Determination of Bioactive Properties, Phenolic Compounds and Mineral Contents of Boiled Fruit Juice Types

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ABSTRACT: *Antioxidant activity, total phenol, total flavonoid, and anthocyanin contents, phenolic compounds, and macro-and microelement contents of concentrated fruit pulp (boiled juices) were determined. While antioxidant activities of marmalade samples varied between 99.6% (Mahaleb) and 117.2% (Cornus), total phenolics of boiled juices changed between 103.52 (Mahaleb) mg GAE/100g and 126.63 mg GAE/100g (Cornus). In addition, while gallic acid contents of marmalade (boiled juices) extracts are determined between 0.91 (Mahaleb) and 6.27 mg/100g (Cornus), (+)-Catechin contents of marmalade samples varied between 3.29 mg/100g (Mahaleb) and 9.61 mg/100g (Cornus). K and P contents of boiled juices were found between 271.37 mg/Kg(Rosa) and 8004.00 mg/kg (Mahaleb) to 55.12 (Cornus) and 415.12 (Mahaleb) mg/kg, respectively. Fe contents of boiled juices changed between 12.97 (Rosa) and 16.92 mg/kg (Cornus). Among samples, the highest Cu (1.75 mg/kg), Mn (1.08 mg/kg), and Zn (7.62 mg/kg) contents were found in mahaleb boiled juice. According to the results, boiled juice samples are rich in bioactive compounds, phenolics, and some minerals (Ca, K, Mg, Na, and P).*

KEYWORDS: *Boiled juice; Antioxidant activity; Anthocyanins; Phenolic compounds; Minerals; ICP-AES.*

INTRODUCTION

Rose hip is a plant that member of the Rosaceae family. The fruits are rarely eaten directly as fresh and dried products. There are several conservation foods used predominantly in the manufacture of canning such as rosehip pulp. *Cornus* and Cornelian cherry fruits have significant amounts of bioactive substances including anthocyanins [1]. Among medicinal plants in Turkey, *Prunus mahaleb* L. is commonly known as mahlep, mehlep, idris ağacı, in Turkey and its kernels are used for medicinal and nutritional purposes, and it is a deciduous small tree or large perennial shrub and found in the Mediterranean region, central and eastern Europe,

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northern Africa, western and central Asia [2]. Hawthorn (*Crataegus* spp) is an endemic member of the Rosaceae family, and its fruit possesses potent antioxidant and free radical scavenging activities, due to the presence of different, bioactive compounds, such as epicatechin, hyperoside, and chlorogenic acid [3,4]. Among many biologically significant properties of anthocyanins, their antioxidant capacity is of major consideration. Anthocyanins are known to play an important role in the prevention of degenerative neuronal disorders, cardiovascular illnesses, cancer, and diabetes [5-7]. Loss of bioactive phenolics may occur during thermal processing and the storage period of processed products such as jams, juices, and puree [8,9]. *Cendrowski et al.* [9] explained that the reduction in the number of bacterial cells in matrices imitating protein food depended on the concentration of the aqueous extract used, and at none of the concentrations used was a complete inhibition of bacterial growth observed. *Hendrich et al.* [10] showed that most of these extracts had effective antioxidants and free radical scavengers, possess reasonable potential anti-inflammatory activity, reduced the adhesion of *E. coli* to uroepithelial cells, and reduced the ability of these bacteria to form biofilm. There is limited information about the antioxidant activity, total phenols, phenolic compounds, and mineral contents of several fruit-boiled juice samples. However, the current study was to determine the effect of boiled juice types on moisture contents, bioactive properties, phenolic compounds, and mineral contents of marmalade samples.

EXPERIMENTAL SECTION

Material

The fruits of *Rosa canina* (rose hip), *Cornus mas* (Cornus), *Mahaleb cerasus* (Mahaleb), and *Crataegus* spp (hawtorn) for concentrated fruit pulp (boiled juice) production were provided in Tokat province, Turkey. The ripened fruits were cleaned in order to remove leaves, flower, and stem, and then washed with clear topwater. The authentication of the plant (reference) and botanical name was identified in the department of horticulture, Faculty of Agriculture, Selçuk University. They were added into hot water in a ratio of 1:1.5 (w/v) at 70 °C for 6 h. After pressing, their pulps were filtered using a sieve with 0.5 cm diameter. After cooking, saccharose was added to pulp at a level of 1:1.5(w/w). After filling into a jar (0.5L), closed hermetically. They were kept at +4 ºC still analyzing. Moisture contents were measured at 100±5 ºC [11].

Methods

Total phenolic content and antioxidant activity

Total phenol contents of boiled juice extracts were determined by using Folin-Ciocalteu (FC) reagent as described by *Yoo et al.* [12]. FC (1 mL) was added and mixed for 5 min, following the addition of 10 mL of 7.5% Na_2CO_3. The solution in the tubes was mixed again and the final volume was adjusted to 25 mL with deionized water. At the end of 1 h, total phenolic content was determined at a wavelength of 750 nm in a spectrophotometer. A calibration curve was made using gallic acid (0-200 mg/mL) as the standard. The results are given as mg gallic acid equivalent (GAE)/100 g of fresh weight. The antioxidant activity values were determined by using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to the method reported by *Lee et al.* [13], respectively. The extract (0.5g) was mixed with 2 mL of a methanolic solution of DPPH. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min and absorbance was recorded at 517 nm using a spectrophotometer.

Determination of Anthocyanins

For anthocyanin analysis, about 0.5 g of Fresh Weight (FW) were mixed in a solution containing propanol, chlorhydric acid, and water (18:1:81). After the homogenates were boiled in a water bath for 3 min, it was kept in a dark place at room temperature for 24 h. Then, the supernatants were centrifuged at 6500 rpm for 10 min. Absorbance was measured at 535 and 650 nm in a spectrophotometer. The absorbance value was calculated and corrected by the following formula [14]:

$$A = A_{535} - A_{650}$$

Determinetion of flavonoid

Total flavonoids contents of boiled juice samples were estimated according to *Dewanto et al.* [15]. The extract (1 mL) was mixed with 0.3 mL of 7.5% $NaNO_2$, 0.3 mL of $AlCl_3$, and 2 mL of NaOH, respectively. The absorbance of the mixture was recorded at 510 nm with a spectrophotometer. The flavonoid content was expressed as mg Catechol Equivalents (CE) per g of dry weight (mg CE/g DW).

Determination of phenolic compounds

Phenolic compounds were extracted according to *Ivanova et al.* [16]. 2 g of ground samples were added to 60 mL of methanol. After the sample was centrifuged

at 5000 rpm for 5 min and the supernatant was collected. The extract was concentrated at 50°C. The dried extracts were dissolved in 2 mL of methanol and filtered. Then, it was injected for analyses. HPLC analyses of phenolic compounds were performed using a Shimadzu-HPLC equipped with a PDA detector and an Inertsil ODS-3 (5µm; 4.6 x 250mm) column. The mobile phase was a mixture of 0.05% acetic acid in water (A) and acetonitrile (B). The flow rate of the mobile phase was 1 mL/min at 30 °C and the injection volume was 20 µL. The peaks were recorded at 280 and 330 nm using a PDA detector. The gradient programme was as follows: 0-0.10 min 8% B; 0.10-2 min 10% B; 2-27 min 30% B; 27-37 min 56% B; 37-37.10 min 8% B; 37.10-45 min 8% B. The total running time per sample was 60 min.

Mineral Analyses

About 0.5 g of marmalade dried in the oven at 70±5 ºC for two days, and ground samples were mineralized by using 5mL of 65% HNO_3 and 2 mL of 35% H_2O_2 in a closed microwave (MARS 5 CEM Corporation Manufacture in USA, NC 28105-5044). Mineral contents were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (Varian-Vista, Australia) [17].

Working conditions of ICP-AES:

Instrument :ICP-AES (Varian-Vista)
RF Power : 0.7-1.5 kW (1.2-1.3 kW for Axial)
Plasma gas flow rate (Ar): 10.5-15 L/min. (radial) 15 " (Axial)
Auxilary gas flow rate (Ar) :1.5 "
Viewing height : 5-12 mm
Copy and reading time :1-5 s (max.60 s)
Copy time : 3 s (max. 100 s)

Statistical analysis

The analyses of variance were performed using JMP version 9.0. All analyses were carried out three times and the results are mean±standard deviation (MSTAT C) of marmalade samples [18].

RESULTS AND DISCUSSION

Moisture contents, antioxidant activity, total phenol, anthocyanins, and flavonoid contents of boiled juice samples are given in Table 1. Moisture contents of samples changed between 86.3% (Rosa) and 89.5% (Hawtorn). While antioxidant activity values of boiled juices vary between 99.6 % (mahaleb) and 117.2% (Cornus), total phenol contents of boiled juices changed between 103.52 (Mahaleb) and 126.63 mg GAE/100g (Cornus).

In addition, the total flavonoid contents of marmalade samples changed between 13.4 mgCE/g (Hawtorn) and 28.3 mg CE/g (Cornus). It was observed a significant correlation between total polyphenols contribute and the antioxidant activity. It was observed statistically significant differences among the amounts of phenolic constituents in marmalade samples ($p<0.05$). In previous study, *Pirone et al.* [19] determined 31 mg/kg of total anthocyanin and carotene 42.6 mg/kg in rose hip nectars. Total phenolic contents of boiled juices was found considerably higher compared to black currant (3.61-4.35 mgGAE/g), blueberry (2.70-3.48 mgGAE/g), strawberry (1.61-2.94 mgGAE/g) and raspberry (2.7-3.03 mgGAE/g) [20]. *Sagdıç et al.* [11] reported that total phenol and antioxidant activity values of rosehip marmalade were determined as 38.5 mg GAE/ g extract and 49.98%. Various researchers determined that the amounts of total phenolic compounds were between 176–960 mg/100 g in ripe rosehips [21-25]. *Guerrero et al.* [26] found that the total anthocyanin content in rosehip fruits was 0.38 mg/100 g, and the total phenolic content was 145.7 mg/100 g. Anthocyanins give color to fruits and they have therapeutic and antioxidant activity. *İlbay et al.* [27] reported that the best total phenolic contents of three different brands of Rose hip (*Rosa canina* L.) tea extracted by the Soxhlet method through 50% MeOH mixture and the UAE method with water were 59.69 ± 0.89 mg GAE/g (dw) and 48.59 ± 0.29 mg GAE/g (dw). Total phenolics, total flavonoids, total anthocyanins, and antioxidant capacities of jams made from fresh fruits of *Rubus coreanus* Miquel extracted in various aqueous methanol concentrations 1147.3 and 1255.8mg GAE/100 g, 257.1 and 287.6mg CE/100 g, 216.2 and 244.8 mg cyanidin 3-O-glucoside equivalents (CGE)/100 g, 817.5 and 1023.7 mg vitamin C equivalent (VCE) /100g [7]. Present results showed partly differences from the literature value. These differences can be probably due to different fruit species, heating, and processing conditions.

Phenolic compounds of boiled juices are shown in Table 2. Generally, gallic acid, (+)-catechin, syringic acid, caffeic acid, rutin trihydrate, quercetin, and kaempferol contents were major phenolics in marmalade samples.

Table 1: Antioxidant activity and total phenol contents of boiled juice samples.

Boiled juices	Moisture content (%)	Antioxidant activity (%)	Total phenolic content (mgGAE/100g)	Total anthocyanin content (μmol g^{-1}DW)	Total flavonoid content (mg CE/g DW)
Cornus	88.6±2.23b*	117.2±1.43a	126.63±3.28a	0.071 ± 0.003a	28.3 ± 0.58a
Rosa	86.3±3.61d**	103.6±2.17b	117.32±2.74b	0.048 ± 0.007c	24.6 ± 0.72b
Hawtorn	89.5±1.97a	101.7±3.56c	109.41±2.67c	0.037 ± 0.001d	13.4 ± 0.39d
Mahaleb	87.9±2.54c	99.6±1.89d	103.52±1.96d	0.069 ± 0.009b	15.8 ± 0.67c

**mean±standard deviation (n:3), **Values within each column followed by different letters are significantly different (p<0.05)*

Table 2: Phenolic compounds of boiled juices (mg/100g).

Phenolic	Cornus		Rosa		Hawtorn		Mahaleb	
Gallic acid	6.27	±0.36*a	1.42	±0.33c	2.35	±0.13b	0.91	±0.09d
3,4-Dihydroxybenzoic acid	2.15	±0.87**a	1.36	±0.28c	1.17	±0.09d	1.49	±0.13b
(+)-Catechin	9.61	±0.49a	8.27	±0.17b	4.62	±0.46c	3.29	±0.21d
1,2-Dihydroxybenzene	3.63	±0.33a	2.56	±0.39b	1.49	±0.32c	1.07	±0.07c
Syringic acid	2.67	±0.09b	3.81	±0.41a	1.56	±0.17c	1.43	±0.17c
Caffeic acid	3.68	±0.21a	2.73	±0.16b	3.48	±0.36a	1.24	±0.09c
Rutin trihydrate	3.37	±0.56a	2.86	±0.47b	1.69	±0.27c	2.77	±0.13b
p-Coumaric acid	1.28	±0.39a	0.96	±0.09b	0.48	±0.09c	0.74	±0.07bc
trans-Ferulic acid	2.17	±0.48a	1.73	±0.21b	1.86	±0.38b	0.68	±0.11c
Apigenin 7 glucoside	0.57	±0.09d	0.98	±0.17c	1.03	±0.16b	2.27	±0.31a
Resveratrol	0.32	±0.07d	0.48	±0.17c	0.76	±0.09b	0.94	±0.09a
Quercetin	4.68	±0.58a	3.36	±0.19b	2.58	±0.27c	1.67	±0.17d
trans-Cinnamic acid	0.34	±0.01bc	0.58	±0.08b	1.17	±0.12a	0.88	±0.09b
Naringenin	0.32	±0.03c	0.48	±0.07b	0.96	±0.09a	0.16	±0.03d
Kaempferol	1.32	±0.19a	0.87	±0.09bc	1.15	±0.09b	1.48	±0.13a
Isorhamnetin	0.67	±0.07c	0.98	±0.11a	0.55	±0.07c	0.84	±0.07b

**mean±standard deviation (n:3), **Values within each row followed by different letters are significantly different (p<0.05)*

While gallic acid contents vary between 0.91 mg/100g (Mahaleb) and 6.27 mg/100g (Cornus), (+)-Catechin contents of boiled juices were determined between 3.29 mg/100g (Mahaleb) and 9.61 mg/100g (Cornus). The highest syringic acid was found in the rosa-boiled juice sample (3.81 mg/100g). Also, the quercetin contents of boiled juice samples ranged from 1.67 mg/100g (Mahaleb) to 4.68 mg/100g (Cornus). The highest rutin trihydrate was determined in cornus marmalade (3.37 mg/100 g). In general, the amounts of phenolic constituents of cornus marmalade were higher compared to the results of other marmalade samples tested. The phenolic compounds, including flavonoids, anthocyanins, and carotenoids are found more in the deep-colored fruits [28]. Geographic location and soil affect the contents of phenolic compoundin fruits [29].

Macro and microelement contents of boiled juice samples are given in Table 3. K contents of boiled juice changed between 271.37 mg/kg (rosa) and 8004.00 mg/kg (Mahaleb). P contents (415.12mg/kg) of Mahaleb marmalade was found higher than those of results of other boiled juice samples. The highest Ca (175.13 mg/kg)was determined in a rose-boiled juice sample.

Table 3: Macro and micro element contents of boiled juices (mg/kg).

Boiled juices						
	P	K	Ca	Mg		
Cornus	55.12±	1311.02±	141.93±	0.00±		
	1.85*d	53.86c	10.37c	0.00		
Rosa	124.76±	271.37±	175.13±	0.00±		
	4.09b**	7.82d	5.06b	0.00		
Hawtorn	116.93±	1569.69±	355.02±	188.95±		
	6.23c	38.02b	21.00a	4.60a		
Mahaleb marmalade	415.12±	8004.00±	132.17±	149.14±		
	7.20a	136.69a	17.43d	3.28b		
Boiled juices						
	Fe	Zn	Mn	B	Cu	Na
Cornus	16.92±	0.58±	0.00±	0.77±	0.99±	248.83±
	0.49a	0.04c	0.00	0.02d	0.06d	10.71c
Rosa	12.97±	0.53±	0.00±	2.71±	1.03±	254.22±
	0.78c	0.11c	0.00	0.51c	0.04c	12.47c
Hawtorn	15.54±	3.50±	0.51±	8.79±	1.46±	536.66±
	0.81b	0.46b	0.03b	0.70b	0.31b	37.78a
Mahaleb	16.01±	7.62±	1.08±	27.54±	1.75±	475.61±
	1.70a	0.54a	0.10a	1.33a	0.14a	36.59b

**mean±standard deviation (n:3); **Values within each column followed by different letters are significantly different ($p<0.05$)*

While B contents of boiled juice samples change between 0.77 mg/kg (Cornus) and 27.54 mg/kg (Mahaleb), Fe contents of boiled juices were found between 12.97 mg/kg (Rosa) and 16.92 mg/kg (Cornus). The highest Cu and Zn were found in mahaleb boiled juices (1.75 mg/kg and 7.62 mg/kg), respectively. It was observed statistically significant differences among mineral contents of marmalade samples ($p<0.05$). In a previous study, concentrated grape juice contained 6216-7120 K, 1398-1782 Ca, 128-163 Na, 36-59 P and 10.58-11.10 mg/kg Fe [30]. *Topçu et al.* [31] established 369.46 Ca, 89.0 P, 12.78 Mg, 7.31 Fe, 18.67 Na, and 1369.40 mg/100g K in boiled grape juice.

Mineral contents varied depending on the different types of fruits. Results showed that marmalade samples are rich in Ca, K, Mg, Na, and P elements. When results were compared with literature values [30], partial variations were observed. These differences can be likely due to variety, processing conditions, fruit parts, growing conditions, and partly analytical factors.

CONCLUSIONS

In conclusion, the chemical composition, and antioxidant activities, nutritional values showed significant differences depending on the marmalade (boiled juice) types. Generally, gallic acid, (+)-catechin, syringic acid, caffeic acid, rutin trihydrate, quercetin, and kaempferol contents were major phenolics in marmalade samples. It was observed a significant correlation between total polyphenols contribute and the antioxidant activity. In general, the amounts of phenolic constituents in Cornu's marmalade were higher compared to the results of other marmalade samples tested. Mineral contents varied depending on the different types of fruits. Results showed that marmalade samples are rich in Ca, K, Mg, Na, and P elements. The Cornus and *Rosa* boiled juices showed the considerable high nutritional value and antioxidant activity which could be chosen for functional food development that benefits human health.

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