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Effect of Cold Storage of Various Pomegranate Cultivars Fruit Juices on Health Promoting Compounds and Their Activities

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Abstract In this study, the total anthocyanins, ascorbic acid and phenolic content in sixteen different pomegranate cultivars are reported as 2.6-11.6, 3.2-13.6 and 956.5-2450.3 mg/100 g, respectively. Total soluble solid content varied between 12.1^0-18.1^0Brix, whereas pH and % titratable acidity values were within the range of 2.87-4.65, and 0.27-3.39, respectively. Total sugar values were ranged 9.6-22.1 g/100g. The total antioxidant capacity of pomegranate cultivars were found to be 2.54-12.4 (ABTS), 5.5-36.2 (DPPH) and 3.9-26.7 (FRAP) µmol TE/g fw. A high correlation between total antioxidant capacity and total anthocyanins (r^2= 0.94), ascorbic acid (r^2=0.75) and phenolic content (r^2=0.94) was observed. Moreover, total anthocyanin, ascorbic acid, total phenolic contents and the antioxidant capacity of four pomegranate cultivars were monitored at the refrigeration temperature for two months. It was shown that ascorbic acid concentrations of the samples significantly decreased during storage, while there was no degradation could be observed in total phenolic concentrations. The degradation of total anthocyanins of Mersin 23 and Mugla 1267 cultivars during storage resulted in a dramatic decrease in the total antioxidant capacity of these samples from 12.36 to 5.6 (µmol TE/g fw) and 9.9 to 5.3 (µmol TE/g fw), respectively. These results demonstrated that ascorbic acid, total anthocyanins and total phenolic compounds are the main contributors for initial total antioxidant capacity and the difference between the cultivars are significant. However, degradation of ascorbic acid was shown to have no significant effect on the total antioxidant capacity unlike the total anthocyanins and phenolic compounds.

Keywords: *pomegranate, antioxidant capacity, phenolic content*

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1. Introduction

Free radicals are molecules with unpaired electrons that are formed via cellular metabolic processes or by environmental stresses such as air pollution, X-ray, or exogenous chemicals [1,2,3]. Due to their unpaired electrons, these compounds are known to be unstable and very reactive. It has been demonstrated that free radicals can lead to DNA damage, protein oxidation and lipid peroxidation in living cells [1,4,5]. The main reason of this damage is the oxidative stress, which is the disruption of the balance between free radicals and antioxidants in the cell [6]. In fact, free radicals such as superoxide anion radical, hydrogen peroxide, hydroxyl radical or nitric oxide radical can lead to oxidative stress that can cause ageing and various diseases including atherosclerosis, liver disease, inflammatory diseases or cancer [2,7,8,9]. Antioxidants play a crucial role in prevention or mitigation of the adverse effects of oxidative stress. Mechanisms by which antioxidants act range from free radical scavenging to enzymatic inhibition or metal chelating [10,11,12]. Although, antioxidants are naturally produced in human body, input of exogenous antioxidants is critical since they work synergistically with the endogenous antioxidants to control the oxidative stress [13,14]. Therefore, antioxidant capacity of numerous alternative exogenous sources have been carefully investigated [11,15,16,17,18]. For example, foods containing vitamin C or E have been implicated to show antioxidant effects since these compounds are capable of neutralizing lipid peroxyl, alkoxyl or hydroxyl radicals, which are normally deleterious to living cells [11]. Nevertheless, despite the copious studies on antioxidant capacities of fruits and vegetables, many other dietary options remain to be studied. Here, we focus on the potential antioxidant components of 16 different pomegranate varieties grown in Turkey. We also address the effect of the cold storage on the antioxidant capacity of this fruit.

Pomegranate (*Punica granatum* L.) is a round fruit with a thick reddish rind in which there are edible arils composed of 80% (w/w) juice and 20% (w/w) seeds [19].

Countries that experience Mediterranean climate such as Afghanistan, Japan, India, or the United States (California) are suitable for cultivation of this fruit [19,20,21,22]. Turkey is, also, well suited for growth of several different types of pomegranate varieties thanks to its climate. Pomegranate has become increasingly popular due to its high content of phytochemicals that might differ depending on the cultivar, growing region, maturity and climate conditions [23,24]. It has been shown that the consumption of pomegranate products reduces the risk of cancer, LDL oxidation, inflammatory and heart diseases [25,26,27,28]. In addition, pomegranate derived products have been used for the treatment of stomachache, inflammation, fever, bronchitis, diarrhea and dysentery [29,30]. An increasing number of various pomegranate supplements and products are also available in the market [31]. Despite the growing attention in red fruit juices due to their high content of anthocyanin and antioxidant compounds [15,32,33,34], few pomegranate species have been studies in terms of their physicochemical characteristics. Therefore, in this current study, we have focused on the analysis and comparison of the physicochemical properties and antioxidant activity of sixteen pomegranate cultivars in Turkey. The correlation of these properties with antioxidant activity was demonstrated to understand their contribution to the total antioxidant capacity. In addition, the compounds associated with antioxidant capacity have been monitored within 2 months at the refrigeration temperature to further explore their impact on the antioxidant activity of pomegranate.

2. Materials and Methods

Sixteen fresh pomegranate cultivars harvested from different regions of Turkey were examined in this study. The varieties and growth regions of the samples are given in Table 1. Fruit samples were processed to fruit juices in laboratory scale and stored at -25 °C prior to analysis.

Table 1. Regional Distribution and Descriptive Values of Pomegranate Samples

Variety	Region	^{0}BRIX	pH	%TA
1267	Mugla	17.0±0.2	4.65±0.04	0.32±0.02
Katirbasi	Mugla	15.1±0.2	3.16±0.01	1.09±0.01
3N26	Mersin	12.1±0.1	4.52±0.02	0.27±0.02
23	Mersin	15.9±0.3	3.12±0.06	1.66±0.04
Hicaz	Mersin	12.9±0.2	3.35±0.01	0.85±0.04
1264	Izmir	14.3±0.4	3.09±0.01	1.78±0.01
Hicaz	Antalya	15.1±0.1	3.45±0.02	1.29±0.01
Ernar	Antalya	15.4±0.2	4.23±0.02	0.46±0.07
1265	Antalya	14.1±0.2	4.23±0.05	0.33±0.07
Tirbey	Antalya	14.1±0.0	3.04±0.02	1.17±0.01
Wonderful	Mersin	18.1±0.2	3.78±0.04	0.79±0.01
Silifke Asisi	Mersin	14.8±0.5	2.87±0.04	3.39±0.01
33N09	Mersin	16.7±0.1	2.99±0.03	1.45±0.04
01N07	Izmir	14.0±0.1	3.65±0.01	0.41±0.05
1479	Izmir	16.3±0.5	3.87±0.02	0.40±0.01
1265	Izmir	16.3±0.1	3.94±0.02	0.29±0.06

%TA: Titratable acidity**.** Values shown are means±S.D. of triplicate measurements.

2.1. Chemical Composition Analysis

Water-soluble solids content (Brix), pH and titratable acidity of samples were determined in order to describe the original characteristics of the fruits. Water-soluble solid contents were determined by Bausch & Lomb, USA, refractometer and expressed as ^{0}Brix at 20 °C [16]. The pH was measured with a digital pH meter (601, Herisau, Switzerland). Following the dilution of 2 ml aliquot of sample with 20 ml distilled water, the total titratable acidity was determined by titrating the sample with 0.1 M NaOH until the pH reached 8.1 and expressed as grams of citric acid per liter [17].

Total sugars were determined as described by Ranganna [35]. Ascorbic acid content was measured by the method described by Ruck [36] with minor modifications and the results were expressed as mg per 100g fw of juice. Total anthocyanins were determined by pH differential method [37], and the results were expressed as mg cyaniding-3-glucoside 100 g of juice.

2.1.1. Total Phenolic Assay

Total phenolic content was determined with the Folin-Ciocalteu reagent according to the procedure described by Singleton and Rossi [38]. Briefly, 0.50 mL of the diluted sample was reacted with 2.5 mL of 0.2 mol/L Folin-Ciocalteu reagent for 4 min, then 2 mL saturated sodium carbonate solution (about 75 g/L) was added into the reaction mixture. The absorbance was measured at 760 nm after incubation at room temperature for 2 h. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent per grams fresh weight (mg GAE/g fw).

2.1.2. Antioxidant Activity

The TEAC (Trolox Equivalent Antioxidant Capacity) assay was carried out according to the method of Re *et al* [39]. Firstly, to produce the radical cation $ABTS^{\bullet+}$, 7 mmol/L ABTS salt and 2.45 mmol/L potassium persulfate were mixed in a volume ratio of 1:1. Next, the reaction mixture was allowed to stay in the dark for 16 h at room temperature and was used within two days of preparation. The $ABTS^{\bullet+}$ radical solution was diluted with ethanol to an absorbance of 0.7 ±0.05 at 734 nm. All samples were approximately diluted to provide 20–80% inhibition of the blank absorbance. One hundred microliters of the diluted sample was mixed with 3.8 mL $ABTS^{\bullet+}$ working solution, and the reaction mixture was left at room temperature to react for 6 min. Then, the absorbance at 734 nm was measured using the ultraviolet spectrophotometer. Trolox solution was used as a reference standard, and the results were expressed as µmoles of Trolox per gram of sample (µmol TE/g fw). The DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay was carried out according to the method of Brandwilliams et al. [40] with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and was stored at -20 ^{0}C. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1±0.02 units at 515 nm. Samples (150 mL) were allowed to react with 2850 mL of the DPPH solution for 24 h in the dark. Then the absorbance was measured at 515 nm. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in µmoles of Trolox per

gram of fresh sample (µmol TE/g fw). Ferric Reducing Antioxidant Power (FRAP) was employed according to the method of Benzie and Strain [41]. A portion of an aqueous 10 mmol/l solution of TPTZ reagent in 40 mmol/l HCl was mixed with the same volume of 20 mmol/l FeCl3·6 H_2O and ten times higher volume of acetate buffer of pH 3.6 (3.1 g sodium acetate and 16 ml acetic acid per litre). The mixture was incubated at 37°C for five minutes. A portion (900 µl) of the Fe^{3+}-TPTZ mixture and the sample or water (for blank) were adjusted to 1 mL with deionized water, incubated for 30 min, and the absorbance at 593 nm was measured.

2.1.3. Statistical Analysis

Analyses were performed in 3 parallel trials and the descriptive statistics were studied employing Minitab 16.0. One-way ANOVA was performed and Tukey test was used for multiple comparisons of the groups at 0.05 significance level.

3. Results and Discussion

Brix values of pomegranate cultivars were found within the range of 12.1-18.1 while % titratable acidity (TA) of the samples were between 0.27-3.39. pH values varied from 2.87 to 4.65. A wide range of brix and % TA values are expected due to the differences in pomegranate varieties studied. These findings are also consistent with other studies [42,43,44]. These results are important for the understanding of the characteristics of different pomegranate cultivars. Especially, different brix and TA value might result in different solid content that might contribute to the antioxidant capacity. The brix, pH and % TA values were shown in Table 2.

Table 2. Antioxidant Capacity of Pomegranate Varieties

Variety	Region	ABTS (µmol TE/g fw)	DPPH (µmol TE/g fw)	FRAP (µmol TE/g fw)
1267	Mugla	9.95±0.2	27.61±1.2	17.65±0.5
Katirbasi	Mugla	6.65±0.4	21.6±0.4	13.75±0.2
3N26	Mersin	7.65±0.1	16.7±0.1	13.45±0.6
23	Mersin	12.36±0.2	36.26±0.7	26.78±0.7
H	Mersin	3.45±0.1	9.96±0.5	5.64±0.1
1264	Izmir	2.63±0.2	5.54±0.2	3.90±0.1
Hicaz	Antalya	4.98±0.1	14.54±0.1	8.86±0.4
Ernar	Antalya	3.65±0.1	7.65±0.3	5.02±0.2
1265	Antalya	10.65±0.5	30.42±0.6	18.76±0.2
Tirbey	Antalya	12.43±0.5	34.34±0.8	22.45±0.6
Wonderful	Mersin	11.23±0.6	29.65±0.6	22.23±0.7
Silifke Asisi	Mersin	5.42±0.6	12.45±0.2	12.34±0.1
33N09	Mersin	3.65±0.1	12.46±0.2	5.64±0.2
01N07	Izmir	2.87±0.1	6.34±0.1	4.16±0.2
1479	Izmir	2.54±0.1	7.54±0.3	4.45±0.5
1265	Izmir	3.89±0.1	7.45±0.4	5.65±0.6

*Values shown represent means ±S.D of triplicate measurements.

Total sugars, total anthocyanins, ascorbic acid and total phenolics values of sixteen pomegranate cultivars are shown in Table 3. Total sugars of pomegranate cultivars varied from 9.6 to 22.1 g/100g, while ascorbic acid values were between 2.9 and 13.6 mg/ 100g. Total anthocyanins of pomegranate cultivars were found with a range of 2.6-11.6 mg/100g. The Mersin 23 cultivar showed the highest total anthocyanin value (11.6 mg/100g), followed by Mugla 1267 (10.9 mg/100g) and Antalya 1265 (10.7mg/100g). Although these values vary in a wide rage, they are consistent with other studies [45,46,47].

Table 3. Total Sugars (TS), Total Anthocyanins (TA), Ascorbic Acid (A) and Total Phenolics (TP) of Juices From Sixteen Pomegranate Cultivars

Variety	Region	TS (g/100 g fw)	TAs (mg/100 g fw)	A (mg/100 g fw)	TPs (mg/100 g fw)
1267	Mugla	16.2^{a}	10.9^{a}	11.5^{a}	2200.7^{a}
Katirbasi	Mugla	16.7^{b}	6.9^{b}	5.6^{b}	1700.4^{b}
3N26	Mersin	17.6^{c}	7.4^{c}	9.6^{c}	1803.3^{c}
23	Mersin	18.4^{d}	11.6^{d}	12.3^{d}	2450.3^{d}
H	Mersin	13.4^{e}	4.2^{e}	5.2^{e}	1235.4^{e}
1264	Izmir	12.6^{f}	4.1^{e}	5.9^{f}	1200.3^{f}
Hicaz	Antalya	11.7^{g}	4.6^{f}	5.8^{f}	1233.3^{g}
Ernar	Antalya	17.2^{h}	4.3^{e}	5.7^{f}	1254.6^{h}
1265	Antalya	13.4^{e}	10.7^{a}	13.6^{f}	2367.9^{i}
Tirbey	Antalya	12.4^{f}	10.2^{g}	9.5^{c}	2306.9^{j}
Wonderful	Mersin	15.8^{i}	10.7^{a}	9.9^{g}	2352.9^{k}
Silifke Asisi	Mersin	15.7^{i}	5.9^{h}	4.2^{h}	1545.9^{l}
33N09	Mersin	19.2^{j}	3.6^{i}	2.9^{i}	1208.9^{m}
01N07	Izmir	22.1^{k}	3.7^{i}	4.5^{j}	1059.2^{n}
1479	Izmir	9.6^{l}	3.1^{j}	4.9^{k}	1232.2^{g}
1265	Izmir	11.2^{m}	2.6^{k}	3.2^{l}	956.5^{o}

* Values (a-o) within a column with the same letter are not significantly different (p = 0.05).

The total phenolic content of the pomegranate cultivars was found to fall within a range of 2450.3-956.5 mg/100g. While Mersin 23, Antalya 1265 and Mersin Wonderful had the highest total phenolic content (2450.3, 2367.9 and 2352.9 mg/100g, respectively), Izmir 1265 had the lowest total phenolic content (956.5 mg/100g). Similar to the extensive research conducted on phenolic content of pomegranate cultivars [48,49,50,51,52] we found that the phenolic content of our samples varied depending on the region and variety. Specifically, the varieties grown in Izmir have lower phenolic content (956.5-1232 mg/100g) compared to those grown in Antalya, Mersin and Mugla.

The differences in anthocyanins, total phenolics, brix and percent titratable acidity is a likely result of the different cultivars analyzed. Disparities between cultivars analyzed here is mostly influenced by the climate as well as the salinity of the irrigation water. Borochov- Neori *et al.* [53] has indicated that temperature significantly affects the antioxidant capacity of pomegranate through modification of anthocyanin content. Despite the pomegranate cultivars analyzed here were subjected to similar climates, temperature fluctuations during handling might change their composition. Likewise, salinity of the irrigation water has been reported to affect the accumulation of phenolic compounds in different pomegranate cultivars [53].

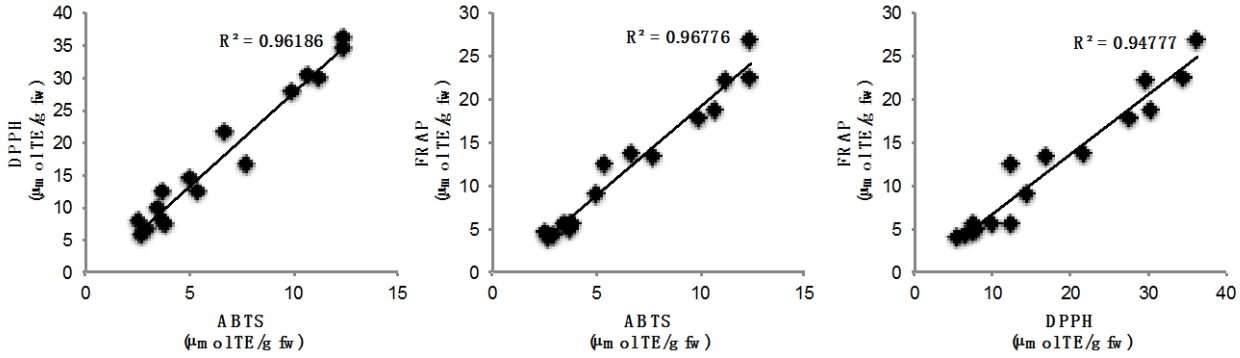

Figure 1. Correlation of ABTS, DPPH and FRAP assays for total antioxidant capacity measurement

ABTS, DPPH and FRAP assays were used to determine the total antioxidant capacity of pomegranate cultivars and the results were between 2.54-12.4, 5.5-36.2 and 3.9-26.7 µmol TE/g fw, respectively (Table 2). According to the results, Antalya Tirbey and Mersin 23 showed the highest antioxidant capacity, followed by Mersin Wonderful and Antalya 1265, whereas Izmir 1264, Izmir 1479 and Izmir 01N07 varieties had the lowest antioxidant capacity. The correlation coefficient between the assays, ABTS-DPPH, ABTS-FRAP and DPPH- FRAP, were found to be 0.96, 0.96 and 0.94, respectively (Figure 1). High correlation between the assays suggest they were consistent in total antioxidant capacity measurement. However, our results are slightly higher than [54,55] the previous studies.

The correlation of total antioxidant capacity with pH, ^{0}BRIX, titratable acidity, total sugars, ascorbic acid, total anthocyanins and total phenolics are shown in Table 4. According to the results, high linear correlation between total antioxidant capacity and total anthocyanins (r^2= 0.94), ascorbic acid (r^2=0.75), and phenolic content (r^2=0.94) were observed. These results demonstrated that total anthocyanins, ascorbic acid and phenolic compounds are the main contributors for total antioxidant capacity. Many studies have been reported on the correlation of antioxidant capacity with these values [16,33,48,56,57,58]. Correlation of antioxidant capacity with anthocyanins and phenolic content determined here are similar to these studies while our regression analysis yielded slightly lower correlation with ascorbic acid.

Table 4. Correlation Coefficients (r) of pH, ^{0}BRIX, titratable acidity (TA), total sugars (TS), ascorbic acid (A), total anthocyanins (TAs), total phenolics (TPs) and antioxidant activity (AA) of juice from sixteen pomegranate cultivars

Variable	pH	^{0}BRIX	TA	TS	A	TAs	TPs
^{0}BRIX	0.07^{NS}						
TA	-0.69^{S}	0.001^{NS}					
TS	0.034^{NS}	0.001^{NS}	0.001^{NS}				
A	0.15^{NS}	0.001^{NS}	-0.05^{NS}	0.002^{NS}			
TAs	0.027^{NS}	0.019^{NS}	0.001^{NS}	0.021^{NS}	0.84^{S}		
TPs	0.019^{NS}	0.019^{NS}	0.001^{NS}	-0.09^{NS}	0.83^{S}	0.98^{S}	
AA	0.012^{NS}	0.021^{NS}	0.001^{NS}	-0.06^{NS}	0.75^{S}	0.94^{S}	0.94^{S}

^{NS}, ^{S} Not significant or significant at P=0.05, respectively.

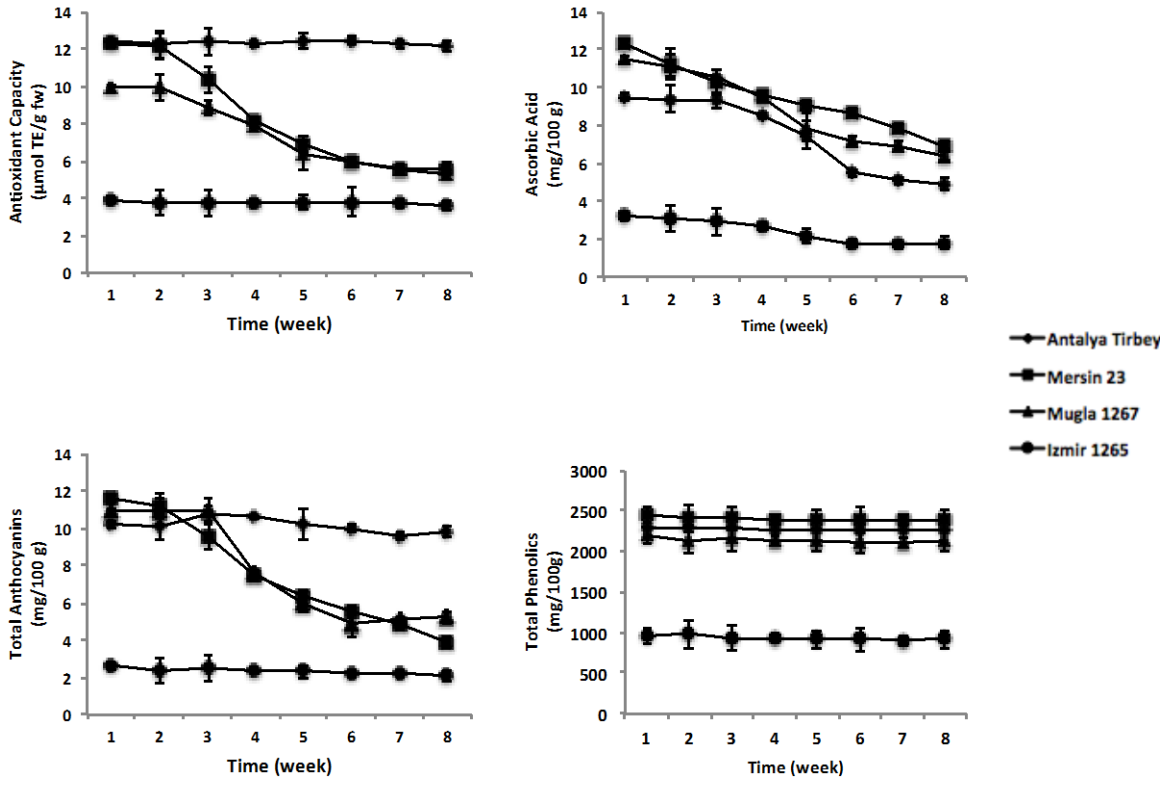

Figure 2. Refrigeration effect on total anthocyanins, phenolics, antioxidant capacity and ascorbic acid values of pomegranate samples for 2 months

The degradation of total phenolics, anthocyanins and ascorbic acid values of Antalya Tirbey, Mersin 23, Mugla 1267 and Izmir 1265 were monitored at refrigerator temperature for two months (Figure 2). The total phenolics concentration of the samples did not significantly change during storage. Stability of total soluble phenolics content was reported by others as well [53]. The ascorbic acid concentration of Antalya Tirbey, Mersin 23, Mugla 1267 and Izmir 1265 decreased from 9.5 to 4.9, 12.3 to 6.9, 11.5 to 6.4 and 3.2 to 1.8, respectively. The total anthocyanin concentration of Mersin 23 and Mugla 1267 cultivars decreased from 11.6 to 3.9 and 10.9 to 5.2 mg/100g, respectively, while the Antalya Tirbey and Izmir 1267 cultivars did not experience significant reduction of total anthocyanins. These results are similar to other studies [23,59]. We also showed that the total antioxidant capacity of Mersin 23 and Mugla 1267 cultivars decreased from 12.36 to 5.6 and 9.9 to 5.3 µmol TE/g fw (ABTS), respectively. However, antioxidant capacity did not significantly change for Antalya Tirbey and Izmir 1267 cultivars. These results demonstrated that the decrease in total anthocyanins had a significant effect on total antioxidant capacity. Moreover, reduced ascorbic acid content did not significantly modulate the total antioxidant capacity despite the high initial correlation between these values.

4. Conclusions

In this study, we investigated the valuable antioxidant compounds of 16 pomegranate cultivars grown in Turkey. The careful compositional analysis strongly recommended that total anthocyanins, phenolics and ascorbic acid are crucial in determining the antioxidant capacity of pomegranate. However, storage of these cultivars at the refrigeration temperature for 2 months interestingly demonstrated that anthocyanins and phenolics had much higher impact on antioxidant capacity than the ascorbic acid. The results of this study can potentially benefit the control of storage during handling and distribution of pomegranate, leading to improved quality of this fruit in the market.

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