

## Antioxidant Capacity, Monomeric Anthocyanin and Total Phenolic Content of Sour Cherry Nectar

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Sour cherry nectar is one of the most popular functional drinks in Turkey due to its taste and positive healthy effects. The aim of this study is to determine the antioxidant capacity of 23 different sour cherry nectars and to investigate relationship between antioxidant capacity and total polyphenolic, monomeric anthocyanin and polymeric colour content. The antioxidant capacity of sour cherry nectars at 13.5 °Bx ranged from 13.93 to 23.53 TEAC mmol/L (TEAC = Trolox equivalent antioxidant capacity). Total phenolic and monomeric anthocyanins content of the nectar samples were found in the ranges of 952.3-1925.4 mg/L and 101.9-361.0 mg/L, respectively. Polymeric colour content varied from 21.13 to 61.69 %. The antioxidant capacity is mainly due to the presence of total phenolic compounds ( $r = 0.716$ ), but the antioxidant capacity is also well supported by the anthocyanins ( $r = 0.710$ ). Polymeric colour was negatively correlated to the antioxidant capacity ( $r = -0.813$ ,  $p < 0.01$ ).

**Keywords:** Sour cherry nectar, Antioxidant capacity, Monomeric anthocyanins, Total phenolic, Polymeric colour content.

### INTRODUCTION

Sour cherry (*Prunusa cerasus* L.) is a fruit that is native to Anatolia. Turkey is the world's top producer of sour cherry and provides for approximately 17 % of the total world production with an annual production of 194,989 tonnes [1]. Although the discriminating purplish red colour fruits are widely consumed as fresh, the important part of the production is processed industrially to juices, jams, marmalades and frozen fruits [2]. However the main driving force for sour cherry production is the fruit juice sector. In 2008, approximately 30 % of sour cherry production was processed to concentrated fruit juice and 24 % of fruit juices consumers in Turkey prefer to sour cherry nectar [3].

Sour cherries and its product have attracted attention due to their high phenolic compounds content and also possible health benefits of anthocyanin demonstrated in numerous clinical studies. Seeram *et al.* [4] found that sour cherry anthocyanins have significant inhibitory activities on the COX-I and COX-II which are known to be responsible from inflammation and pain. Some researchers suggest that anthocyanins have an anti inflammatory affect in cases of rheumatoid arthritis [5-8]. Anthocyanin have been also reported to offer protection against intestinal tumor in <sup>Apc</sup>Min mice and reduce proliferation of human colon cancer cells [9] and type-II diabetes by increasing insulin excretion [10,11]. Recent research

showed that consumption of sour cherry juices prevented the symptoms of muscle damage [12] and reduced muscle pain during running [13]. Pigeon *et al.* [14] claim that sour cherry juice increases the quality of older adults with insomnia and this is mainly attributed to have higher melatonin content [15].

Sour cherry juices are not consumed 100 % fruit juice due to its high level of acidity and excessively sour taste [16]. Therefore, firstly sour cherry juice have been concentrated and later reconstituted with water and sugar suitable for consumption (labeled "juice from concentrate" or "nectar"). Sour cherry nectar should have fruit juices minimum 25-50 % [17]. Producing a nectar product requires evaporation and filtration processes, which have detrimental effects on the antioxidant compounds of the product [18]. Especially, anthocyanins are relatively unstable and often undergo degradative reactions during heating processing which reduce its bioactivity, bioavailability and colour intensity [2,19].

In this study, the antioxidant capacity, total polyphenolic content, monomeric anthocyanin content and polymeric colour content of 23 different sour cherry juices prepared from concentrates were determined to evaluate quality and healthy benefits of the sour cherry nectar. In addition, the other objective of the present study is to investigate possible statistically correlations between antioxidant capacity and these functional parameters.

## EXPERIMENTAL

Research material was consisted of 23 different concentrated sour cherry juice (CSCJ) ( $65 \pm 0.3$  °Bx) samples. The concentrated sour cherry juice samples were collected during the June-July period of 2008 from 7 main industrial-scale fruit juices and concentrate producing companies in Turkey. According to A.I.J.N Code of Practice [20], each concentrated sour cherry juice samples were adjusted to 13.5 °Bx with ultra pure water (PURELAB Option-Q) before the analyses. Fruit juices samples have been stored at  $-20$  °C in glass bottles until used for analysis. The analyses were carried out on these sour cherry nectars (SCN) as two replicates.

**Determination of pH, water soluble solids and total titratable acidity (TTA):** pH was measured at 20 °C with a pH meter (Consort P407, Schott Gerate, Belgium). Water soluble solids were measured by means of a Abbe refractometer (NOW, Nippon Optical Work Co., Ltd., Tokyo, Japan) at 20 °C and expressed as brix degree (°Bx) [21]. Total titratable acidity was determined by potentiometric titration the methods described in the manual of the International Fruit Juice Union (IFU) [22] by using pH-meter (Consort, P407 model, Schott Gerate, Belgium). Acid content (% w/w L-malic acid) is determined by direct titration against standardized alkali solution (0.1 N NaOH) to an end-point at pH 8.1. The results of the samples are given in Table-1.

**Determination of total polyphenol content (TPC):** The total phenolics in nectar was determined spectrophotometrically (Model UV2 Unicam, England) according to the Folin-Ciocalteu method described by Rentscler and Taner [23]. Briefly, all nectar samples were diluted 10 times with deionized water. To 1 mL of diluted samples, 75 mL of distilled water and 5 mL of Folin Ciocalteu reagent (Merck) were added and mixed again. After 3 min, 10 mL of 20 % sodium carbonate was added and mixed. After 1h standing at room temperature, absorbance of the resolution was read at 720 nm at spectrophotometer. Total phenolics in nectar were expressed as mg catechin equivalent in L of fruit juice (mg catechin/L juice) using a standard calibration curve of (+)-catechin (Fluka, Buchs, Switzerland).

**Determination of monomeric anthocyanin content (MAC):** Total monomeric anthocyanin content was determined according to the pH differential method at pH 1.0 and pH 4.5, as described by Fuleki and Francis [24]. Briefly, sour cherry nectars samples were diluted appropriately volume for analysis and determined maximum absorbance point of samples using a UV-visible spectrophotometer (model UV2 UNICAM, England). pH of diluted sour cherry nectars samples were adjusted in pH 1.0 and pH 4.5 using potassium chloride buffer [pH 1.0 (0.025 M)] and sodium acetate buffer [pH 4.5 (0.4 M)]. The absorbance of samples were measured simultaneously at 518 (maximum absorbance point) and 700 nm after 2 h of incubation at  $+4$  °C against distilled water as a blank.

The difference in absorbance between two samples was calculated using the following equation:

$$(A_{518} - A_{700})_{\text{pH } 1.0} - (A_{518} - A_{700})_{\text{pH } 4.5}$$

Total anthocyanin content of samples (mg cyanidin-3-glucoside/L of sour cherry nectars) was calculated by following equation:

$$\text{MAC (mg/L)} = [(A) (10^3) (MW) (DF)] / [(\epsilon) (L)]$$

where A: absorbance; MW: molecular weight ( $449.2 \text{ g mol}^{-1}$ ); DF: dilution factor (50);  $\epsilon$ : molar absorptivity of cyanidin-3-glucoside ( $26\,900 \text{ L cm}^{-1} \text{ mg}^{-1}$ ); L: cell path length (1 cm).

**Determination of polymeric colour index (PCI):** Polymerized colour and colour density of sour cherry nectars samples were determined using the bisulfate bleaching method described by Giusti and Worstad [25]. The sour cherry nectars samples were diluted with distilled water to 20 times. 1 g of potassium metabisulfite was dissolved in 5 mL of distilled water. 2.8 mL of the diluted sour cherry nectars samples were transferred to each of two cuvettes and 0.2 mL of bisulfite solution added to 1 and 0.2 mL distilled water added to the other. The absorbance of bisulfite treated and non-treated solutions were measured at 420 nm for brown pigments, 518 nm ( $\lambda_{\text{max}}$ ) for monomeric anthocyanins and 700 nm for haze correction against distilled water blanks after 45 min. The ratio between polymerized colour and colour density is used to determine the percentage of the colour that is contributed by polymerized material.

This kinetic type was expressed by the following equation:

$$\text{Colour density} = [(A_{420 \text{ nm}} - A_{700 \text{ nm}}) + (A_{518 \text{ nm}} - A_{700 \text{ nm}})] \times \text{DF}$$

$$\text{Polymeric colour} = [(A_{420 \text{ nm}} - A_{700 \text{ nm}}) + (A_{518 \text{ nm}} - A_{700 \text{ nm}})] \times \text{DF}$$

$$\text{Polymeric colour (\%)} = \frac{\text{Polymeric colour}}{\text{Colour density}} \times 100$$

where the DF is the dilution factor.

**Determination of antioxidant capacity (AOX):** Trolox equivalent antioxidant capacity (TEAC) assay on antioxidant capacity was determined using the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ABTS method described by Miller *et al.* [26]. Phosphate buffer solution (PBS) was prepared by dissolving 7.14 g (41 mmol) di-potassium hydrogenphosphate and 1.23 g (9 mmol) potassium dihydrogen phosphate with water in a total volume of 1 L (50 mM/L final concentration). To prepare the ABTS stock solution, 77 mg of ABTS (FLUKA, 11557) and potassium persulfate (13 mg) were dissolved with phosphate buffer solution to a 7 mM/L concentration. ABTS radical cation (ABTS<sup>•+</sup>) was generated by reacting ABTS stock solution with phosphate buffer and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. After 16 h, this stock solution was diluted with phosphate buffer (pH 7.2-7.4) to give  $0.700 \pm 0.005$  absorbance at 734 nm. Sour cherry nectars samples were also diluted with the PBS by 1:200 (v:v). 0.1 mL of diluted samples were mixed with 1.9 mL of diluted ABTS<sup>•+</sup> solution and absorbance was measured against blank which was a mixture of 0.1 mL PBS and 1.9 mL of diluted ABTS<sup>•+</sup> solution, by spectrophotometer at 734 nm after 6 min of incubation. All determinations were carried out in triplicate.

A calibration curve was performed with TROLOX (0.050-0.400 mmol/L) from FLUKA (no: 52976), a water-soluble vitamin E analogue and the results were reported as TEAC mmol/L.

**Statistical analysis:** The data were performed by using the statistical software program MINITAB 15 (Minitab Inc., State College, PA, USA). The results are reported as an average of two replicates and are given as mean  $\pm$  standard deviation

(SD). The possible correlation between the antioxidant capacity, total phenolic contents, monomeric anthocyanin and percent polymeric colour of the sour cherry nectars was analyzed by using MINITAB 15 statistic program and differences at  $p < 0.01$  were considered significant [27].

## RESULTS AND DISCUSSION

### Description of concentrated sour cherry juice (CSCJ):

Results of pH, °Bx and total titratable acidity measurements are given in Table-1 for the identifying characteristics of the concentrated sour cherry juice. °Bx/acid ratio which is the main criterion for determining the tasting value of fruit juice [28] is also shown in Table-1.

TABLE-1  
DESCRIPTION OF CSCJ SAMPLES

No.	°Bx	Total titratable acidity (%) <sup>a</sup>	Ratio (°Bx/Acid) <sup>b</sup>	pH <sup>b</sup>
1	64.8	1.40 ± 0.007	9.6	3.06
2	64.9	1.39 ± 0.007	9.7	3.03
3	64.8	1.28 ± 0.007	10.5	3.16
4	64.8	1.18 ± 0.013	11.4	3.18
5	65.0	1.27 ± 0.000	10.6	3.13
6	65.0	0.97 ± 0.013	13.9	3.14
7	64.8	1.55 ± 0.020	8.7	2.99
8	64.9	1.34 ± 0.000	10.1	3.06
9	64.9	1.30 ± 0.027	10.4	3.16
10	65.0	1.11 ± 0.007	12.2	3.16
11	65.2	1.35 ± 0.007	10.0	2.94
12	65.2	1.35 ± 0.007	10.0	2.98
13	65.1	1.37 ± 0.007	9.9	3.05
14	65.1	1.35 ± 0.007	10.0	3.01
15	65.3	1.09 ± 0.007	12.4	3.18
16	64.9	1.35 ± 0.000	10.0	2.91
17	65.0	1.35 ± 0.007	10.0	2.90
18	64.9	1.08 ± 0.007	12.5	3.03
19	64.8	1.30 ± 0.000	10.4	3.08
20	64.9	1.41 ± 0.020	9.6	3.27
21	65.0	1.40 ± 0.007	9.6	3.20
22	65.2	1.24 ± 0.007	10.9	3.16
23	65.3	1.28 ± 0.007	10.5	3.05

<sup>a</sup>Determined in sour cherry nectar with 13.5 °Bx and calculated as L-Malic acid; <sup>b</sup>In sour cherry nectar with 13.5 °Bx

The °Bx of concentrated sour cherry juice was found to vary within the narrow range of 64.8-65.3, because the minimum °Bx of concentrate are reported by the A.I.J.N code of Practice of fruit and vegetable juices for facilitating the testing for minimum quality requirements at industry is 65 °Bx [20]. Titratable acidity of sour cherry nectars samples adjusted to 13.5 °Bx was found to vary within the range of 0.97-1.41 % as (L-malic acid). The °Bx/acid ratio of samples ranged from 8.7 to 13.9.

**Total polyphenol, monomeric anthocyanin content and polymeric colour index:** Total polyphenol (TPC), monomeric anthocyanin (TMA) and polymeric colour index (PCI) analysis of sour cherry nectars are given in Table-2. The mean value of total polyphenol content was found 1501 mg/L. The lowest total phenolic levels were detected in sample 22 and the highest in sample 8 in parallel to the results of antioxidant capacity (Fig. 1). The results of this study are lower than reported by Kim *et al.* [29] (161.7-312.4 mg/100 g), Bonerz *et al.* [19]

TABLE-2  
TOTAL POLYPHENOL CONTENT, MONOMERIC ANTHOCYANIN CONTENT AND POLYMERIC COLOUR INDEX OF SOUR CHERRY NECTARS

Number of sour cherry nectars samples	Total polyphenol content (mg/L)	Monomeric anthocyanin content (mg/L)	Polymeric colour index (%)
1	1591 ± 9.62	248 ± 0.84	25.2 ± 1.24
2	1674 ± 34.60	245 ± 10.90	29.7 ± 1.20
3	1414 ± 25.00	230 ± 2.50	32.0 ± 1.55
4	1435 ± 15.40	223 ± 1.25	31.0 ± 0.03
5	1360 ± 5.77	190 ± 0.84	37.6 ± 0.31
6	999 ± 21.20	102 ± 0.00	48.6 ± 0.69
7	1631 ± 7.69	284 ± 0.42	30.0 ± 0.47
8	1925 ± 17.30	340 ± 5.43	23.6 ± 0.12
9	1562 ± 34.60	232 ± 0.84	30.0 ± 0.56
10	1766 ± 30.80	361 ± 2.17	29.2 ± 1.07
11	1866 ± 42.30	336 ± 0.84	27.0 ± 0.28
12	1902 ± 1.92	283 ± 1.67	31.0 ± 0.63
13	1756 ± 36.50	289 ± 2.09	28.5 ± 0.79
14	1583 ± 40.40	295 ± 0.84	29.2 ± 0.03
15	987 ± 44.20	107 ± 0.42	61.7 ± 0.88
16	1685 ± 7.69	238 ± 1.25	27.9 ± 1.04
17	1216 ± 38.50	227 ± 2.09	28.9 ± 0.16
18	1241 ± 9.62	207 ± 2.09	30.7 ± 1.61
19	1277 ± 44.20	184 ± 1.25	28.0 ± 0.37
20	1441 ± 32.70	254 ± 1.25	24.2 ± 0.34
21	1781 ± 50.00	268 ± 3.34	21.1 ± 0.42
22	952 ± 25.00	147 ± 0.84	42.0 ± 1.01
23	1000 ± 7.69	175 ± 0.00	33.6 ± 0.38

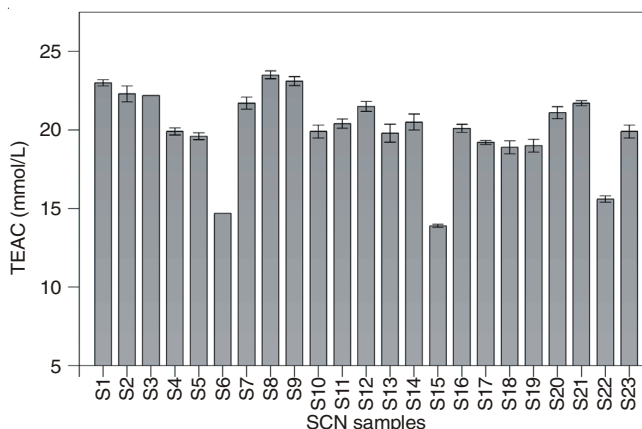


Fig. 1 . Antioxidant capacity of sour cherry nectars (n = 23)

(2704-4998 mg/L) and Kirakosyan *et al.* [2] (6742-12665 µg/g). The discrepancies probably occurred to study with several different forms of cherry samples such as row cherry fruit, frozen cherry and hot filled (85 °C) sour cherry juices.

The total anthocyanin content of the 23 samples ranged from 102 to 361 mg/L (Table-1). Ochoa *et al.* [30] found the anthocyanin content in a variety of sour cherries as 252 mg/kg, while Blando *et al.* [7] determined the anthocyanin content as 278-804 mg/kg in six different varieties of sour cherries. In this research, the anthocyanin content of sour cherry nectars was lower than the values reported in the literature and our previous studies [31]. Anthocyanin content can be affected by many factors like cultivar, maturity, geographic location and environmental factors such as light, temperature and various stresses [32,33]. However this is mainly attributed to heating in concentration process before the production of sour cherry

nectars. Anthocyanins can be easily degraded due to temperature in concentrated products, this cause decrease of lower monomeric anthocyanin content of nectar than 100 % sour cherry juices [34].

Polymeric content (degradation index) of the sour cherry nectars samples was found to vary within the wide range of 21.1-61.7 %. The main reason for the high results of the degradation index can be time and intensity of temperature during evaporation and lost or modify forms with anthocyanins [35]. Chaovanalikit and Wrolstad [36] reported that the degradation index 12.5-47.7 % in canning fresh cherry and stored cherry syrup at 2 and 22 °C. Thermal degradation of anthocyanins leads to colour loss and appearance of brown coloured compounds. For example, after 2 h at 98 °C, the polymeric colour value was 50, 26, 35 and 18 % for purple-flesh potato, red-flesh potato, grape and purple carrot extracts, respectively [37].

**Total antioxidant capacity:** The antioxidant capacities of all sour cherry nectar samples can clearly be observed in Fig. 1. The results show that the sample 8 has the highest antioxidant capacities (23.5 TEAC mmol/L) and sample 15 has the lowest antioxidant capacities (13.9 TEAC mmol/L) in the all samples. Total antioxidant capacity of sour cherry nectar samples was between 13.9-23.5 TEAC mmol/L.

The results are in accordance with reported the antioxidant capacity found in whole tart cherry fruit extracts by Blando *et al.* [7]. However it is lower than the results of Bonerz *et al.* [19] who reported the antioxidant activities against ABTS cation radicals in juices from five sour cherry cultivars as 27.5 and 54.6 TEAC mmol/L and Damar and Eksi [31] who reported the antioxidant capacities of the eleven sour cherry juice samples obtained from Turkish sour cherry varieties as 20.0-37.9 TEAC mmol/L. These findings clearly indicate that antioxidant capacity is affected by the examples of different forms used in the analysis. Similar results have been presented by Kirakosyan *et al.* [2] who found the crude extracts from frozen cherries have higher antioxidant capacity than other products obtained from tart cherry fruits. Reduce of monomeric anthocyanin content causes the decrease of the antioxidant capacity due to the heat treatment in the concentration process.

**Correlations between antioxidant capacity, total polyphenol, total monomeric anthocyanin and polymeric colour index:** The correlation coefficient between antioxidant capacity, total polyphenol, total anthocyanin and polymeric colour index are shown in Table-3. Total polyphenol content and total monomeric anthocyanin content were highly correlated ( $r = 0.822$ ), it is not surprising due to containing high level anthocyanin in sour cherry. The antioxidant capacity and total polyphenol, monomeric anthocyanin of sour cherry nectar samples were showed a positively correlation ( $r = 0.716$ ,  $r = 0.710$ ) and this correlation was statistically significant ( $p < 0.01$ ). Total phenolics content were correlated with antioxidant capacity higher than monomeric anthocyanin content. Not only sour cherry monomeric anthocyanins contribute to antioxidant capacity, but also colourless phenolics compounds are effective in antioxidant capacity.

In addition, polymeric colour content (%) were significant negatively correlated to the antioxidant capacity of sour cherry

TABLE-3  
CORRELATION COEFFICIENT BETWEEN TOTAL ANTIOXIDANT CAPACITY, TOTAL POLYPHENOL CONTENT, TOTAL MONOMERIC ANTHOCYANIN, POLYMERIC COLOUR INDEX

	Total polyphenol content	Total monomeric anthocyanin	Polymeric colour index
Total antioxidant capacity	0.716 <sup>a</sup>	0.710 <sup>a</sup>	-0.813 <sup>a</sup>
Total polyphenol content	–	0.822 <sup>a</sup>	-0.677 <sup>a</sup>
Total monomeric anthocyanin	–	–	-0.737 <sup>a</sup>

<sup>a</sup>Significant at  $p < 0.01$

nectar samples ( $r = -0.813$ ,  $p < 0.01$ ). When the polymeric colour content increases or monomeric anthocyanins are transformed into polymeric anthocyanins the antioxidant activity of sour cherry juice decreases. It shows that antioxidant capacity of polymeric anthocyanins is lower than antioxidant capacity of monomeric anthocyanins. A significant negatively correlation was found between total polyphenol content, monomeric anthocyanins and polymeric colour content as expected ( $p < 0.01$ ), because polymeric anthocyanins consist from monomeric anthocyanins. The results showed that concentration process that including heat treatment should be noticed to protect antioxidant capacity of sour cherry juices during sour cherry nectar processing.

## Conclusion

The antioxidant capacity, total phenolics, monomeric anthocyanins and polymeric colour index of sour cherry nectar was investigated in this study. These results clearly showed that antioxidant capacity measurements (23.5-13.9 TEAC mmol/L) of sour cherry nectar are lower than freshly sour cherry juices. In spite of this sour cherry nectar is still a promising source of antioxidants. The linear correlations ( $r = 0.716$  and  $r = 0.710$ ,  $p < 0.01$ ) among antioxidant capacity, total polyphenol content and monomeric anthocyanins were found. On the other hand, the negatively correlation ( $r = -0.813$ ,  $p < 0.01$ ) was determined between antioxidant activity and polymeric colour content. The antioxidant capacity is well supported by the polyphenol and monomeric anthocyanins content, though the antioxidant capacity is adversely affected by the anthocyanin degradation due to heating.

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