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Original Article

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# Quality Evaluation, Total Phenolic Content, Organic Acid Profiles and Antioxidant Activity of Soft Drinks with Koruk (Sour Grape) Concentrate

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# Abstract

**Objective:** Koruk (sour grape) juice has a tart flavor and a strong acidity, owing to the acidifying flavoring properties and has a significant antioxidative agent.

**Material and Methods:** In this study; koruk juice utilizing in the production of new soft drinks including carbonated drinks, sherbet and ice tea products and their total phenolic contents, antioxidant activities and organic acid profiles were investigated.

**Results:** Depending on the proportion of koruk juice concentrate addition, the phenolics (TP) and DPPH antioxidant activity (AA) changed in the range of 27.30-365.64 mg/L, 18.42-83.33%, respectively. It was determined that the most abundant organic acid type in soft drinks was malic acid and it represented more than 90% of total acidity in carbonated drinks and ice tea and 85% in sherbet.

**Conclusion:** Koruk juice is a potential antioxidant alternative for various range of products in soft drink sector.

Key Words: Antioxidant, soft drinks, koruk juice, phenolic, organic acid

# Introduction

Grape (*Vitis viniferia*) is one of the most produced fruits in the world that has been using for wine, juice, raisin, koruk juice, vinegar and molasses. Especially, it has a very common usage in wine and juice production. Koruk is called as unripened grape and is defined as a stage between berry set and veraison. It may also expressed as a berry near the average size of the cultivar previous veraison. It has been reported that the grapes undergo different stages until maturation and berry composition rapidly alter during this period. It has been described as the koruk formation in the stage where berry size is rapidly changed; as a result, sugar aggregation is stable, acidity is high and berries are firm (1).

Currently, beverage in food sector has an important sharing and it describes all consumable drinks like alcoholic and soft drinks including alcohol free, carbonated or non-carbonated (2). In recent manufacturing of fruit drinks and some ice drinks, the consumer demand goes to the utilizing of various fruit flavor for competition in sector. For this reason, studies on innovative drinks and additives has been performing by many researchers (3-8).

Koruk (sour grape) juice has a tart flavor and a strong acidity (10), owing to the acidifying and flavoring properties for commonly consumed salads and processed vegetables in Turkey and its neighboring countries. Besides, it is traditionally consumed as a drink after sweeteners (9). Koruk and koruk juice have high antioxidant property because of its phenolic profiles. Moreover, these are sources of organic acid and have antimicrobial effects. For this reasons, different researchers carried out various studies on defining the composition, properties and the clarification of koruk juice (9-15), on the quality of grape juice and its variability of different storage conditions (16-23), although there are limited studies concerning the usability of koruk juice for soft drinks manufacturing.

No study could be found regarding phenolic content, antioxidant activity and organic acid profiles of koruk (sour grape) juice based soft drinks. In the current study, the concentration process and its different concentration of koruk juice concentrate usability and their phenolic content, antioxidant activity and organic acid profiles in soft drinks including carbonated and non-carbonated drinks and ice tea were investigated.

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# **Materials and Methods**

#### 1. Koruk juice concentrate

Koruk samples belongs to Sultani seedless (*Vitis viniferia*) grape variety which used for koruk juice production were harvested from Manisa Viticulture Research Institute vineyards at season 2013. Following the harvest, clusters were rinsed for removing of dust, soil and other impurities. Then stalks were discarded and clusters were passed through a crusher destemmer machine (Türköz Makine). The mash was pressed in a hydraulic press (Türköz Makine) and cleared koruk juice was obtained. Juice then was kept in 2-4°C at cold room in Manisa Viticulture Research Institute research laboratory for 24 h to precipitate and to remove rough residue. Pectolicytic enzyme application (Shazym Claro Pectolytic Enzyme, 10.500 PGNU/g polygalakturonase, 0.15 g/L) was performed in 50°C for 2 h. Bentonite and gelatin were applied during clarification process. 10 ml/L from 10% bentonite solution and 25 ml/L from 1% gelatin solution were used at 20 °C and then koruk juice was kept in 4 °C for 24 h. In the same temperature, 5g/L potassium bitartrate (KC<sub>4</sub>H<sub>5</sub>O<sub>6</sub>) was added and left for 7 days for detartarization. The final clarified koruk juice was concentrated in evaporator at 50 °C and 600 mmHg vacuum. The obtained concentrates were kept in 125 ml glass jars at -24 °C at cold refrigerator (*Vestel*, *Turkey*) until use.

#### 2. Manufacturing of soft drinks

In this study, three different soft were manufactured by using different amounts of koruk juice concentrate as additive agents. One of these products was carbonated whereas the remains were non-carbonated. These soft drinks are classified as carbonated drinks, sherbet, and ice-tea and also three sub-groups were manufactured for each main soft drinks.

For carbonated beverages, 1.75% (called as carbonated drinks1), 2.0% (called as carbonated drinks2), and 2.25% (called as carbonated drinks3), koruk juice concentrates were added. During the production, 50% sugar syrup with different koruk juice concentration rates was prepared and dilutions were made to reach the desired sugar/acid balance, then the sugar syrup was manipulated as to 10°brix in the final product. Following the adding of carbonated water on syrup mixture, the filling/ sealing process were carried out. Glass bottles were closed with crown caps. The preservative agent (250 mg/l sorbic acid + 150 mg/L benzoic acid) was used in carbonated drinks.

For the production of non-carbonated sotf drink (sherbet), 10% sugar syrup was prepared and koruk juice concentrate was added to reach 20, 25 and 30 sugar/acid balance of the soft drinks. These different sherbet groups (sherbet1, 2, 3) were filled 250 cc glass bottles and closed with crown caps, then all applications were pasteurized at 85 °C for 20 min at once.

Ice tea production was made by infusing of 750 g black tea (brand *Çaykur Kamelya*) for 15 min and then diluting 2 times with boiled water. Three different ice tea groups were manufactured and the koruk juice concentrate was added to 2 g/L acid in the each end products. The sugar concentrations of ice tea were adjusted to 6,7 and 8 °brix with prepared sugar syrup and final groups had three different sugar/acid balances (ice tea 1, 2, 3). These mixtures were filled 250 ml glass bottles and closed with crown caps. All applications were pasteurized at 85 °C for 20 min at once.

#### 3. pH, acid and total soluble solid

The pH value of concentrate and soft drinks was measured with a pH meter (Hanna 211) (24). The titratable acidity by titrating 10 ml sample with 0.1 N NaOH to pH 8.1 and acidity was expressed as tartaric acid %. The soluble solid (TSS) of the samples was determined as °brix by using a refractometer (24) and sugar balance was calculated as TSS/acid.

### 4.Determination of antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) method was used to determine the antioxidative properties of the samples (25). The principle of the method is regarding the measurement of the reduction ability of the DPPH• radical on samples. 3ml of the 1 mM DPPH• solution was transferred and 200, 400, 600, 800 and 1000  $\mu$ l of diluted samples were added and standardized to 4 ml solution with methanol and incubated at room conditions (24±1°C) in dark. Methanol was used as blank solvent. Then, the absorbance was measured at 517 nm wavelength by spectrophotometer (*Thermo scientific, Multiskango*, Finland). Percent inhibition values were calculated according to blank absorbance as described the formula as shown below.

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#### Inhibition% = $((A_{DPPH}-A_{SAMPLE})/A_{DPPH}) \times 100.$

Calculated inhibitions and sample volumes were subjected to linear regression on the graphic and slope of each sample and equilibrium of these slopes were obtained. By using those equation of obtained slope values (necessary volume of equate for elimination the 50% of DPPH•) and EC<sub>50</sub> values were calculated. EC<sub>50</sub> = ((a × sample volume)  $\pm$  b) / dilution factor

#### 5. Determination of total phenolic content

Total phenolics in the samples were determined according to Folin-Ciocalteu colorimetric method (26). 100  $\mu$ l of Folin-Ciocalteu solution was added to each samples and those were completed to 4 ml of solution volume and then 500  $\mu$ l of 20% saturated sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) was added to final solution after 3 min and all was shaked. Then the samples were incubated at room temperature (24±1°C) for 30 min. At the end of the duration, 350  $\mu$ l samples were transferred in a 96 well of microplate and absorbance was measured at 760 nm. 50, 100, 200, 300, 400 and 500 mg/L of standard concentrations were used for calibration curve. Results were expressed as gallic acid equivalent in lt (mgGAE/L).

#### 6. Organic acid analysis by HPLC

High pressure liquid chromatography (HPLC) method was used for organic acid analysis (27). Samples were diluted a certain amounts of mobile phase and filtrated through 0.45  $\mu$ m syringe filter and directly injected to machine. Diode Array Detector (DAD) and ODS C18 (250 x 4.6 mm 5 $\mu$ m) column were used in the HPLC system (Agilent 1260 infinity). Column temperature was 30°C, elution time was 12 min, wavelength 210 nm and injection volume was 10  $\mu$ l. The mobile phase consisted of 0.005 N H<sub>2</sub>SO<sub>4</sub>. Flow was isocratic and rate was 1ml/min.

# **Results and Discussion**

In current study, the phenolic content, antioxidant activity and organic acid profiles of koruk (sour grape) juice based soft drinks including carbonated and non-carbonated drinks and ice tea were carried on and the concentration process and its different concentration of koruk juice concentrate usability was performed.

#### 1. Physicochemical Parameters of Soft Drinks

Physiochemical properties of three different groups of soft drinks have shown in Table 1. The pH of the samples was between 3.17-3.76, acidity was in the range of 0.22-0.48% and TSS was 6.07-10.90 °brix. TSS/acid ratio of all soft drinks was also calculated between 21.55 and 44.16.

The above-mentioned ratio was higher in carbonated drinks than that of the sherbet and ice tea. TSS and acidity of ice tea samples were similar with the results of Plestenjak *et al* (28) whereas pH values were slightly high. This may be owing to the differency of the fruits utilized for the ice tea production. It was seen that pH of the carbonated drinks was similar to the results of Jooyandeh (8) while higher than that of given by Verma *et al* (7). TSS values of carbonated drinks were lower than the other published studies (6-8). Balaswamy *et al* (4) reported that pH values, acidity, and TSS values of drinks with koruk juice additive were 2.45-3.25; 0.75-0.14% and 15-20° brix, respectively and our results showed some differencies from those literature studies.

Drinks	pН	Acid%	TSS (°brix)	TSS/acid
Carb.Drinks1	3.49±0.02 <sup>a</sup>	$0.23 \pm 0.02^{\circ}$	10.33±0.06 <sup>b</sup>	44.16±0.45 <sup>a</sup>
Carb.Drinks2	$3.42{\pm}0.02^{b}$	$0.28{\pm}0.01^{b}$	$10.90{\pm}0.10^{a}$	39.31±0.67 <sup>b</sup>
Carb.Drinks3	$3.37{\pm}0.01^{\circ}$	$0.30{\pm}0.03^{a}$	$10.27{\pm}0.06^{b}$	$34.00\pm0.74^{\circ}$
Sherbet1	3.17±0.03 <sup>b</sup>	$0.48{\pm}0.01^{a}$	10.27±0.10	21.55±0.34°
Sherbet2	$3.26{\pm}0.02^{a}$	$0.39{\pm}0.04^{\rm b}$	$10.30 \pm 0.06$	$26.47 \pm 0.30^{b}$
Sherbet3	$3.31{\pm}0.03^{a}$	$0.30{\pm}0.01^{\circ}$	10.33±0.06	33.92±0.36ª
Ice Teal	3.73±0.01 <sup>b</sup>	0.23±0.02	$6.07{\pm}0.06^{\circ}$	26.50±0.30°
Ice Tea2	3.75±0.01 <sup>a</sup>	0.22±0.01	$7.03{\pm}0.06^{b}$	31.60±0.65 <sup>b</sup>
Ice Tea3	$3.76 \pm 0.01^{a}$	0.23±0.01	$8.07{\pm}0.04^{a}$	35.75±0.23ª

Table 1. Physicochemical properties of soft drinks

Values indicated with different letters within each group and column are significantly different for p < 0.05

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#### 2. Total phenolic content in soft drinks

TP contents of studied soft drinks were determined as shown in Table 2. Among the all studied soft drink samples including carbonated drinks and sherbets, the significant statistical differences were determined regarding the TP (p<0.05) while no significant differences in that of the data for ice tea samples (p>0.05). It has been observed that TP increment was changed depending on the koruk juice concentrate level increment in carbonated drinks and sherbets although these increments differency were not observed in ice tea samples (p<0.05). This situation may be owing to the constant concentrate level fortification for ice tea production.

Brenna et al. (29) reported that the TP content classical cola carbonated drinks 98.47- 79.29 mg/L, in diet colas 59.31-56.38 mg/L and in cola with lemon juice 72.19 mg/L. TP content of carbonated drinks and sherbets were lower than those results of Brenna et al. (29). Lugasi and Hovari (30) reported that TP content in green tea was 583 mg/ and our ice tea TP results were lower than the results given by Lugasi and Hovari (30). These results were lower than the average of green tea samples, but higher than the average of black tea samples obtained by Wu et al. (31).

Drinks	%Inhibition	EC <sub>50</sub>	TP (mg/L)	
Carb.Drinks1	$18.42 \pm 2.50^{\circ}$	$1770 \pm 98^{a}$	32.70±2.24 <sup>b</sup>	
Carb.Drinks2	$26.78 \pm 1.21^{b}$	$1467 \pm 58^{b}$	$35.66 \pm 0.51^{b}$	
Carb.Drinks3	34.69±1.47 <sup>a</sup>	1113±37 <sup>b</sup>	$40.82{\pm}3.42^{a}$	
Sherbet1	$67.84{\pm}2.17^{a}$	598±13 <sup>b</sup>	35.00±3.35 <sup>a</sup>	
Sherbet2	$59.32 \pm 0.85^{b}$	$720{\pm}19^{a}$	$30.29 \pm 0.41^{b}$	
Sherbet3	53.57±0.83°	757±63ª	27.30±0.51 <sup>b</sup>	
Ice Tea1	82.61±2.00	20.20±0.12	357.08±15.2	
Ice Tea2	83.33±1.52	20.30±0.20	365.64±9.76	
Ice Tea3	$79.59 \pm 0.98$	20.43±0.25	363.55±7.08	

Values indicated with different letters within each group and column are significantly different for p < 0.05

#### 3. DPPH inhibition and antioxidant activity of soft drinks

DPPH• inhibitions and  $EC_{50}$  values of the soft drinks were recorded and each studied soft drink groups were evaluated in their assessment. The obtained data concerning inhibition and  $EC_{50}$  for carbonated drinks and sherbets was found as significantly statistical different from each other. No significantly statistical differency was obtained on inhibition and  $EC_{50}$  results for ice tea samples (p>0.05) and the highest inhibition and the lowest  $EC_{50}$  values were recorded in ice tea. This may be due to the high antioxidant profiles of both ice tea and concentrate. Detected  $EC_{50}$  values of ice tea were similar with the result of green tea and mixed fruit drink in Lugasi and Hovari's (30) study. The inhibition values of ice tea were higher than the green and black tea results reported by Wu et al. (31). According to obtained results; it was determined that when the additive concentrate level increased in soft drinks, the antioxidant properties also increased.

#### 4. Organic acid profile of soft drinks

Organic acid profile of the soft drinks was evaluated by chromatographically. Five organic acids including tartaric, malic, citric, acetic and fumaric were determined in the soft drinks samples and the results were shown in Table 3.

**Table 3.** Organic acids in soft drinks.

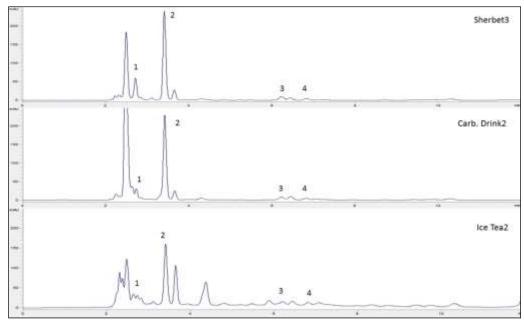
Drinks	Tartaric acid (mg/L)	Malic acid (mg/L)	Acetic acid (mg/L)	Citric acid (mg/L)	Fumaric acid (mg/L)
Carb.Drinks1	$67.02 \pm 0.42^{\circ}$	2164±0.47°	n.d.	69.77±0.72°	4.53±0.01°
Carb.Drinks2	$80.14{\pm}0.05^{b}$	2640±2.04 <sup>b</sup>	n.d.	$75.44 \pm 0.32^{b}$	$4.58 \pm 0.01^{b}$
Carb.Drinks3	$88.48{\pm}0.17^{a}$	$2904{\pm}2.30^{a}$	n.d.	$84.53{\pm}0.58^{a}$	4.63±0.01 <sup>a</sup>
Sherbet1	416.53±0.54 <sup>a</sup>	4122±5.39 <sup>a</sup>	n.d.	$151.60 \pm 1.84^{a}$	$4.86{\pm}0.01^{a}$
Sherbet2	$347.33{\pm}0.53^{b}$	3423±6.17 <sup>b</sup>	n.d.	126.73±0.41 <sup>b</sup>	$4.72 \pm 0.01^{b}$
Sherbet3	259.45±0.16°	2604±0.59°	n.d.	97.16±0.24 <sup>c</sup>	$4.58 \pm 0.02^{\circ}$
Ice Teal	$60.54{\pm}0.46^{a}$	1823±15.9 <sup>b</sup>	n.d.	120.35±16.1	4.91±0.01
Ice Tea2	$60.38 \pm 1.12^{a}$	1873±5.21ª	n.d.	$141.44 \pm 0.26$	$4.94{\pm}0.01$
Ice Tea3	$56.90{\pm}0.30^{\rm b}$	$1872 \pm 9.70^{a}$	n.d.	136.74±4.75	$4.92 \pm 0.04$

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Values indicated with different letters within each group and column are significantly different for p<0.05 \*n.d. : not detected

Tartaric, malic, citric and fumaric acid levels were found in those range as 56.90-416.53 mg/L; 1823-4122 mg/L, 69.77-151.60 mg/L, 4.53-4.94 mg/L, respectively while acetic acid was not detected (p<0.05). In carbonated drinks and sherbets, significant statistical differences were observed for studied organic acids whereas in ice tea samples, these statistical differences was only found for tartaric and malic acids (p<0.05). No significantly statistical alterations was achieved for citric and fumaric acid levels in ice tea samples (p>0.05).

It is known that the most abundant organic acid in grapes is tartaric acid. The organic acid profile of koruk juice depends on the maturation period of grape (12,13). When koruk juice concentrate was utilizing in soft drink production, organic acid distribution in soft drinks also as altered as in the concentrate level. It was found that the major organic acid was malic acid in studied soft drinks and it represented more than 90% of total acidity in carbonated drinks and ice tea and 85% of that of sherbet. It was reported that the organic acid distribution related to concentrate additive was affected by various factors including koruk maturity, variety, climate, location and processing technology (12,17,21,32).



**Figure1.** Organic acid HPLC chromatograms of samples (1: tartaric acid; 2: malic acid; 3: citric acid; 4: fumaric acid)

## Conclusion

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In current study, the usability of koruk juice in the production of ice tea, carbonated drink and sherbet was evaluated. It was also revealed that the novel soft drinks had significant organic acid profile. Besides, the total phenolic content and the antioxidant activity increased by adding level of koruk juice concentrate. As a result, it may be concluded that koruk juice is a potential antioxidant alternative for various range of products in soft drink sector.

**Conflict of interest:** The authors declare they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article, and declare study has ethical permissions if required.

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