acidity regulators and colorants unlike PM, so the change in composition affects the sensory properties of these products and consumer appreciation as well. The astringency taste of PM, which contains nothing but pomegranate juice, was more perceptible. The higher phenolic substances, which take charge in taste and aroma formation and low total acidity of PM 2, caused sensory preference for this sample. Also, PJ 1 was more acceptable than PJ 2.

4. Conclusion

The PM samples were found to have the highest ascorbic acid and phenolic contents with antioxidant capacity. Although the samples of PM 1 and PM 2 were the same product type, the total phenolic content of PM 2 was about 7 times higher and the antioxidant capacity values were approximately 21 times higher in the DPPH assay and 15 times higher in the CUPRAC assay than the PM 1. The lowest phenolic content and antioxidant capacity were observed in PS 1.

PM 1 did not exceed the legal limit of HMF (50 mg kg^{-1}) specified by TS 4953. The differences in the production techniques, storage conditions, heat treatment parameters, sugar contents, pH levels, water activities *etc*. of these products was thought to effect the HMF content and many other physicochemical properties. As sensorial, PM 2, PS 1 and PJ 1 were the most appreciated products by the panelists.

The total phenolic contents of PM 2, PS 1, PJ 1 and PJ 2 were increased after *in-vitro* GI digestion. The antioxidant capacity results obtained by CUPRAC method were generally increased after digestion and the maximum increment was seen in PJ samples. This was thought to be due to the differentiation of the antioxidant properties after the interaction of food matrices of the products with the digestive enzymes and other chemicals under the *in-vitro* digestion conditions and the increase of the release of bioactive components from the cells. There is a limited research in the literature about the changes in the total phenolic content and antioxidant capacity of commercial pomegranate products in *in-vitro* or *in-vivo* digestion conditions. Therefore, the data obtained through this study will be important for further studies on the *in-vitro* bioaccessibility of total phenolics and antioxidants and the effects of these commercial products on health and nutrition.

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Table 1. The results of the physicochemical analysis of samples* (mean ± standard deviation)

	pH	**Titratable acidity (g 100 g^{-1})	Brix (g 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	HMF (mg kg^{-1})
PM 1	1.69±0.01d	10.25±0.05a	75.17±0.33a	3.07±0.12b	9.20±3.11c
PM 2	2.83 ±0.01a	7.14±0.05b	73.8 ±0.06b	19.78±0.66a	118.68±3.19b
PS 1	1.71±0.01c	3.29±0.00d	72.3 ±0.29c	1.42±0.27c	117.15±6.91b
PS 2	2.01±0.00b	3.73±0.01c	70.6 ±0.20d	1.06±0.18c	387.32±66.36a
PJ 1	3.13±0.00b	0.53±0.00a	75.3±0.03b	0.89±0.20a	479.63±2.15a
PJ 2	3.40±0.01a	0.44±3.925e	78.5 ±0.22a	1.65±0.26a	175.11±2.67b

* Different letters means significantly different at $p < 0.05$ according to the LSD test. ** Citric acid

PM: Pomegranate molasses, PS: Pomegranate sour sauce, PJ: Pomegranate jam

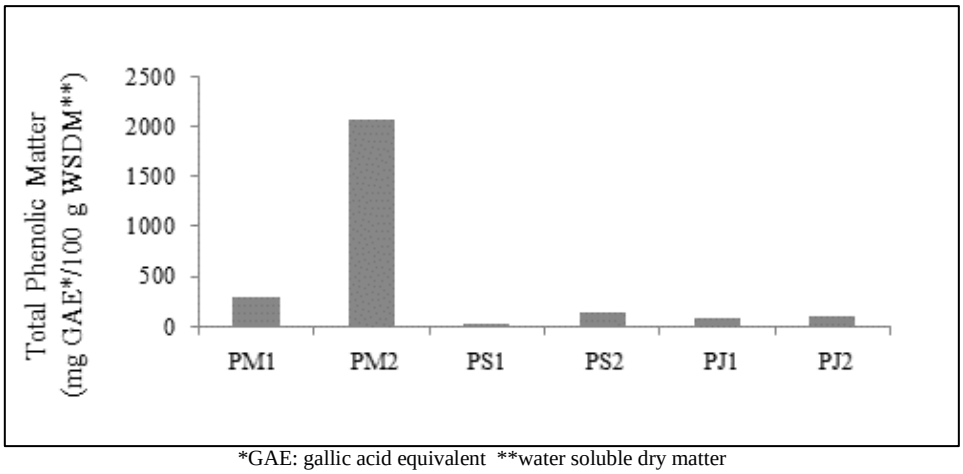
Table 2. Color values of samples* (mean ± standard deviation)

	L	*a*	*b*	*C**ab	*h*°
PM 1	31.69±0.00a	44.42±0.01a	53.79±0.02a	69.76±0.02a	50.45±0.01b
PM 2	0.00±0.00d	0.07±0.04d	-0.07±0.03d	0.10±0.05d	298.84±15.16 b
PS 1	6.99±0.30c	26.30±0.01c	12.02±0.02c	28.92±0.01c	24.56±0.05a
PS 2	18.19±0.01b	32.69±0.01b	31.30±0.02b	45.25±0.02b	43.76±0.01b
PJ 1	5.24±0.02a	7.18±0.02a	6.15±0.03a	9.45±0.02a	40.56±0.17a
PJ 2	2.85±0.01b	3.55±0.04b	1.92±0.02b	4.04±0.04b	28.47±0.19b

* Different letters means significantly different at $p < 0.05$ according to the LSD test.

Table 3. Sensory analysis results of concentrated pomegranate products* (mean ± standard deviation)

	Color	Appearance	Consistency	Odor	Taste	Flavor	Average
PM 1	4.2±0.92	4.2±1.03	3.2±1.32	3.5 ±1.35	3.1±1.29	3.0±1.33	3.53
PM 2	3.8±0.63	4.1±0.74	4.1±0.99	3.6±1.43	3.6±0.97	3.5±1.08	3.78
PS 1	3.7±0.82	4.3±0.67	4.3±0.82	3.8±0.79	3.9±0.74	3.7±1.1	3.95
PS 2	3.5±1.18	4.2±0.92	3.1±1.29	3.4±0.97	3.4±1.07	3.3±1.06	3.48
PJ 1	3.9±0.93	4.0±0.87	4.2±0.67	4.3±0.1	3.8±1.09	3.9±1.35	4.01
PJ 2	3.4±1.13	3.3±1.00	3.7±1.12	4.0±0.2	3.6±1.24	3.6±1.2	3.60



*GAE: gallic acid equivalent **water soluble dry matter

Figure 1. Total phenolic content of samples.

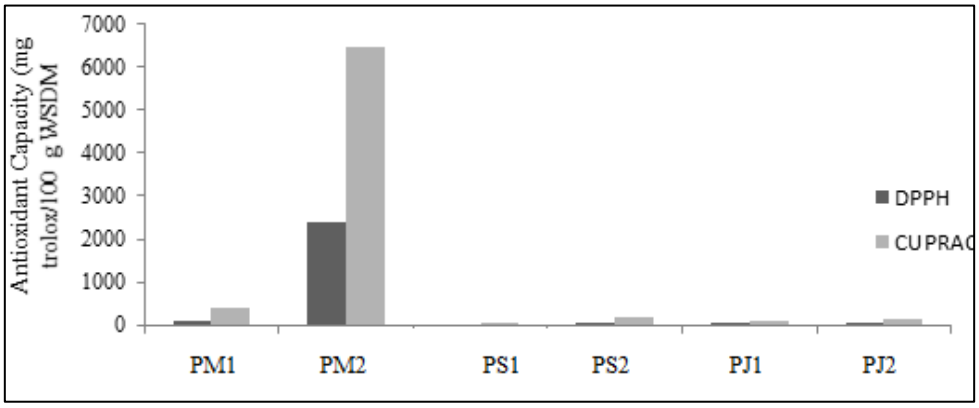


Figure 2. Antioxidant capacity of samples.

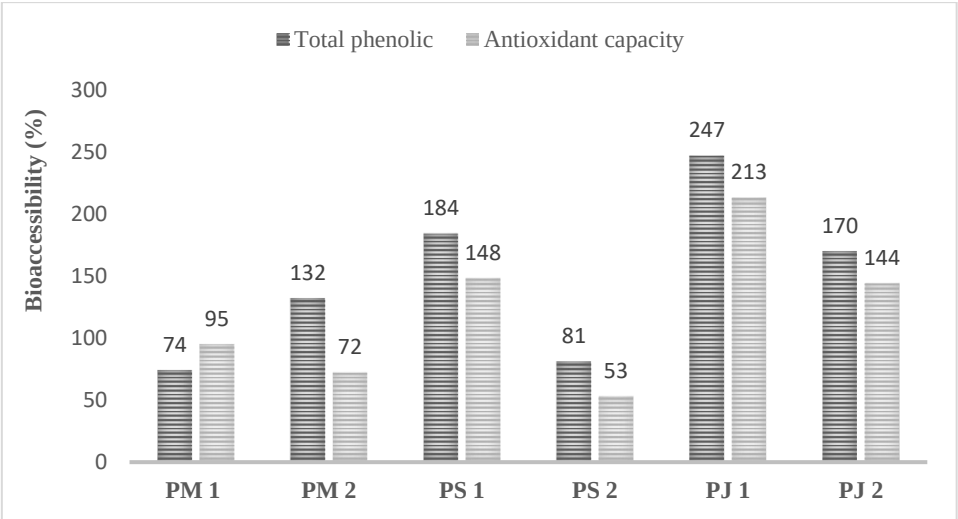


Figure 3. *In-vitro* bioaccessibility of total phenolics and antioxidants of concentrated pomegranate products.