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Determination of in-vitro phenolics, antioxidant capacity and bio-accessibility of Kombucha tea produced from black carrot varieties grown in Turkey

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Abstract

Black carrot, which is an economically important product, is produced extensively in Konya and Hatay regions in Turkey. Kombucha tea is a symbiotic system, comprises of bacteria and yeasts cultures called as SCOBY, and produced by fermentation of sugar and tea leaves. Kombucha samples contained black carrot juice in two concentrations (10 and 20%) and compared with the Kombucha contained green tea leaves. Black carrot varieties were used for enrichment of Kombucha production and investigated in terms of physico-chemical properties and antioxidant capacity. Black carrot samples belonging to the Hatay region, have higher anthocyanin content (660.26 mg C3G/100 g-dw), antioxidant capacity ($TEAC_{ABTS}$ and $TEAC_{CUPRAC}$; 15.33 ± 0.39 and 21.91 ± 0.28 µmole Trolox/g), total phenolic content (67.22 ± 0.24 mg/g GAE) and bioaccessibility (36.48 ± 0.78 mg/g GAE) comparing to Hatay black carrot variety. The highest anthocyanin content (71.05 mg C3G/100 mL), antioxidant capacity ($TEAC_{ABTS}$ and $TEAC_{CUPRAC}$; 3.67 ± 0.15 and 12.33 ± 0.11 µmole Trolox/g) were obtained from Kombucha tea containing black carrot juice (20%). According to the sensorial evaluation, panelists stated that the Kombucha samples with Hatay Black carrot juice had a fresh and pleasant taste and flavor due to green tea aroma.

Keywords: black carrot; kombucha tea; antioxidant capacity; anthocyanin; bioaccessibility.

Practical Application: Anthocyanin rich black carrot was utilized in Kombucha tea production.

1 Introduction

Awareness about the health benefits of foods and nutrition cause to increase the demand for new and healthy food products. Kombucha tea is one of the remarkable products, with its increasing market share and consumption especially in the USA and other countries. Kombucha tea, which draws attention to its positive effects on health and metabolism. It has antioxidant and anti-inflammatory effects and found to have positive effects on gastrointestinal and immune systems to prevent certain types of cancers (Martínez Leal et al., 2018; Villarreal-Soto et al., 2018).

Kombucha tea is originated in China, Korea and named by Dr. Kombu who took tea mushroom (SCOBY) from Korea to Japan. 'Kombu', a Japanese name, is a broad-leaved seaweed (*Laminaria japonica*), and 'Cha' means tea in Japanese (Ishida, 1999; Lončar et al., 2006). It is a symbiotic system, comprise of bacteria (Acetobacter and gluconobacter) and yeasts; slightly sweet, acidic, carbonated beverage produced by fermentation of sugar and tea leaves (black, green, white or oolong) (Martínez Leal et al., 2018). Yeasts that is available in the symbiotic culture (SCOBY) and convert sucrose to ethyl alcohol with CO_2 using the enzyme invertase, while acetobacteria convert yeast-formed ethyl alcohol to acetic acid by aldehyde dehydrogenase enzymes. On the other hand, gluconobacteria produce gluconate and cannot oxidize acetic acid due to the absence of succinate and α-ketoglutarate enzymes. Gluconic acid is produced from glucose by gluconobacteria and acetobacteria, while fructose and ethanol are used to produce acetic acid (Goh et al., 2012; Martínez Leal et al., 2018). The fermented Kombucha tea consists of sugars, organic acids, ethanol, CO_2, dietary fiber, amino acids (lysine, etc.), essential elements (Cu, Fe, Mn, Ni, Zn, etc.), vitamin C, vitamin B derivates, antibiotic substances, hydrolytic enzymes, and polyphenols that are conveyed from green tea leaves (Jayabalan et al., 2014; Miranda et al., 2016). Antioxidants are the major compounds responsible from the health benefits of Kombucha tea. In terms of phenolic compounds, the most abundant groups were flavonoids and phenolic acids available in all Kombucha varieties (Cardoso et al., 2020). Catechins as a sub-group of polyphenols, main group of flavonoids and are present in green tea leaves, caused to increase antioxidant properties including free radical scavenging activity (Jayabalan et al., 2007; Srihari & Satyanarayana, 2012). Kombucha contain organic acids such as glucuronic acid, acetic acid are other important compounds (Cardoso et al., 2020).

Kombucha including black tea leaves were found to have higher antioxidative potential due to high amount of phenolic compounds, while Kombucha incorporated green tea leaves were found to exhibit greater antibacterial activity and anti-tumoral activity (Cardoso et al., 2020).

Black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef) members of the *Apiaceae* family have been cultivated for more than 3000 years and originated from the Middle and

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Far East (Montilla et al., 2011). They are rich source of vitamin (C and E), mineral, fiber and anthocyanin (Kammerer et al., 2004). In addition to anthocyanins content, the black carrot is a significant source of phenolic acids especially caffeic acid and hydroxycinnamates (Kammerer et al., 2004).

It has also been used as a natural colorant in the production of milk products such as ice cream, jam, marmalade, pastry, fruit-vegetable juices, alcoholic beverages, and canned foods (Agcam & Akyıldız, 2015). Black carrot is also the raw material of a traditional lactic acid fermented beverage with turnip (*Brassica rapa* L.), known as shalgam and has been mainly produced in Southern Turkey (Erten et al., 2008). Fermented products have determined to have important potential with antioxidative compounds (Jayabalan et al., 2014, Gurbuz & Yildiz, 2019)

The antioxidant properties of foods are among the popular issues to sustain healthy living nowadays. Bioavailability and bioaccessibility are the concepts interrelated to evaluate antioxidant properties of foods. Health benefits of antioxidants depend on their digestion capability. Moreover, their digestion capability is limited by the production of new compound by other food components.

Polyphenols are presented in complex mixtures immersed to food matrix, which exposed to a digestion process in the gut (Bermúdez-Soto et al., 2007). Therefore, it is not possible to digest all antioxidants during the digestion process. On the other hand, the absorbed components are not fully able to reflect their beneficial effects on human body. Bioaccessibility is a level of ingested component that is able to owe biological impact when included in the systemic circulation, while bioavailability is a term that can be expressed as the amount of antioxidant that has a biological impact, available in blood and urine, after absorption intestinally (Bermúdez-Soto et al., 2007; Porrini & Riso, 2008). Bioaccesibility is a method that is able to determine by artificial intestinal systems including digestion enzymes.

Black carrot Konya variety is one of the important agricultural products of the Eregli Region in Konya and is used to produce "shalgam drink" in the domestic market. More than half of the production is exported. In this study, two black carrots cultivated in two different regions of Turkey (Konya and Hatay) were used. Kombucha samples were produced by these black carrot varieties in two concentrations (10% and 20%) in order to evaluate in terms of physico-chemical, antioxidant and sensorial properties.

2 Materials and methods

2.1 Materials

Black carrot samples

Black carrot samples were obtained from Döhler - Natural Food & Beverage Ingredients Inc. Company. Black carrot varieties have been produced in and supplied from Hatay (36°28'18" N36°22'58" E) and Konya (37°35'50" N 33°56'50" E) regions of Turkey. Organic green tea leaves were supplied from Caykur Co, Turkey.

Kombucha production

The black carrot juice was obtained from black carrot samples by juice extractor (Tefal, Easy Fruit model, France) for Kombucha production. Organic green tea (14 g/L) was brewed for 15 min and cooled down to 30 °C. Sucrose (30 g/L) was added either into the tea with SCOBY or green-tea Kombucha. The level of pH was adjusted by the acetic acid solution to 3.0. The tea mixture was started fermentation at pH 3.0. Kombucha fermentation was sustained at 30 °C in 12 days by water-bath. Two different control samples were prepared as *Control-A* contained sugar and SCOBY, and *Control-B* contained green tea, sugar, and SCOBY. Black carrot juices obtained from Konya and Hatay varieties were added to the Kombucha samples prepared in two different concentrations (10 and 20%). According to preliminary trials, fermentation was stopped at the day 12. Because, the best sensorial results were obtained on that day in terms of flavor and acidity production.

2.2 Methods

Physicochemical properties

Physico-chemical properties of black carrot and black carrot Kombucha samples were determined according to Association of Official Analytical Chemists (2000) in terms of pH, total titratable acidity (citric acid equivalent), brix, dry matter content, ash content. Total anthocyanin of content of samples was determined by pH-differentiation method spectrophotometrically (UV Mecasys Optizen 3220, Daejeon, Republic of Korea) proposed by Lee et al. (2005) and expressed as cyanidin-3-glucoside (C3G) equivalents.

Antioxidant capacity and total phenolic content

Extraction

Extractable, hydrolysable and bioaccessible phenolics of black carrot and black carrot Kombucha samples were extracted according to Vitali et al. (2009) and Bouayed et al. (2012) with some minor modifications. The carrot (2 grams) and Kombucha samples (2 mL) were mixed with HCl conc/methanol/water (1:80:10, v/v) and shaken in a water bath (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 20 °C (250 rpm, 2 h). The extracts were centrifuged (Sigma centrifuge 3 K 30, Germany) at 3500 rpm and 4 °C for 10 min at first. The residue of extractable phenolics was mixed with methanol/H_2SO_4 (10:1), shaken in a water bath at 85 °C (250 rpm, 2 h) and centrifuged (4 °C, 3500 rpm, 10 min). Mimic digestion procedure including enzymatic extraction was applied according to Bouayed et al. (2012). For this reason; 2 grams for carrot samples-2 mL for kombucha samples were treated with the pepsin enzyme (40 mg/mL in 0.1 M HCl) at 37 °C and 250 rpm for 2 h. Then, the intestinal digestion procedure was applied with a porcine pancreatic enzyme (2 mg/mL) and porcine bile mixture (12 mg/mL) at 37 °C and 250 rpm for 2 h, then centrifuged (15 °C, 3500 rpm, 10 min). The extracts were stored at -18 °C.

Antioxidant capacity analysis

Antioxidant capacities of black carrot and Kombucha samples were evaluated according to CUPRAC (Cupric reducing antioxidant capacity), DPPH (2,2diphenyl-1-picrylhydrazyl) and ABTS (2,20-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt). The analytical procedures were applied according to methods mentioned by Boskou et al. (2006) and Apak et al. (2008) with after slight modifications. Absorbance values of the extract were determined spectrophotometrically. The results were determined as µmole Trolox equivalent (TE) per g/mL sample weight and expressed as mean ± SD for triplicates.

Total phenolic content

Total phenolic content was evaluated by the Folin-Ciocalteu method according to procedures Apak et al. (2008). Absorbance of the extracts was measured by a spectrophotometer and the results were expressed as mg gallic acid equivalents (GAE) per g/mL sample weight.

Color measurements

Color measurements of black carrot and black carrot Kombucha samples were determined by the Minolta Spectrophotometer (CM-3600d; Osaka, Japan) in order to measure L^*, a^* and b^* values. According to the Commission Internationale de l'Eclairage (CIE), L indicates lightness 0 (darkest) to 100 (lightest), a is the red/green coordinate (+ redder, - greener), and b is the yellow/blue (+ yellower, - bluer) coordinate.

Sensorial evaluation

Sensorial evaluation of black carrot Kombucha samples was done by 27 untrained panelists whose ages were between 17 to 53. The hedonic scale with 9-points was used for sensorial evaluation. Samples evaluated in terms of color, clarity, odor, flavor, taste, sourness and overall acceptability. Furthermore, consumption frequency of panelists was also asked.

Statistical evaluation

JMP software was used to perform the statistical analyses. Differences among means were analyzed by the one-way analysis of variance (ANOVA). The level of significance among the means ($p \leq 0.05$) were determined by the least significant difference (LSD) test.

3 Results and discussion

3.1 Physicochemical properties

Some physicochemical properties of the black carrots were determined in two varieties and are given in Table 1. The dry matter content of the black carrots was high as approximately 96% and no significant differences were seen between those two varieties, statistically ($p \leq 0.05$). But, the differences between two varieties in terms of acidity, pH, ash, brix and anthocyanin contents were significant, statistically ($p \leq 0.05$). Anthocyanin contents of the Hatay variety of black carrots were higher than Konya variety and changed between 418.27 and 660.26 mg C3G/100 g-dw. When Hunter Lab color values were considered, the L^* (lightness) value of the Hatay variety was lower than the Konya variety. This means that Hatay variety has a darker color. Also, high "+ a^*" values of the varieties reflect the red color in the black carrot samples. As a parallel to Hunter color values, anthocyanin content of the Hatay variety (660.26 mg C3G/100 g-dw) was higher than the Konya variety as seen in Table 2. There is an interaction between the acidity and pH values of black carrot samples, affecting the color properties. Anthocyanin content of Hatay variety was higher than Konya variety as about 70%. The anthocyanin content of Konya variety was close to the previous researchers and found as 536 mg C3G/100 g-dw by Kamiloglu et al. (2015) and 486 ± 43 mg C3G/100 g-dw

Table 1. Physicochemical properties of black carrot samples.

Sample	Dry Matter (%)	Ash (%)	pH	Total Acidity (%)***	Brix	Anthocyanin (mg/100 g dw)****
Konya-BC*	89.45 ± 0.51^{a**}	0.96 ± 0.02 ^{a}	5.35 ± 0.03^a	0.26 ± 0.00^b	8.97 ± 0.15 ^{a}	418.27 ± 9.38 ^{a}
Hatay-BC	89.66 ± 0.11^a	0.82 ± 0.53^b	4.88 ± 0.10^b	0.51 ± 0.00 ^{a}	8.40 ± 0.10^b	660.26 ± 11.81^b

*Hatay-BC: Hatay variety black carrot sample; Konya-BC: Konya variety black carrot sample; **Mean values ± standard deviation (n = 3) with different superscript in the same column are significantly different (p ≤ 0.05); ***meq expressed as citric acid equivalents; ****Anthocyanin content expressed as cyanidin-3-glucoside (C3G).

Table 2. Antioxidant capacity and total phenolic content of black carrot samples.

	Extractable Phenolics				Hydrolysable Phenolics			
	Total Phenolic Content (mg/g GAE)**	Antioxidant Capacity (µmole Trolox/g)			Total Phenolic Content (mg/g GAE)	Antioxidant Capacity (µmole Trolox/g)		
		ABTS	CUPRAC	DPPH		ABTS	CUPRAC	DPPH
Hatay-BC*	27.54 ± 0.15^a	15.33 ± 0.39^a	21.91 ± 0.28^a	15.13 ± 0.22^b	67.22 ± 0.24^a	7.09 ± 0.15^a	18.70 ± 0.13^a	27.54 ± 0.64^a
Konya-BC	14.91 ± 0.12^b	9.03 ± 0.24^b	4.25 ± 0.15^b	20.98 ± 0.33^a	41.35 ± 0.06^b	2.71 ± 0.12^b	8.49 ± 0.58^b	27.71 ± 0.86^a
	Bioaccessible Phenolics				Bioaccessibility %			
	Total Phenolic Content (mg/g GAE)	Antioxidant Capacity (µmole Trolox/g)			Total Phenolic Content	ABTS	CUPRAC	DPPH
		ABTS	CUPRAC	DPPH				
Hatay-BC	36.48 ± 0.78^a	12.06 ± 0.49^a	15.61 ± 0.19^a	14.89 ± 0.79^a	38.50 ± 1.58^a	53.72 ± 1.29^b	38.44 ± 0.64^b	34.89 ± 1.73^a
Konya-BC	20.54 ± 0.25^b	8.53 ± 0.01^b	7.11 ± 0.46^b	12.04 ± 0.79^b	36.52 ± 1.39^b	72.68 ± 0.32^a	55.90 ± 2.98^a	24.74 ± 1.83^b

*Hatay-BC: Hatay variety black carrot sample; Konya-BC: Konya variety black carrot sample; GAE: Gallic acid equivalent; ABTS: (2,20-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) antioxidant capacity assay; CUPRAC: Cupric reducing antioxidant capacity assay; DPPH: (2,2diphenyl-1-picrylhydrazyl) antioxidant capacity assay; **Mean values ± standard deviation (n = 3) with different superscript in the same colon are significantly different (p ≤ 0.05).

by Suzme et al. (2014). According to Kamiloglu et al. (2015) contents of ferulic acid, coumaric acid, sinapic acid, and cyanidin were found to be major anthocyanins in black carrot samples, and 57% of the total anthocyanin in this variety consisted of cyanidin-3-xylosyl-feruloyl-glucosyl-galactoside.

3.2 Antioxidant Properties

Black carrot samples

Antioxidant capacity and total phenolic content of black carrot samples were evaluated in terms of extractable, hydrolysable and bioaccessible phenolics, results are given in Table 3 ($p \leq 0.05$). In general, higher values of extractable, hydrolysable and bioaccessible phenolics were obtained from Hatay-BC. $TEAC_{ABTS}$ and $TEAC_{CUPRAC}$ values in the extractable phenolics of Hatay and Konya varieties were 15.33 ± 0.39 and 21.91 ± 0.28 and, 9.03 ± 0.24 and 4.25 ± 0.15µmole Trolox/g, respectively and were higher in Hatay variety. The same proportional similarities were observed in the bioaccessible phenolics as seen in Table 3. $TEAC_{ABTS}$ and $TEAC_{CUPRAC}$ values of bioaccessible phenolics were (12.06 ± 0.49 and 15.61 ± 0.19 µmole Trolox/g) in Hatay variety and (8.53 ± 0.01 and 7.11 ± 0.46 µmole Trolox/g) in Konya variety. Similar results were also determined by Kamiloglu et al. (2015) for Konya variety, while Hatay variety in our study showed higher antioxidative potential. The total phenolic content of bioaccessible phenolics was determined 20.54 ± 0.25 and 36.48 ± 0.78 mg/g GAE as in Konya and Hatay varieties respectively. Algarra et al. (2014), found similar results. The total anthocyanin content of black carrot variety (Antonina) was determined to contain 50% of total phenolics. Bioaccessible phenolics are the sensible compounds to the external conditions and thus, can interact with other food components. The slight differences observed in bioaccessible phenolics compared to the other investigators can be explained by the extraction method used and those interactions. As mentioned by Bouayed et al. (2012), the polyphenols are the compounds that may either interact with other food constituents, or be further degraded by hydrolysis and enzymes. On the other hand, the constituents of the black carrots change according to seasonal differences and harvesting period.

Black carrot Kombucha samples

Black carrot Kombucha samples include three groups of components are significant in terms of antioxidant capacity and health benefits. The antioxidant capacity of our Kombucha samples are based on anthocyanins that are included from enrichment with black carrot. The catechins are contained due to incorporation of green tea used for Kombucha production. In addition, the acidic substrates are obtained because of SCOBY fermentation.

Total acidity and pH values of black carrot Kombucha samples are given in Figure 1 ($p \leq 0.05$). After 3rd day of fermentation, a significant increase in pH and total acidity values was observed. As reported by Rahmani et al. (2019), a large portion of sugar content was hydrolyzed after 3rd day of fermentation by yeasts, while the sucrose was converted into glucose and fructose by invertase enzyme (Harkness Troy & Arnason Terra, 2014). At the same time, a visible thickening with a sticky texture was observed due to SCOBY activity. The main reason of the

Table 3. Antioxidant capacity and total phenolic content of black carrot kombucha samples.

Extractable Phenolic

	Total Phenolic Content (mg GAE/mL)**		Antioxidant Capacity (µmole Trolox/mL)					
			ABTS		CUPRAC		DPPH	
	BF***	AF	BF	AF	BF	AF	BF	AF
Control-A*	3.24 ± 0.11^{e}	5.27 ± 0.10^{d}	1.24 ± 0.17^{d}	1.75 ± 0.03^{e}	3.12 ± 0.01^{e}	3.50 ± 0.04^{e}	1.24 ± 0.37^{d}	1.32 ± 0.37^{d}
Control-B	7.43 ± 0.25^{d}	10.19 ± 0.37^{c}	2.34 ± 0.18^{c}	$2.56 \pm 0.37^{c,d}$	6.32 ± 0.75^{d}	7.83 ± 0.05^{d}	$1.98 \pm 0.25^{c,d}$	2.11 ± 0.05^{b}
KomH10	12.65 ± 0.35^{c}	$14.79 \pm 0.11^{b,c}$	2.98 ± 0.02^{b}	3.38 ± 0.25^{b}	$9.23 \pm 0.37^{b,c}$	10.33 ± 0.21^{b}	3.14 ± 0.12^{c}	$3.35 \pm 0.23^{b,c}$
KomH20	19.54 ± 0.37^{a}	21.63 ± 0.37^{a}	3.46 ± 0.23^{a}	3.67 ± 0.15^{a}	11.54 ± 0.23^{a}	12.33 ± 0.11^{a}	$3.87 \pm 0.12^{a,b}$	$4.15 \pm 0.04^{a,b}$
KomK10	10.55 ± 0.34^{c}	12.39 ± 0.23^{c}	$2.67 \pm 0.32^{b,c}$	2.89 ± 0.01^{c}	7.65 ± 0.32^{c}	8.67 ± 0.01^{c}	3.67 ± 0.37^{b}	4.07 ± 0.12^{b}
KomK20	15.09 ± 0.23^{b}	16.76 ± 0.22^{b}	2.91 ± 0.11^{b}	$3.25 \pm 0.01^{b,c}$	9.34 ± 0.25^{b}	$9.83 \pm 0.21^{b,c}$	4.12 ± 0.23^{a}	4.34 ± 0.23^{a}

Hydrolysable Phenolics

	Total Phenolic Content (mg GAE/mL)*		Antioxidant Capacity (µmole Trolox/mL)					
			ABTS		CUPRAC		DPPH	
	BF	AF	BF	AF	BF	AF	BF	AF
Control-A	4.62 ± 0.37^{e}	4.34 ± 0.12^{e}	1.14 ± 0.08^{d}	1.26 ± 0.25^{d}	4.34 ± 0.25^{e}	4.80 ± 0.08^{d}	1.67 ± 0.12^{d}	1.87 ± 0.05^{d}
Control-B	9.61 ± 0.23^{d}	9.93 ± 0.11^{d}	2.16 ± 0.23^{c}	2.28 ± 0.20^{c}	7.65 ± 0.07^{d}	8.43 ± 0.31^{c}	2.76 ± 0.23^{c}	2.97 ± 0.04^{c}
KomH10	$12.30 \pm 0.07^{b,c}$	15.67 ± 0.30^{b}	$2.78 \pm 0.38^{b,c}$	3.12 ± 0.07^{b}	14.23 ± 0.37^{b}	15.76 ± 0.23^{b}	$3.53 \pm 0.25^{b,c}$	3.87 ± 0.05^{b}
KomH20	16.42 ± 0.11^{a}	18.70 ± 0.23^{a}	3.24 ± 0.37^{a}	3.42 ± 0.12^{a}	15.37 ± 0.23^{a}	17.67 ± 0.12^{a}	3.87 ± 0.37^{a}	4.05 ± 0.37^{a}
KomK10	10.48 ± 0.37^{c}	10.91 ± 0.08^{c}	$2.56 \pm 0.23^{b,c}$	$2.68 \pm 0.23^{b,c}$	10.65 ± 0.02^{c}	$12.65 \pm 0.09^{b,c}$	3.67 ± 0.12^{b}	3.82 ± 0.03^{b}
KomK20	13.29 ± 0.23^{b}	$14.34 \pm 0.01^{b,c}$	2.89 ± 0.08^{b}	$2.76 \pm 0.07^{b,c}$	$13.76 \pm 0.05^{b,c}$	14.71 ± 0.07^{c}	3.76 ± 0.07^{a}	$3.60 \pm 0.09^{b,c}$

*Control-A: Sugar fermentation with SCOBY; Control-B: Green tea Kombucha sample; KomH10: 10% Hatay variety black carrot juice added Kombucha sample; KomH20: 20% Hatay variety black carrot juice added Kombucha sample; KomK10: 10% Konya variety black carrot juice added Kombucha sample; KomK20: 20% Konya variety black carrot juice added Kombucha sample; GAE: Gallic acid equivalent; ABTS: (2,20-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) antioxidant capacity assay; CUPRAC: Cupric reducing antioxidant capacity assay; DPPH: (2,2diphenyl-1-picrylhydrazyl) antioxidant capacity assay; **Mean values ± standard deviation (n = 3) with different superscript in the same colon are significantly different ($p \leq 0.05$); ***BF: Before Fermentation; AF: After Fermentation.

Table 3. Continued...

	Bioaccessible Phenolics							
	Total Phenolic Content (mg GAE/mL)*		Antioxidant Capacity (µmole Trolox/mL)					
			ABTS		CUPRAC		DPPH	
	BF	AF	BF	AF	BF	AF	BF	AF
Control-A	4.32 ± 0.12^{d}	4.65 ± 0.43^{d}	0.93 ± 0.01^{d}	1.45 ± 0.08^{d}	2.54 ± 0.23^{d}	3.65 ± 0.25^{d}	1.13 ± 0.05^{d}	1.33 ± 0.08^{d}
Control-B	9.54 ± 0.23^{c}	9.91 ± 0.23^{c,d}	1.78 ± 0.12^{c}	2.12 ± 0.04^{c,d}	4.10 ± 0.23^{c,d}	5.77 ± 0.23^{c,d}	1.68 ± 0.12^{b,c}	1.97 ± 0.09^{c}
KomH10	11.73 ± 0.01^{b,c}	12.35 ± 0.25^{b,c}	2.16 ± 0.43^{b,c}	2.76 ± 0.07^{b}	6.09 ± 0.04^{b}	8.69 ± 0.05^{b}	2.65 ± 0.09^{b,c}	2.76 ± 0.08^{b}
KomH20	15.69 ± 0.44^{a}	15.99 ± 0.17^{a}	2.71 ± 0.25^{a}	3.21 ± 0.54^{a}	8.22 ± 0.07^{a}	10.74 ± 0.09^{a}	3.11 ± 0.02^{a}	3.23 ± 0.18^{a}
KomK10	9.95 ± 0.45^{c}	10.25 ± 0.54^{c}	1.85 ± 0.54^{c}	2.26 ± 0.33^{c}	4.76 ± 0.10^{c}	6.14 ± 0.02^{c}	2.57 ± 0.08^{c}	2.80 ± 0.04^{b}
KomK20	14.73 ± 0.44^{b}	15.30 ± 0.54^{b}	2.31 ± 0.34^{b}	2.55 ± 0.09^{b,c}	5.66 ± 0.12^{b,c}	7.43 ± 0.07^{b,c}	2.98 ± 0.12^{b}	3.12 ± 0.09^{a}
	% Bioaccessibility							
	Total Phenolic Content (%)		Antioxidant Capacity (%)					
			ABTS		CUPRAC		DPPH	
	BF	AF	BF	AF	BF	AF	BF	AF
Control-A	54.96±1.43^{a}	56.58 ± 0.12^{a}	39.13 ± 1.00^{b}	48.28 ± 1.33	28.57 ± 2.34	43.37 ± 2.35	39.29 ± 0.12	41.94 ± 1.36
Control-B	55.98±2.34^{a}	57.23 ± 2.07^{a}	38.64 ± 0.12^{c}	44.68 ± 0.07	30.77 ± 2.09	35.11 ± 0.07	34.78 ± 1.45	38.00 ± 1.66
KomH10	47.01±2.55^{c}	48.15 ± 1.07^{c}	37.50 ± 2.00^{d}	42.19 ± 1.33	25.86 ± 0.12	33.03 ± 1.65	39.39 ± 1.43	38.03 ± 1.39
KomH20	43.63±2.88^{d}	44.07 ± 1.12^{d}	40.91 ± 1.24^{a}	45.71 ± 1.43	30.42 ± 2.77	35.75 ± 2.55	40.79 ± 3.24	39.51 ± 1.35
KomK10	47.31±1.54^{c}	48.53 ± 1.43^{c}	35.29 ± 2.54^{e}	40.74 ± 2.45	27.65 ± 1.23	29.52 ± 1.45	34.72 ± 1.33	35.90 ± 1.23
KomK20	51.90±2.34^{b}	52.13 ± 2.45^{b}	40.35 ± 2.00^{a}	42.37 ± 3.22	25.45 ± 2.08	31.05 ± 2.66	37.18 ± 1.28	39.24 ± 1.73

*Control-A: Sugar fermentation with SCOBY; Control-B: Green tea Kombucha sample; KomH10: 10% Hatay variety black carrot juice added Kombucha sample; KomH20: 20% Hatay variety black carrot juice added Kombucha sample; KomK10: 10% Konya variety black carrot juice added Kombucha sample; KomK20: 20% Konya variety black carrot juice added Kombucha sample; GAE: Gallic acid equivalent; ABTS: (2,20-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) antioxidant capacity assay; CUPRAC: Cupric reducing antioxidant capacity assay; DPPH: (2,2diphenyl-1-picrylhydrazyl) antioxidant capacity assay; **Mean values ± standard deviation (n = 3) with different superscript in the same colon are significantly different (p ≤ 0.05); ***BF: Before Fermentation; AF: After Fermentation.

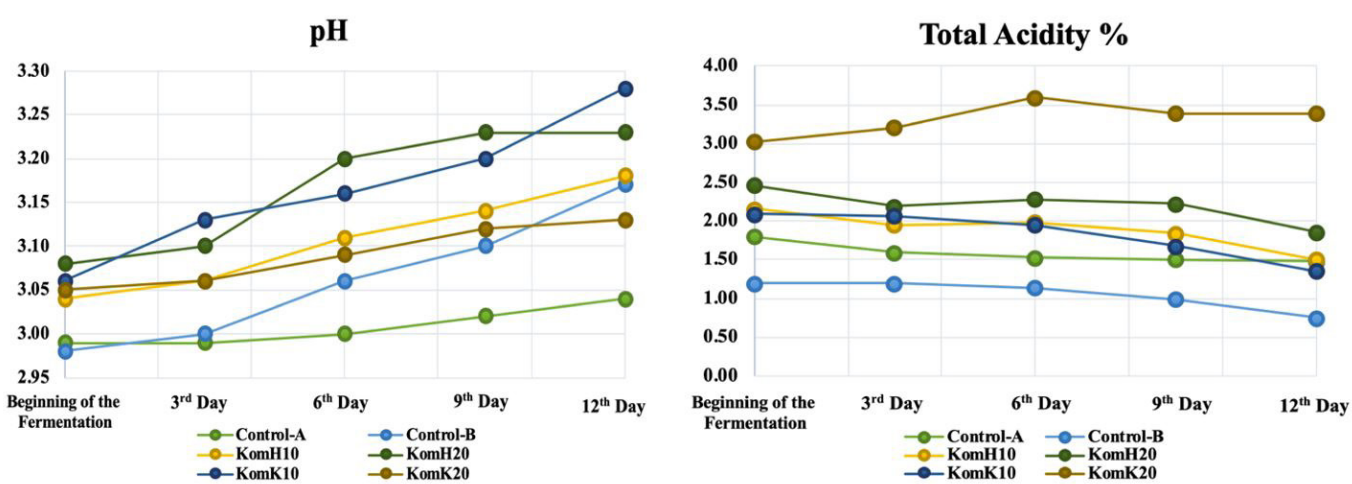

Figure 1. Total acidity and pH values of black carrot Kombucha samples. Control-A: Sugar fermentation with SCOBY; Control-B: Green tea Kombucha sample; KomH10: 10% Hatay variety black carrot juice added Kombucha sample; KomH20: 20% Hatay variety black carrot juice added Kombucha sample; KomK10: 10% Konya variety black carrot juice added Kombucha sample; KomK20: 20% Konya variety black carrot juice added Kombucha sample.

increasing acidity was probably associated with the production of some organic acids (such as acetic acid, glucuronic acid and gluconic acid) during the fermentation. Acetic acid was determined as the major acid (3 g/L) in the Kombucha samples by Cardoso et al. (2020). The products such as CO_2 and the acids are mainly produced during the fermentation of the sucrose incorporated. The high acidity and low pH value are due to these acids and CO_2 that were solubilized in the liquid phase. Jayabalan et al. (2007) determined that concentrations of acetic acid, glucuronic acid, and lactic acid in green tea Kombucha samples were 3.0, 1.39 and 0.13 g/L, respectively.

Extractable, hydrolysable and bioaccessible phenolics regarded to antioxidant capacity and total phenolic content of control and black carrot Kombucha samples, before and after fermentation, are given in Table 3 ($p \leq 0.05$). When the antioxidant capacity essays ($TEAC_{ABTS}$, $TEAC_{CUPRAC}$ and $TEAC_{DPPH}$) are considered, the values obtained from $TEAC_{CUPRAC}$ are relatively broad-ranged. According to $TEAC_{CUPRAC}$, extractable phenolics of *Control-B* including green tea fermentation were 12% higher than *Control-A* including sugar fermentation only; while hydrolysable phenolics increased almost 11% from 4.34 ± 0.25 to 4.80 ± 0.08 μmole Trolox/mL in the same samples. Cardoso et al. (2020) determined that flavonoids and phenolic acids were the major phenolic compounds in green tea and black tea Kombucha samples and their availability changes depending on the fermentation conditions. The phenolic compounds that are 70.2% of flavonoids, were identified in green tea and black tea Kombucha samples.

The content of phenolic compounds of Kombucha was increased by the fermentation. With the increase in microorganism kinetics in Kombucha by SCOBY inoculation, an enhancement in phenolic compounds occurs after 3 days (Jayabalan et al., 2007). They expressed that antioxidant potential of the Kombucha samples increased due to splitting of the complex phenolic into minor molecules during the fermentation. Enzymes such as α-galactosidase, phytase and tannase are responsible from degradation of complex polyphenols and increasing in total phenolic content by the fermentation (Dueñas et al., 2007). Ivanišová et al. (2019) reported that the Kombucha samples have significant antioxidative potential due to their total phenolic and flavonoid contents.

The antioxidant potential of the black carrot juice added Kombucha samples was found to increase due to fermentation process. In addition, Hatay carrot variety exhibited higher antioxidant potential versus to Konya carrot variety (Table 3, $p \leq 0.05$). In terms of $TEAC_{CUPRAC}$ antioxidant capacity, bioaccessible phenolics of *KomH20* sample (10.74 ± 0.09 μmole Trolox/mL) increased 138% and 50% comparing to *Control-A* (3.65 ± 0.25 μmole Trolox/mL) and *Control-B* (5.77 ± 0.23 μmole Trolox/mL). $TEAC_{CUPRAC}$ values in terms of bioaccessible phenolics for *KomH20* samples increased 23% from 8.22 ± 0.05 to 10.74 ± 0.09 μmole Trolox/mL by fermentation process. In terms of total phenolic content, same sample (*KomH20*, 15.99 ± 0.17 mg GAE/g) increased 243% comparing to *Control-A* (4.65 ± 0.43 mg/g GAE) and 61% comparing to *Control-B* (9.91 ± 0.23 mg/g GAE). In general, Hatay variety was found to have higher antioxidant potential.

Total anthocyanin content of Kombucha samples was increased by black carrot juice incorporation and the fermentation process. Kombucha is a beverage which normally does not contains anthocyanin. In our research, it was showed that Kombucha beverage was able to enrich with black carrot juice in order to increase anthocyanin content. In total, anthocyanin content of Kombucha beverage containing Hatay black carrot variety was increased to 71.05 mg C3G/100 mL from 68.12 C3G/100 g-dw after fermentation (Figure 2, $p \leq 0.05$). Anthocyanins were defined as the richest phenolic compounds (33.81 mg/100 g FW of which 33.051 mg/100 g FW, glycosylated) in black carrot (Smeriglio et al., 2018) that is utilized as a raw material in Kombucha production.

Change in phenolic acids of black carrots by *in-vitro* digestion evaluated in study of Padayachee et al. (2013). They determined that stay bound to plant cell walls during the process. On the other hand, Kamiloglu (2016) reported that ferulic acid content of black carrot samples was increased at the end of the *in-vitro* small intestinal digestion depending upon the partition of the major anthocyanin, cyanidin-3-xylosyl-feruloyl-glucosyl-galactoside to ferulic acid. Moreover, Correa-Betanzo et al. (2014) expressed that caffeic acid determined to be the major component resulting from the hydrolysis of chlorogenic acid by intestinal microbiota.

Sensorial evaluation

Sensorial evaluation scores of Kombucha samples are given in Table 4 ($p \leq 0.05$). The most preferred Kombucha sample is evaluated as *KomH10* sample by the panelists. The highest

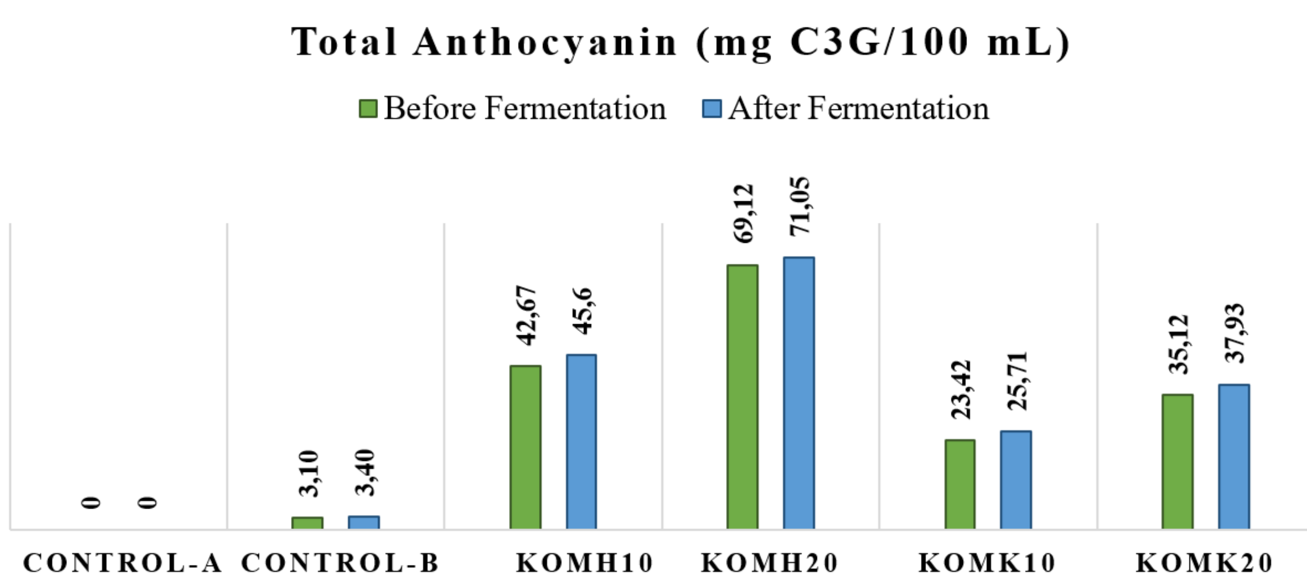

Figure 2. Total anthocyanin contents of black carrot Kombucha samples. Control-A: Sugar fermentation with SCOBY; Control-B: Green tea Kombucha sample; KomH10: 10% Hatay variety black carrot juice added Kombucha sample; KomH20: 20% Hatay variety black carrot juice added Kombucha sample; KomK10: 10% Konya variety black carrot juice added Kombucha sample; KomK20: 20% Konya variety black carrot juice added Kombucha sample

Table 4. Sensorial evaluation.

Sample*	Color	Clarity	Odor	Flavor-taste	Sourness	Overall Acceptability
Control-A	$5.23 \pm 0.34^{e**}$	8.54 ± 0.45^{a}	5.90 ± 0.56^{c}	6.34 ± 0.67^{c}	5.67 ± 0.56^{d}	5.21 ± 0.34^{e}
Control-B	6.45 ± 0.87^{d}	7.23 ± 0.76^{b}	7.23 ± 0.67^{b}	7.34 ± 0.23^{b}	7.54 ± 0.34^{a}	$7.12 \pm 0.56^{b,c}$
KomH10	8.03 ± 0.34^{b}	6.65 ± 0.47^{c}	$7.44 \pm 0.23^{a,b}$	7.87 ± 0.67^{a}	$7.45 \pm 0.94^{a,b}$	7.89 ± 0.69^{a}
KomH20	8.43 ± 0.56^{a}	6.45 ± 0.23^{c}	7.88 ± 0.98^{a}	7.77 ± 0.67^{a}	7.34 ± 0.12^{b}	7.34 ± 0.65^{b}
KomK10	7.01 ± 0.34^{c}	5.78 ± 0.95^{d}	$5.55 \pm 0.45^{c,d}$	6.43 ± 0.65^{c}	6.74 ± 0.41^{c}	6.87 ± 0.23^{c}
KomK20	6.45 ± 0.87^{d}	5.03 ± 0.65^{e}	5.38 ± 0.78^{d}	6.12 ± 0.85^{d}	6.34 ± 0.67^{e}	6.34 ± 0.22^{d}

*Control-A: Sugar fermentation with SCOBY; Control-B: Green tea Kombucha sample; KomH10: 10% Hatay variety black carrot juice added Kombucha sample; KomH20: 20% Hatay variety black carrot juice added Kombucha sample; KomK10: 10% Konya variety black carrot juice added Kombucha sample; KomK20: 20% Konya variety black carrot juice added Kombucha sample; **Mean values ± standard deviation with different superscript in the same colon are significantly different ($p \leq 0.05$).

sensorial scores were belonged to flavor-taste. The highest points were given to *Control-B* and Kombucha with black carrot Hatay variety in terms of overall acceptability. If the results are evaluated according to the frequency of consumption, it was seen that 44% of the panelists consumed Kombucha beverage at first time and they highly liked Kombucha samples prepared with black carrot as seen in Table 4. Panelists also stated that the Kombucha samples with Hatay Black carrot juice were expressed as a fresh and pleasant taste due to green tea aroma. Black carrot was accepted as suitable and attractive substrate for Kombucha beverage by the panelists. Comparing to Kombucha included black carrot belonged to Konya variety, Kombucha included black carrot belonged to Hatay variety were expressed to have more intense and pleasant flavor by the panelists.

The foods that are described as healthy food, is generally not preferred by the consumers due to their sensory properties that are expressed unpleasant. Therefore, beyond its composition and nutritional value, the taste is a more preferable factor by the consumers. Kombucha beverage with black carrot juice is a new product that either has a pleasant taste and healthy content. In this sense, in our research, it is important that each of the Kombucha samples exceeds the limit of acceptability in the sensory evaluation.

In subsequent studies, the effects of fermentation on phenolic compositions of Kombucha beverages containing Hatay black carrot variety and its effects on the metabolism should be examined with further analyses and *in-vivo* studies.

4 Conclusion

Hatay and Konya black carrot varieties which are cultivated in Anatolian Region were investigated for Kombucha beverage production. It was observed that anthocyanin rich black carrot vegetable is suitable substrate for the Kombucha fermentation. Hatay variety was preferred to use Kombucha beverage production due to its pleasant flavor and better color. This supplementation with black carrot Hatay variety caused to significant increase in anthocyanin content (71.05 mg C3G/100 mL), total phenolic content and antioxidant capacity ($TEAC_{CUPRAC}$ and $TEAC_{ABTS}$) in terms of extractable, hydrolysable and bioaccessible phenolics.

Kombucha beverage, having increasing consumption in our era, possessing anti-inflammatory, anti-microbial, anti-inflammatory and antioxidant effects, can be used as a significant functional food to reduce risk of some chronical diseases such as diabetes, obesity, cardiovascular diseases. Black carrot as a supplement in Kombucha production which is a rich substrate of anthocyanin and phenolic compounds, therefore can be used in various food formulations due to rich nutrient content.

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