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Research Article

Study of the Physiochemical and Nutraceutical Properties of Sour and Sweet Pomegranate Juice in Northern Jordan

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Pomegranate juice (PJ) is the major pomegranate product that offers a simple way to consume pomegranate's biologically active compounds, obtained from arils. In this study, we objectively investigate the physiochemical properties such as pH value, total soluble solids, color parameters, fructose and glucose contents, hydroxymethyl furfural (HMF) content, and viscosity. Also, the phytochemical content includes total phenols content, antioxidant activity, flavonoid, anthocyanin content, and phenolic quantification. In addition, the alpha-amylase and angiotensin-converting enzyme inhibitory activities among sweet and sour varieties of pomegranate juice obtained from different regions in Northern Jordan (Ajloun, Dair Abi Said, and Kufur Soum) where the significant differences at $P \leq 0.05$ appeared among sweet and sour varieties in different pomegranate juice samples. The pH values for pomegranate juice range from 2.87 to 3.77, and TSS ranges from 15.36 to 16.9 Brix. The total phenol content of pomegranate juice ranged from 105.8 to 238.63 mg/g while the total flavonoid content was present in the range of 135.53–184.9 mg/g. The DPPH inhibition (%) of pomegranate juice ranged between 20.66% and 50.63%, and the anthocyanin content range was 3.66–11.02 mg/g. Ellagic acid, delphinidin, 3,4-dihydroxyphenethyl alcohol, 2-hydroxyphenethyl alcohol, catechin, epicatechin, vanillic acid, caffeic acid, P-coumaric acid, chlorogenic acid, gallic acid, ferulic acid, and syringic acid are phenolics present predominantly in pomegranate juice. Pomegranate juice exhibits high alpha-amylase and angiotensin-converting enzyme inhibition activity. All results indicate good quality and health properties for pomegranate juice.

1. Introduction

Pomegranate (*Punica granatum* L.) is an ancient, deciduous shrub or a small fruit tree, belonging to the Punicaceae family [1, 2]. Its name emanates from "Pomuni granatum," in which pomum means apple and granatus means grainy, translated as "seeded apple" [3–5]. The pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits that originated in Central Asia (Iran, Turkmenistan) to the Himalayas in northern India in 3000–4000 BC. Pomegranate was cultivated and naturalized over the Mediterranean region thousands of years ago [3, 4, 6–8]. The pomegranate is a nutritious fruit with different cultivars (sweet, sour, or sweet-sour) and is composed of organic acids, sugars, vitamins, polysaccharides, polyphenols, and minerals [2]. It is consumed fresh or processed into juices, canned beverages, jelly, jam, syrup, sauce, molasses, and paste [6].

Pomegranate juice (PJ) is the major pomegranate product that offers a simple way to consume pomegranate's biologically active compounds. It is obtained from arils, which account for about 50% of the fruit weight and contain about 78% juice and 22% seeds [9–11]. The reddish-purple, moderately acidic juice contains 85.4% water and 15.6% dry substance, composed of 10.6% sugars, 1.4% pectin, 0.2–1.0% polyphenols, organic acids, anthocyanins (potent antioxidants provide pomegranate juice with its brilliant color), and other compounds include fatty acids, amino acids, indoleamines, sterols, triterpenoids, α-tocopherol, vitamins, and minerals (Fe, Ca, Cl, Cu, K, Mg, Mn, Na, and Zn). These compounds vary in correlation to the pomegranate variety and juice production technology [10, 12]. Several steps are included in the production process of pomegranate juice (PJ), such as washing, crushing, deshelling, pressing, clarification, and pasteurization. Juice production increased in recent years, thus as a healthy beverage and a novel flavor for new product development [10, 13]. Generally, pomegranate juice (PJ) provides a sweet and sour taste, musty/earthy and fruity odors, and an astringent mouthfeel [7]. It is considered a "superfood" where routine consumption of pomegranate juice (PJ) is associated with improved cardiovascular well-being through cholesterol and blood-pressure-reducing effects, preventing some cancer types such as skin, breast, and prostate, anti-inflammatory, antidiarrheal, and astringent activities [9]. Pomegranate juice (PJ) showed 20% higher antioxidant activity than other polyphenol-rich juices and beverages such as apple, acai, black cherry, blueberry, cranberry, concord grape, orange juices, red wines, iced tea, green tea infusion, organic elderberry, and cranberry juices. It is enriched with antioxidants including anthocyanins, ellagic acid, ellagitannins, vitamin C, and vitamin E [9, 14].

Customer preferences for pomegranate fruits showed that sweet cultivars were appropriate for fresh consumption and juice production due to their sweetness and other characteristics (seed hardness/astringency level/bitterness), whereas sour cultivars showed several characteristics that could be of great interest for food and nutraceutical industries [7, 15].

This work aims to determine the nutritional value of the sweet and sour varieties of pomegranate juice (PJ) obtained from different regions in Northern Jordan (Ajloun, Dair Abi Said, and Kufur soum) to investigate their physiochemical properties (pH value, total soluble solid, color parameters, fructose and glucose content, hydroxymethyl furfural (HMF) content, and viscosity) and the phytochemical content (total phenols content, antioxidant activity, flavonoid, anthocyanin content, and phenolic quantification). The results of this study will increase the awareness of people about the benefits of eating pomegranate fruit or drinking the pomegranate juice and encourage the investment in the pomegranate juice industry.

2. Materials and Methods

2.1. Chemicals. Folin–Ciocalteau reagent, sodium carbonate Na_2CO_3, gallic acid, methanol, sodium nitrite ($NaNo_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), 2,2-diphenyl-2-picrylhydrazyl (DPPH), HCL, DNS reagent (dinitrosalicylic acid), Hip-His-Leu (hippuryl-L-histidine-leucine), and HPLC grade acetonitrile, and all other chemicals were purchased from local agents (Irbid, Jordan).

2.2. Sample Collection. Pomegranate fruit was collected during the summer of October 2021 from the main local producer farms (Ajloun, Deir Abi Said, and Kufur Soum) in the Northern part of Jordan.

Juice Processing: the pomegranate fruits were washed by submerging them in tap water, drained, and manually cut up, and the outer leathery skin, which encloses hundreds of fleshy arils, was removed. The arils were manually collected and pressed using an electric fruit juicer machine and extracted and centrifuged (1,500 g), collected in sterile bottles, and quickly refrigerated at 4°C until further analysis [16].

2.3. Physiochemical Properties

2.3.1. Total Soluble Solids (Brix). The total soluble solids were determined at room temperature (25C) using a digital refractometer (ATAGO HTT, ILLUMINATOR, Fukuoka, Japan) using a scale from 0 to 95%.

2.3.2. Determination of pH Value. pH measurement was directly measured at room temperature using a pH meter (CyberScan pH510—Eutech Instruments). A sample solution of 5 g/50 g was used, and the results were expressed as pH to the nearest 0.01 degree.

2.3.3. Sugar Profile Analysis

(1) Preparation of Pomegranate Juice Samples. Glucose and fructose were measured according to AOAC [17] with some modifications. Each sample (5 g) was weighed and dissolved in 50 ml of distilled water. From each sample, 1 ml was transferred to a 5 ml glass tube, and then, 1 ml of acetonitrile was added. The final solution was filtered through a 0.45 μm filter and transferred to sample vials.

(2) HPLC Analysis of Pomegranate Juice Sugars. This method is based on AOAC [17] with minor modifications, a 10 μL portion of each prepared sample was injected into the HPLC, and the sugar content was determined by HPLC (high-pressure liquid chromatography) equipped with RI detection (SHIMADZU refractive index (RID-10A)) and separation column (Shim-pack SCR-101N) (250 mm·L × 4.6 mm I.D., 10 μm) was used. The column temperature was held at 30°C. The mobile phase was a mixture of water/acetonitrile (80 : 20 v/v). The flow rate was 1.3 ml/min. Sugar was identified according to their retention times by comparing it with sugar standards. Quantitation is performed according to the external standard method on peak areas or peak heights [18].

2.3.4. Hydroxymethylfurfural Determination. The HMF content was determined according to the official AOAC method (AOAC official method 980.23, 1983) [19]. Five grams of each sample was dissolved in 25 ml of water and transferred quantitatively into a 50 ml volumetric flask, then added 0.5 ml of $K_4Fe\ (CN)_6.3H_2)$ and 0.5 ml of Zn

$(CH_3COO)_2$, and made up to 50 ml with water. The solution was filtered through paper discarding the first 10 ml of the filtrate. Aliquots of 5 ml were put in two test tubes; 5 ml of distilled water was added to one tube (sample solution); 5 ml of sodium bisulfite solution was added to the second (reference solution). The absorbance of the solutions at 284 and 336 nm was determined using a spectrophotometer (Varian Cary, model 1E UV/Visible Spectrophotometer). The HMF content was calculated by the following equation:

$$\text{HMF}\left(\frac{\text{mg}}{\text{kg}}\right) = \left(\frac{((A284 - A336) * 14.97 * 5)}{\text{wet sample}}\right) * 10, \quad (1)$$

where A284 is the absorbance at 284 nm, A336 is the absorbance at 336 nm, and 14.97 is a factor calculated by the molecular weight of HMF.

2.3.5. Color Measurement. The color of pomegranate juice (PJ) samples was measured by a colorimeter (12MM Aperture U 59730 Inc., Pittsford, New York, USA) and recorded in the L^*, a^*, and b^* color system according to [20]. This color system consists of a luminance or lightness component L^* and a^* which is the component for greenness and redness and the b^* component for blue to yellow. The colorimeter was calibrated by utilization of a standard white ceramic reference (Commission Internationale de I`Eclairage $L^* = 97.91$, $a^* = -0.68$, and $b^* = +2.45$). In addition, the total color difference (ΔE) and Chroma were calculated using the following equations: $\Delta E = [(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2]^{1/2}$

$$\text{Chroma} = \left[(a)^2 + (b)^2\right]^{1/2}, \text{three observations were used to calculate the mean value.} \quad (2)$$

2.3.6. Viscosity Determination. The viscosity of pomegranate samples was conducted according to a method described by Ereifej et al. [21]. Haake falling ball viscometer (Haake Mess Technik, "Falling Ball Viscometer" Manual, Dieselstr. 6–7500 Karlsruhe 41, Germany) was used to determine the viscosity of pomegranate juice (PJ) samples at 25°C. Five ml from each sample was used to measure the viscosity. The viscosity was expressed as follows:

$$\text{Viscosity} = A(K1 - K2) * t, \quad (3)$$

where (i) viscosity is in Pa · s, A = ball constant, $K1$ = ball density kg/m^3, $K2$ = sample density kg/m^3, and t = time (sec). (ii) The nominal size of balls is 1/16 inch and 3/32 inch. Duran borosilicate glass specifications are length: 362 mm, inner diameter: 50 mm, and outer diameter: 53 mm.

2.4. Phytochemical Determination

2.4.1. Extraction. Pomegranate juice (PJ) samples (5 g) were diluted in 50 ml distilled water in the ratio of 1 : 10 (w/v), filtered through Whatman No. 1 filter paper, and stored in the dark until further analysis [22].

2.4.2. Determination of Total Phenolics. Total phenolic was determined according to the Folin–Ciocalteu procedure known by Singleton and Rossi [23], with minor modifications that 100 μl of the sample extract (triplicate) was transited into a test tube and mixed with 0.4 ml of 10% Folin–Ciocalteu reagent. After 3 min, 0.8 ml of a 1% Na_2CO_3 solution was added. Tubes were allowed to stand for 1 h at room temperature, and the absorption was defined at 725 nm using a spectrophotometer (CELL, model CE 1020, Cecil Instruments, Cambridge, U.K.) against a blank, which contained 100 μl of distilled water. Gallic acid was used as a calibration standard, and the results were expressed as gallic acid equivalent (mg GAE/100 g of pomegranate).

2.4.3. Determination of Total Flavonoids. The total flavonoid content was determined using a colorimetric method as described by Zhishen et al. [24]. Shortly, 0.5 ml of each sample was mixed with 2 ml of distilled water and then with 0.15 ml of a $NaNO_2$ solution (15%). After 6 min, 0.15 ml of an $AlCl_3$ solution (10%) was added and allowed to stand for 6 min, and then, 2 ml of NaOH solution (4%) was added to the mixture. The volume was brought to 5 ml, and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance was determined at 510 nm versus a water blank using a spectrophotometer (CELL, model CE 1020, Cecil Instruments). Results were expressed as catechin equivalents (mg catechin/g of juice sample). All measurements were carried out in triplicates.

2.4.4. Determination of DPPH Radical Scavenging Activity. DPPH radical scavenging effect was determined using a procedure described by Matthäus [25]. Five grams of each pomegranate juice (PJ) sample was dissolved in 50 ml methanol, centrifuged at 4350 × g, and then filtered through Whatman No. 1 filter paper. Juice extracts (0.5 ml) were reacted with 0.2 ml of DPPH solution. The mixture was made up of a total volume of 4.0 ml with methanol, and the mixture was mixed completely and allowed to stand in the dark for 60 min at room temperature. Absorbance (A) was then determined at 515 nm using a spectrophotometer (CELL, model CE 1020) against the blank. The radical scavenging activity was expressed as % of inhibition according to the following formula [26]:

$$\text{Inhibition of control of sample}\,(\%) = \left(\frac{(A\text{ of control} - A\text{ of sample})}{A\text{ of control}}\right) * 100. \tag{4}$$

2.4.5. Determination of Anthocyanin

(1) Anthocyanins Extraction. The sample extract was determined as described by Rabino and Mancinelli [27]. Five grams of each pomegranate juice (PJ) sample was diluted in 50 ml of 1% HCL methanol (w/v) solution. Then, extraction was carried out by shaking for 60 min at 60°C in a water bath and then filtered with Whatman No. 1 filter paper.

(2) Determination of Anthocyanin. The anthocyanin content was conducted according to Rabino and Mancinelli [27], with minor modifications. Absorbance (A) of the extract was determined at 657 nm and 530 nm using a spectrophotometer (CELL, model CE 1020, Cecil Instruments). Net absorbance was calculated based on cyanidin 3-glycoside by the following equation:

$$\text{Net Abs.} = \text{Abs. at 530 nm} - 0.25\,(\text{Abs. at 657 nm}),\ \text{where anthocyanin content in}\left(\frac{\text{mg}}{g}\right) = \left(\frac{\text{net }Abs}{29,600}\right) \times \text{MW} \times \text{DF} \times \left(\frac{V}{\text{Wt.}}\right), \tag{5}$$

where 29,600 = molar extinction coefficient, MW = 449.1 molecular weight of cyanidin 3-glycoside, DF = dilution factor, V = total volume (ml), and Wt. = sample weight (g). Three replicates were used to calculate the mean value.

2.4.6. RP/UHPLC of Phenolic Quantification. The quantification of the selected phenolic standards (gallic acid, 3,4-dihydroxyphenethyl alcohol, catechin, 2-hydroxyphenethyl alcohol, chlorogenic acid, vanillic acid, epicatechin, caffeic acid, syringic acid, P-coumaric acid, sinapic acid, ferulic acid, rutin, rosmarinic acid, quercetin, thymol, ellagic acid, and delphinidin) was studied in the pomegranate juice (PJ) using a reversed-phase UHPLC (Thermo Scientific Ultimate 3000, USA) instrument utilizing a binary gradient elution. The UHPLC instrument is equipped with a diode array detector (DAD). The column used for reversed-phase was a Venusil SCX column (C18 column, 4.6 mm × 250 millimeter, 5 μm). The mobile phase was a gradient of solvent (A) made up of 0.2% (v/v) TFA in water and solvent (B) made up of 100% methanol with a linear gradient. Each run takes 58 min with a flow rate of 0.75 ml/min. The column was washed before and after each run. The volume of 20 μL of each sample was injected into the column using the above mobile phase, and the UHPLC was run at a wavelength of 280 nm. Data acquisition and chromatographic analysis are carried out by Chromeleon software (c) Dionex Version 7.2.10.23925.

2.5. Enzymatic Assay Determination

2.5.1. Determination of Alpha-Amylase Inhibitory Activity. The α-amylase inhibitory activity of the pomegranate juice (PJ) samples was conducted by a method described by Mccue et al. [28] with modifications. A 0.03% (w/v) porcine pancreatic α-amylase (10080, Sigma Chemical Co, USA) mixture was prepared in 100 ml of distilled water. Then, 0.5 ml of sample, 0.5 ml of α-amylase solution, and 0.5 ml of phosphate buffer (pH 7) were mixed and incubated at 25°C for 10 min, and 0.5 ml of water was used as a control. Next, 0.5 ml of starch solution (0.5 g of starch powder in 100 mL of distilled water incubated at 65°C for 20 min) was added and mixed well, followed by incubation at 25°C for 10 min in a water bath. A 1 ml of colorimetric reagent 3,5-dinitrosalicylic acid (DNS) was added, and the mixture was heated in a water bath at 95°C for 5 min and cooled to room temperature. The mixture was brought to 10 ml with distilled water. Stock solutions were prepared at concentrations of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml. Then, the absorbance was measured at 540 nm using a spectrophotometer (CELL, model CE 1020, Cecil Instruments) against the blank.

The inhibitory activity of α-amylase was calculated according to the following equation: inhibitory activity of α-amylase $(\%) = (100 \times [Abs_{(C)} - Abs_{(S)}]/Abs_{(C)})$, where $Abs_{(C)}$ is the absorbance of the control at 540 nm and $Abs_{(s)}$ is the absorbance of the sample at 540 nm.

2.5.2. Determination of Inhibitory Activity of Angiotensin 1-Converting Enzyme (ACE). The inhibitory activity of ACE was determined according to [29] with some modifications described by [30]. HEPES-HCL from (Sigma Chemical Co.) was used to prepare buffer. A buffer was prepared by adding 1.3014 g HEPES sodium salt and 1.75329 g sodium chloride in 100 ml distilled water. This buffer was used in the preparation of Hippuryl-histidyl-leucine (HHL) (H1635, Sigma Chemical Co., Ltd., USA) by dissolving 6 μl of HHL in 2 ml HEPES-HCl buffer. An ACE enzyme from A6778, Sigma Chemical Co., Ltd., USA was prepared by mixing 0.33 U in 1 ml of distilled water. A 100 μl of pomegranate juice (PJ) samples was mixed with 200 μl of HHL followed by adding 50 μl of ACE, and the mixture was incubated at 37°C. To stop the reaction, 0.25 ml of HCL was added. After 15 minutes, 2 ml of ethyl acetate was added to extract the liberated hippuric acid. The mixture was centrifuged at 3000 rpm for 3 min, and 1 ml of ethyl acetate was collected and evaporated by using a boiling water bath. After 15 minutes, 3 ml of distilled water was added. The amount of liberated hippuric acid was quantified by measuring the absorbance at a wavelength of 228 nm using UV 1800, UK. The preparation of control was done by adding 200 μl HHL and 50 μl ACE in 100 μl distilled water instead of the sample. The 100% ACE activity was defined as the amount of hippuric acid liberated in control. The ACE inhibition was measured in triplicate for each sample and calculated using the following equation:

$$\text{Inhibitory}\,\% = \frac{(\text{ABc} - \text{ABs})}{(\text{ABc})} * 100, \qquad (6)$$

where ABc is the absorbance of control at 228 nm and ABs is the absorbance of the sample.

2.6. Statistical Analysis. Data were analyzed using the SAS version 8.2 software package [31] for data analysis, and ANOVA was applied to observe the existence of significant differences among the means. Means were separated by LSD analysis at a least significant difference of 0.05 P value.

3. Results and Discussion

3.1. Physiochemical Properties of Pomegranate Juice

3.1.1. Total Soluble Solids (Brix). The mean values of TSS of the sweet and sour pomegranate juice from different regions of Northern Jordan are given in Table 1. The results indicate that the TSS values for the different pomegranate juice (PJ) samples of sweet and sour varieties range from 15.36 to 16.9 °Brix, where Ajloun sour juice ($A1$) has the lowest value and Kufur Soum sweet juice ($K2$) is the highest. There is no significant difference in the TSS values for the $A1$ (15.36), Ajloun sweet juice ($A2$) (15.43), and Dair Abi Said sweet juice ($D2$) (15.43). Also, Dair Abi Said sour juice ($D1$) (15.93) and Kufur Soum sour juice ($K1$) (15.9) showed no significant differences whereas $K2$ (16.9) had a significant difference compared to the other samples. Our result is quite similar to those reported by Zaouay et al. [32] who found that the lowest mean of total soluble solids content is 14.08 °Brix, and the highest is 16.28 °Brix. Another study by Fernandes et al. showed that different values of TSS ranged from 14.87 to 18.04 °Brix for nine pomegranate cultivars in Spain [33]. In addition, Tehranifar et al. [34] found that the TSS values of the Iranian pomegranate cultivars ranged from 11.37 °Brix to 15.07 °Brix, which is slightly lower than our range. The differences in TSS values are attributed to the effect of genotypes, variety, maturity level, cultural and environmental practices, and the region of growth [35, 36].

3.1.2. pH Value. The mean pH values of different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan are given in Table 1. For pomegranate juice (PJ), the pH values are significantly different and range from 2.87 to 3.77. The lowest PH was found in $A1$, and the highest was in $D2$. Sour cultivars have a lower value than sweet cultivars in PJ thus indicating that they are more acidic resulting in fewer customers' preferences in the case of juice production [7]. Tehranifar et al. [34] found that the pH values ranged between 3.16 and 4.09 for Iranian pomegranate cultivars while the results obtained by Fernandes et al. [33] ranged from 2.56 to 4.31. In addition, Legua et al. [37] obtained a pH range of 3.94–4.07 for Spanish pomegranate cultivars. Beaulieu et al. [38] found that the range of pH values for California pomegranate cultivars is 2.76–3.48. Several factors such as fruit variety, maturity status, organic acid content, genotypes, the growing region, and postharvest handling will contribute to differences in pH values [33].

3.1.3. Fructose and Glucose Contents. The mean values of fructose and glucose contents from total sugar content for different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan are given in Table 1. Fructose content in pomegranate juice (PJ) samples shows significantly different values ranging from 2.11% to 7.37%, and the lowest value (2.11%) is for $D1$, whereas the $A2$ has the highest. The fructose content of $A2$ (7.37%) and $D2$ (6.86%) as sweet cultivars is higher than $A1$ (3.56%) and $D1$ (2.11%), which are sour cultivars. The highest glucose content in pomegranate juice (PJ) samples was $D1$ (9.76%), followed by $D2$ (9.48%), $A2$ (9.15%), and $K2$ (8.86%), significantly followed by 4.19% for $A1$, and the lowest is 3.49% for $D1$. Generally, sweet cultivars are higher in glucose content than the sour ones. Due to the customer's preference regarding juice consumption, sweet varieties are better by having a sweet taste [7]. Fadavi et al. [39] reported that the fructose content for ten different pomegranate juice (PJ) samples in Iran ranged from 3.50% to 5.96%, and the glucose content varied from 3.40% to 6.40%. Furthermore, Pasricha [36] obtained fructose content in the range of 1.07–5.01 and glucose in the range of 1.03–5.93. Legua et al. [40] mentioned that glucose and fructose were the main sugars in pomegranate juice (PJ). It seems that the fructose percentage in the PJ from Jordan has higher values than that in the PJ from some other countries. However, the differences in the sugar composition of pomegranate depend on the genotype, variety, agro-climatic conditions, extraction technique, and degree of maturation [15, 41].

3.1.4. Hydroxymethylfurfural (HMF). The hydroxymethylfurfural (HMF) content of different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan is given in Table 1. The results of the HMF content of different pomegranate juice (PJ) samples ranged from 160.58 mg/kg to 181.39 mg/kg. The highest content was in $A1$, and the lowest was in $D2$. All sour cultivars $A1$ (181.39 mg/kg), $D1$ (167.56 mg/kg), and $K1$ (179.74 mg/kg) show higher HMF content than the sweet ones $A2$ (178.44 mg/kg), $D2$ (160.58 mg/kg), and $K2$ (174.85 mg/kg). INCEDAYI [42] found that the PJ sample had the highest HMF level (479.63 mg/kg) in concentrated

Table 1: Total soluble solids, pH, fructose and glucose contents, and HMF content of different pomegranate juice samples.

Varieties	Sample name	Parameters				
		TSS	pH	Fructose and glucose content		HMF (mg/kg)
				Fructose (%)	Glucose (%)	
Sour	*A*1	15.36 ± 0.06[c]	2.87 ± 0.002[f]	3.56 ± 0.08[d]	4.19 ± 0.56[b]	181.39 ± 0.74[a]
	*D*1	15.93 ± 0.55[b]	3.026 ± 0.002[e]	7.37 ± 0.02[a]	9.76 ± 0.77[a]	167.56 ± 0.43[d]
	*K*1	15.9 ± 0.01[b]	3.77 ± 0.002[a]	2.11 ± 0.10[e]	3.49 ± 0.73[b]	179.74 ± 0.77[b]
Sweet	*A*2	15.43 ± 0.06[c]	3.26 ± 0.002[d]	6.86 ± 0.23[b]	9.15 ± 0.29[a]	178.44 ± 0.91[b]
	*D*2	15.43 ± 0.05[c]	3.55 ± 0.003[c]	6.96 ± 0.19[b]	9.48 ± 0.38[a]	160.58 ± 1.08[e]
	*K*2	16.9 ± 0.17[a]	3.62 ± 0.002[b]	6.07 ± 0.26[c]	8.86 ± 0.39[a]	174.85 ± 0.65[c]

[#]All values are calculated as a wet basis and means of three replicates. *Means ± SD in the same column with the same letter are not significantly different ($P \leq 0.05$). **A*1: Ajloun sour cultivar, *D*1: Deir Abi Said sour cultivar, *k*1: Kufur Soum sour cultivar, *A*2: Ajloun sweet cultivar, *D*2: Deir Abi Said sweet cultivar, and *k*2: Kufur Soum sweet cultivar.

pomegranate products. It was mentioned that the composition of pH, dry matter, and reducing sugar affects the amount of HMF.

3.1.5. Color Measurement. The mean color values of different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan are given in Table 2. The results were expressed as L^* for darkness/lightness (0 black, 100 white), a^* ($-a$ greenness, $+a$ redness), and b^* ($-b$ blueness, $+b$ yellowness) and showed differences that exist between the juice samples. Pomegranate juice (PJ) L^* values ranged from 49.84 to 64.21, where the increase of L^* indicates more lightness. The a^* values varied from 2.39–17.88, and the b∗ values ranged from 10.69–18.19. The highest lightness value was found in the *D*2 (sweet cultivar) (64.21), followed significantly by *K*2 (61.47) and *A*2 (60.42), and both are sweet, whereas for the sour samples, it was as follows: *A*1 (58.14), *K*1 (57.43), and *D*1 (49.84). However, sweet cultivars showed more lightness than sour ones. The redness is higher in the sour cultivar *D*1 (17.88), followed significantly by *K*1 (8.53), *A*1 (6.78), *D*2 (4.21), *A*2 (3.47), and *K*2 (2.39), which indicates that red pigment or anthocyanins are more abundant [43]. The *A*2 showed the highest yellowish value (18.19), followed significantly by *D*1 (16.69), *K*2 (14.24), *A*1 (14.11), *D*2 (11.83), and *K*1 which showed the lowest yellowness value (10.69). Passafiume et al. [44] studied L^* values for pomegranate juice (PJ) among three cultivars and found that the highest value was 40.8, the highest a^* value was 60.6, and the highest b^* value was 12.8. In a similar study, Mditshwa et al. [35] found that lower L^* values than ours from pomegranate cv. Bhagwa fruit that has been grown in three microclimates in the Western Cape, South Africa, ranged from 22.88 to 27.12 while the redness values (a^*) were higher and ranged from 18.65 to 24.34. b^* values and the yellowness values range from 10.42 to 13.09. The differences in color values arise from different pomegranate fruit composition, climate, and processing steps, while the red color is related to the anthocyanin content [45].

3.1.6. Viscosity. The viscosity at 25°C of different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan are shown in Table 3. The results exhibit that the pomegranate juice (PJ) viscosity ranged from 145.04 to 294.47 MPa s. The thicker was *K*2, whereas the lightest was *A*1. Sour juice samples *A*1 (145.04 MPa·s) and *K*1 (155.66 MPa·s) were less viscous than *A*2 (234.67 MPa·s) and *K*2 (294.47 MPa·s), respectively. The *D*1 viscosity (235.34 MPa·s) was thicker than *D*2 (169.24 MPa·s). It seems that the viscosity of PJ from Jordan is higher than what Salehi [46] reported in his study that the viscosity values ranged between 7.4 and 106 MPa·s at different concentrations using a rotational viscometer. The variation is related to different TSS values [47].

3.2. Phytochemical Contents of Pomegranate Juice

3.2.1. Total Phenolics Content (TPC). The mean values of TPC of different pomegranate juice (PJ) samples from sweet and sour varieties of different regions of Northern Jordan are illustrated in Table 4. The phenolic content of sweet and sour pomegranate juice (PJ) samples ranged significantly between 105.8 and 238.63 mg GAE/g. The *A*2 juice shows the highest value, and the *D*2 juice is the lowest. The *D*1 and *K*1 juices as sour cultivars are higher in TPC than the sweet cultivars *D*2 and *K*2, but in the case of Ajloun samples, the sour sample (*A*1) has lower TPC than the sweet sample (*A*2). These findings were lower than the finding of [34], who found that total phenolics concentration ranged from 295.79 to 985.32 (mg GAE/g) with significant variation among twenty Iranian pomegranate varieties, and higher than the values of [48] who found that the TPC varied from 11.62 to 21.03 mg GAE/g for Parisian pomegranate cultivars. Zaouay et al. [32] reported that the total phenol range is 164.47–181.84 mg gallic acid/100 ml. In addition, the authors of [49] obtained a significantly varied range from 25.96 to 30.25 μg GAE/mg for the TPC of different pomegranate juice (PJ) samples while Derakhshan et al. [50] showed that the range was 12.4–23.8 mg GAE/g by using methanol for the extraction method.

That variation in the total phenolic contents of the pomegranate can be affected by the solvent used for extraction. The differences in TPC values depend on the fruit variety, development, maturation, agriculture, climate, and growing regions [51]. According to these results, the Jordanian PJ can be considered a good source of total phenolics and an important source of nutrients for human health.

Table 2: Color measurements (L^*, a^*, b^*, ΔE, and Chroma) for different pomegranate juice samples.

Varieties	Sample name	Color parameters of pomegranate juice				
		L^*	a^*	b^*	ΔE	Chroma
Sour	A1	58.14 ± 1.09^c	6.78 ± 1.15^c	14.11 ± 1.08^c	60.22 ± 0.75^c	15.66 ± 1.32^c
	D1	49.84 ± 0.85^d	17.88 ± 0.67^a	16.69 ± 0.42^b	55.53 ± 0.51^d	24.46 ± 0.74^a
	K1	57.43 ± 1.41^c	8.53 ± 0.48^b	10.69 ± 0.15^d	59.04 ± 1.33^c	13.68 ± 0.36^{de}
Sweet	A2	60.42 ± 0.11^b	3.47 ± 0.25^d	18.19 ± 0.68^a	63.19 ± 0.28^b	18.52 ± 0.67^b
	D2	64.21 ± 0.18^a	4.21 ± 0.14^d	11.83 ± 0.42^d	65.43 ± 0.16^a	12.56 ± 0.39^e
	K2	61.47 ± 0.63^b	2.39 ± 0.08^e	14.24 ± 0.99^c	63.15 ± 0.39^b	14.44 ± 0.98^{cd}

[#]All values are calculated as a wet basis and means of three replicates. [*]Means ± SD in the same column with the same letter are not significantly different ($P \leq 0.05$). [*]*A*1: Ajloun sour cultivar, *D*1: Deir Abi Said sour cultivar, *k*1: Kufur Soum sour cultivar, *A*2: Ajloun sweet cultivar, *D*2: Deir Abi Said sweet cultivar, *k*2: Kufur Soum sweet cultivar.

Table 3: The viscosity of different pomegranate juice samples.

Varieties	Sample name	Viscosity (mPa*) 25°C
Sour	*A*1	145.04 ± 2.08^e
	*D*1	235.34 ± 2.55^b
	*K*1	155.66 ± 1.41^d
Sweet	*A*2	234.67 ± 2.79^b
	D2	169.24 ± 1.21^c
	*K*2	294.47 ± 1.78^a

[#]All values are calculated as a wet basis and means of three replicates. [*]Means ± SD in the same column with the same letter are not significantly different ($P \leq 0.05$). [*]*A*1: Ajloun sour cultivar, *D*1: Deir Abi Said sour cultivar, *k*1: Kufur Soum sour cultivar, *A*2: Ajloun sweet cultivar, *D*2: Deir Abi Said sweet cultivar, *k*2: Kufur Soum sweet cultivar.

Table 4: Total phenolic content, total flavonoid content, antioxidant activity, and anthocyanin content of different pomegranate juice samples.

Varieties	Sample name	TPC (mg GAE/g)	TFC (mg catechin/g)	Antioxidant activity	Anthocyanin content (mg/g)
Sour	*A*1	161.2 ± 0.82^c	135.63 ± 1.69^d	47.97 ± 0.68^{bc}	6.63 ± 0.01^b
	*D*1	176.87 ± 1.51^b	157.76 ± 0.35^b	50.63 ± 0.55^a	11.02 ± 0.02^a
	*K*1	108.6 ± 1.05^d	184.9 ± 1.3^a	25.4 ± 1.68^d	4.84 ± 0.02^e
Sweet	*A*2	238.63 ± 2.13^a	135.53 ± 0.85^d	47.3 ± 1.49^c	6.05 ± 0.02^d
	*D*2	105.8 ± 1.14^e	149.5 ± 2.31^c	49.6 ± 1.32^{ab}	3.66 ± 0.01^f
	*K*2	107.66 ± 1.21^{de}	183.5 ± 0.46^a	20.66 ± 1.21^e	6.24 ± 0.02^c

[#]All values are calculated as a wet basis and means of three replicates. [*]Means ± SD in the same column with the same letter are not significantly different ($P \leq 0.05$). [*]*A*1: Ajloun sour cultivar, *D*1: Deir Abi Said sour cultivar, *k*1: Kufur Soum sour cultivar, *A*2: Ajloun sweet cultivar, *D*2: Deir Abi Said sweet cultivar, *k*2: Kufur Soum sweet cultivar.

3.2.2. Total Flavonoids Content. The mean values of TFC of different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan are shown in Table 4. The results of TFC of pomegranate juice (PJ) indicate that the highest TFC is found in *K*1 at 184.9 mg catechin/g, where *A*2 exhibits the lowest value at 135.53 mg catechin/g, with no significant difference at $P > 0.05$ between sweet and sour varieties for Ajloun (*A*1, *A*2) and Kufur Soum (*K*1, *K*2) samples, whereas for Deir Abi Said (*D*1) samples, the sour variety has 157.76 mg catechin/g TFC, and the sweet one (*D*2) has 149.5 mg catechin/g TFC. Fernandes et al. [33] studied nine different cultivars in Spain and found that the highest content of flavonoids in the Katirbasi cultivar was 189.4 mg QE/100 ml juice, whereas the lowest content was 20.8 mg QE/100 ml juice. Furthermore, the authors of [49] studied the TFC of juice samples, and the range is found to be within 0.92–1.78 μg QE/mg. Moreover, the authors of [48] by studying Persian pomegranate cultivars found that their TFC ranged from 0.84 to 2.14 mg catechin equivalents/g, which are much lower than our range. The juice samples studied by Derakhshan et al. [50] used methanol for extraction given 8.7–1.8 mg rutin/g for TFC. The flavonoid amount variations could be explained due to cultivar type, climate, growing region different maturity levels, genetic factors, and total phenolic contents [51–53].

3.2.3. Antioxidant Activity. The mean values of antioxidant activity of different pomegranate juice (PJ) samples from sweet and sour varieties of different regions of Northern Jordan are given in Table 4. The results indicate that the antioxidant activity varied between all pomegranate juice

Table 5: Individual phenolic contents (ppm) for different pomegranate juice samples.

Individual phenol	PJ					
	Sour			Sweet		
	*A*1	*D*1	*K*1	*A*2	*D*2	*K*2
Gallic acid	2.68 ± 0.83^{c}	3.37 ± 1.87^{c}	11.55 ± 1.52^{a}	4.44 ± 0.85^{bc}	2.94 ± 0.84^{c}	7.14 ± 1.41^{b}
3,4-Dihydroxyphenethyl alcohol	91.95 ± 1.38^{c}	101.53 ± 0.07^{a}	74.17 ± 0.07^{d}	95.33 ± 1.74^{b}	75.46 ± 1.07^{d}	93.16 ± 1.30^{bc}
Catechin	65.15 ± 1.07^{d}	88.22 ± 2.16^{b}	51.24 ± 1.36^{e}	75.52 ± 1.37^{c}	151.48 ± 1.89^{a}	41.96 ± 0.41^{f}
2-Hydroxyphenethyl alcohol	31.22 ± 1.42^{f}	352.86 ± 0.07^{a}	144.60 ± 0.14^{b}	103.46 ± 0.96^{d}	52.91 ± 0.47^{e}	118.82 ± 0.27^{c}
Chlorogenic acid	15.76 ± 0.86^{e}	30.03 ± 0.98^{b}	20.24 ± 1.09^{d}	44.89 ± 1.20^{a}	26.80 ± 0.39^{c}	17.18 ± 0.51^{e}
Vanillic acid	4.93 ± 1.24^{e}	13.43 ± 0.06^{d}	4.64 ± 0.23^{e}	59.83 ± 0.21^{b}	84.30 ± 0.07^{a}	31.75 ± 0.07^{c}
Epicatechin	81.98 ± 0.44^{a}	13.42 ± 1.94^{d}	47.12 ± 1.44^{b}	8.39 ± 0.92^{e}	23.23 ± 0.81^{c}	16.06 ± 0.98^{d}
Caffeic acid	16.76 ± 0.79^{e}	36.46 ± 0.09^{b}	23.03 ± 0.69^{d}	43.67 ± 0.87^{a}	34.35 ± 0.42^{c}	22.95 ± 1.20^{d}
Syringic acid	2.55 ± 0.79^{a}	0.68 ± 0.52^{bc}	0.34 ± 0.47^{c}	0.58 ± 0.26^{c}	2.25 ± 1.02^{ab}	1.39 ± 0.49^{abc}
P-Coumaric acid	22.33 ± 0.21^{b}	20.23 ± 0.26^{c}	24.58 ± 0.12^{a}	15.26 ± 0.76^{e}	16.95 ± 0.12^{d}	14.82 ± 0.47^{e}
Sinapic acid	26.29 ± 0.01^{b}	38.75 ± 0.12^{a}	18.58 ± 0.35^{c}	n.d	n.d	n.d
Ferulic acid	4.25 ± 0.87^{b}	8.92 ± 0.18^{a}	3.72 ± 0.82^{b}	4.99 ± 0.55^{b}	3.58 ± 0.13^{b}	4.09 ± 0.53^{b}
Rutin	n.d	68.71 ± 0.35^{a}	31.83 ± 0.35^{b}	12.32 ± 0.14^{d}	21.48 ± 0.28^{c}	n.d
Rosmarinic acid	27.49 ± 0.21^{a}	28.52 ± 0.41^{a}	n.d	n.d	27.47 ± 0.79^{a}	27.49 ± 0.71^{a}
Quercetin	2.94 ± 0.01^{a}	n.d	n.d	n.d	n.d	n.d
Thymol	n.d	n.d	n.d	n.d	n.d	n.d
Ellagic acid	85.09 ± 0.62^{d}	90.98 ± 0.85^{b}	88.02 ± 0.74^{c}	99.37 ± 0.14^{a}	86.25 ± 0.91^{cd}	84.84 ± 0.97^{d}
Delphinidin	809.15 ± 1.15^{b}	1667.61 ± 1.41^{a}	759.22 ± 0.56^{c}	627.81 ± 1.05^{d}	213.76 ± 1.04^{f}	267.03 ± 1.43^{e}

[#]All values are calculated as a wet basis and means of three replicates. *Means ± SD in the same row with the same letter are not significantly different ($P \leq 0.05$). **A*1: Ajloun sour cultivar, *D*1: Deir Abi Said sour cultivar, *k*1: Kufur Soum sour cultivar, *A*2: Ajloun sweet cultivar, *D*2: Deir Abi Said sweet cultivar, *k*2: Kufur Soum sweet cultivar.

(PJ) samples and ranged from 20.66% to 50.63%, where the highest value is for the *D*1 sample, and the lowest is for the *K*2. In general, the sour cultivars from Ajloun (*A*1), Deir Abi Said (*D*1), and Kufur Soum (*K*1) samples exhibit slightly higher antioxidant activity than sweet cultivars *A*2, *D*2, and *K*2, respectively. Similar results were obtained by Akhavan et al. [54] who studied that the DPPH in Iranian pomegranate juice (PJ) samples obtained from arils and found that the range of antioxidant content for the samples was 18.8%–46.8% which agrees with our finding. In addition, Tehranifar et al. [34] found that the differences in antioxidant activity among the studied pomegranate cultivars were statistically significant, and the values ranged from 15.59% to 40.72%. The difference in antioxidant activity of pomegranate can be related to the ascorbic acid content, and total phenolic compounds [2].

3.2.4. Anthocyanins Content. The mean values of anthocyanin content of different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan are given in Table 4. The results of pomegranate juice (PJ) samples for anthocyanins content exhibit a significantly different range from 3.66 to 11.02 mg/g, the highest amount was for *D*1, and *D*2 has the lowest content. *A*1 and *D*1 sour samples have higher anthocyanins content than *A*2 and *D*2 sweet juice samples, respectively, and *K*2 was higher than *K*1. Tehranifar et al. [34] found that the highest amount of total anthocyanin among twenty Iranian pomegranate cultivars is (30.11 mg cy-3-glu 100 g-1). Additionally, Hasnaoui et al., 2011 found that the total anthocyanin content ranged from 9 to 115 mg/L juice among the studied pomegranate varieties. Akhavan et al. [54] found that the anthocyanins content ranged between 1.8 and 175.4 mg/L. Anthocyanin content varies among varied species or cultivars and can be affected by genetic makeup, light, temperature, and agronomic factors [54].

3.2.5. Individual Phenolic Contents of Pomegranate Juice. Individual phenolic contents (ppm) for different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan are displayed in Table 5. The results show that the individual phenolic contents in the pomegranate juice (PJ) samples are delphinidin (213.76–1667.61 ppm) as the highest content, and 3,4-dihydroxyphenethyl alcohol (74.17–101.53 ppm), ellagic acid (84.84–99.37 ppm), 2-hydroxyphenethyl alcohol (31.22–352.86 ppm), catechin (41.96–151.48 ppm), epicatechin (8.39–81.98 ppm), vanillic acid (4.64–84.30 ppm), caffeic acid (16.76–43.67 ppm), P-coumaric acid (14.82–24.58 ppm), chlorogenic acid (15.76-44.89 ppm), gallic acid (2.68–11.55 ppm), ferulic acid (3.58–8.92 ppm), and syringic acid (0.34–2.55 ppm) were prominent and have significant contents. Thymol as an individual phenol is not found in any sample of pomegranate juice (PJ). The presence of sinapic acid is missing in *A*1, *D*2, and *K*2 while the highest content is found in *D*1 (38.75 ppm). The *A*1 and *K*2 have no rutin content, but it was 68.71 ppm in *D*1 (the highest), and *A*2 12.32 ppm (the lowest). The *A*2 and *K*1 missed the presence of rosmarinic acid, and quercetin was missed in all PJ except in A1 which shows 2.94 ppm. Akhavan et al. [54] studied the contents of individual phenolic compounds in pomegranate juice (PJ) of ten Iranian pomegranate cultivars and found that the content of ellagic acid ranged from 17.4 to 155.9 mg/L. Furthermore, Alsataf et al. [55] found that the

Table 6: The inhibitory activity of alpha-amylase and ACE of different pomegranate juice samples.

Varieties	Sample name	Inhibitory activity (%) of pomegranate juice	
		Alpha-amylase inhibitory activity (%)	ACE inhibitory activity (%)
Sour	*A*1	439.84 ± 2.33^{a}	65.39 ± 2.27^{B}
	*D*1	403.77 ± 2.08^{b}	53.75 ± 0.60^{c}
	*K*1	133.34 ± 2.21^{e}	45.65 ± 0.90^{d}
Sweet	*A*2	263.23 ± 1.68^{c}	47.68 ± 0.56^{D}
	*D*2	173.17 ± 1.88^{d}	91.03 ± 2.01^{a}
	*K*2	64.95 ± 2.33^{f}	66.37 ± 0.00^{b}

#All values are calculated as a wet basis and means of three replicates. *Means ± SD in the same column with the same letter are not significantly different ($P \leq 0.05$). *A1: Ajloun sour cultivar, *D*1: Deir Abi Said sour cultivar, *k*1: Kufur Soum sour cultivar, *A*2: Ajloun sweet cultivar, *D*2: Deir Abi Said sweet cultivar, *k*2: Kufur Soum sweet cultivar.

gallic acid content is 2.5 μg/g, catechin is 19.0 μg/g, ellagic acid is 26.5 μg/g, and vanillic acid is 2.1 μg/g for pomegranate juice (PJ) among the other pomegranate tissues. The difference in phenol component and content among juice samples may be related to the agronomic and genetic factors or environment.

3.3. Enzymatic Assay

3.3.1. Alpha-Amylase Inhibitory Activity of Pomegranate Juice. The inhibitory activity of alpha-amylase of different pomegranate juice (PJ) samples from sweet and sour varieties and different regions of Northern Jordan is shown in Table 6. The results exhibit that the alpha-amylase inhibitory activity of pomegranate juice (PJ) samples significantly varied from 64.95% to 439.84%. *A*1 has the strongest inhibitory activity of alpha-amylase, and *K*2 was the weakest. The *A*1 (439.8%), *D*1 (403.7%), and *K*1 (133.3%), which are the sour cultivars, show the highest activity for alpha-amylase inhibition among the sweet cultivars *A*2 (263.2%), *D*2 (173.15%), and *K*2 (64.95%).

3.3.2. ACE Inhibitory Activity of Pomegranate Juice. The inhibitory activity of ACE of different pomegranate juice (PJ) samples from sweet and sour varieties of different regions of Northern Jordan is shown in Table 6. The results show that the ACE inhibitory activity of pomegranate juice (PJ) samples ranged from 45.65% to 91.03%. The highest inhibition activity against ACE is shown by sweet variety *D*2 grown in Dair Abi Said, whereas Kufur Soum sour pomegranate exhibits the lowest ACE inhibitory activity, and this value has no significant differences with Ajloun sweet pomegranate *A*2 (47.68%). Also, *A*1 (66.39%) and *K*2 (66.37%) show no significant differences. Significant differences are found between the sour variety and sweet ones in the same region of growing, where the sour Dair Abi Said and Kufur Soum pomegranate (*D*1 and *K*1) have a lower ACE inhibitory activity than sweet ones *D*2 and *K*2, respectively, but the sour Ajloun pomegranate shows higher activity than the sweet Ajloun pomegranate.

4. Conclusions

We conclude in this study of physiochemical and nutraceutical properties of sweet and sour pomegranate juice (PJ) from different regions in northern Jorden (Ajloun, Dair Abi Said, and Kufur Soum) that the sweet pomegranate juice (PJ) has higher TSS, fructose, and glucose contents than the sour ones, thus the sweet is more appropriate for juice consumption, while the sour cultivars were more acidic, redder, and darker than the sweet ones. Also, sour pomegranate juice (PJ) is higher in TPC, anthocyanin content, DPPH activity, and activity for alpha-amylase inhibition which indicates a good health property for the sour PJ. The alpha-amylase and ACE inhibitory activity of both pomegranate juice (PJ) cultivars exhibit good values which reflect the good health properties of the PJ in general. Pomegranate juice (PJ) has high HMF content in general. Also, it has a high content of ellagic acid, delphinidin, 3,4-dihydroxyphenethyl alcohol, 2-hydroxyphenethyl alcohol, catechin, epicatechin, vanillic acid, caffeic acid, P-coumaric acid, chlorogenic acid, gallic acid, ferulic acid, and syringic acid.

Data Availability

Data are available upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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