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Health Benefits of Kombucha Tea Enriched with Olive Leaf and Honey

Nurcan Değirmencioğlu[1*], Elif Yildiz[2], Metin Guldas[3] and Ozan Gurbuz[4]

[1]*Department of Food Processing, Bandirma Vocational High School, University of Bandirma Onyedi Eylul, Bandirma-Balıkesir, Turkey*

[2]*The College of Agriculture Urban Sustainability and Environmental Sciences, University of the District of Columbia, Washington, DC 20008, USA*

[3]*Department of Nutrition and Dietetics, Faculty of Health Sciences, University of Bursa Uludag, 16059 Bursa, Turkey*

[4]*Department of Food Engineering, Faculty of Agriculture, University of Bursa Uludag, 16059 Bursa, Turkey*

[*]Correspondence to:
Nurcan Değirmencioğlu
Department of Food Processing
Bandirma Vocational High School
University of Bandirma Onyedi Eylul
Bandirma-Balıkesir, Turkey
Tel: +902667149302
E-mail: ndegirmencioglu@bandirma.edu.tr, nurcan.degirmencioglu@gmail.com

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Abstract

Kombucha is a fermented beverage that is produced by fermenting tea and beet sugar solution under aerobic conditions by adding symbiotic culture (yeasts and acetic acid bacteria) named as SCOBY. The health benefits of Kombucha are mainly associated with the phenolic content of substrate and the microbiota of Kombucha cultures. Olive leave and honey contain various antioxidant phytochemicals and have been used as folk medicine for long time in the form of infusion or can also be used directly. Phenolic compounds play role as antioxidant to reduce cardiovascular and neurodegenerative diseases. They also cause to weight loss, reduce the dimension of adipose tissue and blood triglyceride level, promote digestion, and lower the inflammation.

The main objective of this research was to develop a beverage using dried olive leaf (0-5%) and honey (20 °Brix) as an alternative to beet sugar required for the Kombucha fermentation. Phenolic contents of extractable and hydrolysable extracts and the antioxidant activity of the fermented Kombucha beverages supplemented with beet sugar were higher following to the fermentation. The maximum phenolic contents were determined at the 12th day. However, the bioavailability of phenols in the beverages supplemented with honey and olive leaves was much higher than the samples supplemented with beet sugar only.

Keywords

Kombucha tea, Fermentation, Antioxidant capacity essays, Phenolics, Bioaccessibility

Introduction

"Kombucha" is a beverage made by fermenting tea (generally black tea or sometimes green and oolong tea) and sugar, with a Symbiotic Culture of Bacteria and Yeast (SCOBY) [1]. The SCOBY is a selulo comprises yeasts (*Saccharomyces cerevisiae*, *Saccharomyces ludwigii*, *Saccharomyces apiculatus*, *Schizosaccharomyces pombe, Torulaspora delbrueckii, Zygosaccharomyces bailii, Z. rouxii, Z. kombuchaensis sp.nov., Brettanomyces bruxellensis, B. lambicus, B. custersii*, *Pichia fermantans, P. membranaefaciens, Candida crusei, C. albicans, C. stellata, Kloeckera apiculata, Kluyveromyces africanus, Medusomyces gisevii, Torulopsis sp.,Dekkera sp.*), acetic acid bacteria (*Acetobacter xylinum, A. xylinoides, A. aceti, A. pasteurianus, Komagataeibacter europaeus, Bacterium gluconicum, Gluconobacter oxydans, G. hansenii*), lactic acid bacteria (*Lactobacillus sp., Lactococcus sp., Leuconostoc sp., Bifidobacterium sp.*),

and others (*Actinobacteria, Bacteroidetes, Deinococcus-Thermus, Firmicutes* and *Proteobacteria)* which varies depending on the climatic and geographic conditions [2-16].

Kombucha is extensively consumed for its prophylactic and beneficial properties such as anticarcinogenic, antimicrobial, antioxidant, antihyperglycemic, antihyperlipidemic [11, 17], and it contains many metabolites, such as organic acids (glucuronic, gluconic, lactic, acetic, succinic, malic, oxalic etc.), water-soluble vitamins (C, B_1, B_2, B_{12}), sugars, tea polyphenols, ethanol, amino acids (lysine, folic acid), minerals (Cu, Fe, Mn, Ni, Zn, etc.), carbon dioxide, hydrolytic enzymes, are produced by this complex symbiotic consortium [8, 14, 15, 18-21].

Phytochemicals as natural sources, even presenting in small quantities, improve the nutritional value of food products and has great health effects in human body such as preventing or reducing diabetes, hypertension, cardiovascular diseases, etc. [22, 23].

Olea europaea L. subsp. *europaea* is considered one of the oldest agricultural crops in the world and is widely used for the production of its products (olive oil, table olive, olive paste etc.). Olive leaves are a by-product of olive harvesting, and a cheap raw material in order to evaluate the valuable components (phenolic compounds) [24]. Honey is a natural food product, and the biologically active components (antioxidant and antibacterial) of its have nutritional, health-promoting and therapeutic properties. The antioxidant and antibacterial activity of honey is correlated with phenolic compounds, vitamins (C and E), enzymes (catalase, peroxidase etc.) and trace elements [25]. Phenolic compounds, with assorted biological properties such as antimicrobial, antioxidant, anti-inflammatory, anticancer, radioprotective, preventing neurodegenerative disorders (Alzheimer, Parkinson etc.), regulating blood pressure and cholesterol levels [24, 26, 27]. Olive leave and honey serve as a source of antioxidant phytochemicals such as phenolic compounds, and have been used as folk medicine for long time in the form of infusion or can also be used directly, in pharmaceutical industry as a dietetic tablets, in medicine, or as a functional food supplements [24, 25].

The aim of the study was to evaluate of total phenolic content, antioxidant capacity and their bioaccessibility of Kombucha tea, supplemented with dried olive leaf (0-5.0%) and honey (20 °Brix), by an *in vitro* gastrointestinal (GI) digestion.

Material and Methods

Materials

Black tea (Lipton Brand) sachets was purchased from local market in Balikesir, Turkey and used as a substrate for the fermentation. SCOBY was provided from previously research of authors. The sucrose (beet sugar obtained from Torku Co., Turkey) and honey (Highland honey obtained from Balparmak Co., altitude 1000 m, water soluble dry matter 81 °Bx) used as a carbon source. Samples of dried organic olive leaf were obtained from İlhan Sarı Organic Olive Farm from Manisa, Turkey).

Kombucha production

The Kombucha production was adapted from Greenwalt et al. [2] with slight modifications. Briefly, for Kombucha fermentation one-liter sterile water was boiled at 95 °C for 15 min before adding of carbon sources (commercial crystalline sucrose, 20.0% or honey which has been diluted to 20 °Bx with sterile water) and 6 g/L of black tea sachets. The mixture was steeped to infusion of tea phenols for 15 mins. After removing tea bags, the teas were filled in sterile glass jars, allowed to cool down to room temperature and then was added SCOBY, liquid old Kombucha (10%, v/v) as inoculums, and supplemented with dried olive leaf (1.0%, 2.5%, and 5.0%, w/v), respectively. All glass jars were covered with cheesecloth and kept at 30 ± 2 °C in dark condition for 12 days. It was used as a control samples that does not contain dried olive leaf (Table 1).

Table 1: Abbreviations of Kombucha samples.

Samples	Olive Leaf	Beet Sugar 20%	Honey 20 °Bx
OSC	-	+	-
OS-1.0	1.0 %	+	-
OS-2.5	2.5 %	+	-
OS-5.0	5.0 %	+	-
OHC	-	-	+
OH-1.0	1.0 %	-	+
OH-2.5	2.5 %	-	+
OH-5.0	5.0 %	-	+

Methods

Kombucha samples evaluated as in terms of extractable, hydrolysable, bioaccessible phenolics according to Vitali et al. [28] and Bouayed et al. [29] with some modifications. The total phenol content (TPC) and antioxidant capacity of extracts were determined by using a modified Folin-Ciocalteu colorimetric method and ABTS (2,20 -azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) antioxidant capacity by Apak et al. [30]. The results are expressed as mg gallic acid equivalents (GAE) per 100 mL sample for TPC and as micro mole (µmol) Trolox equivalent (TE) per mL sample for antioxidant capacity. The bioaccessibility % extracts were calculated according to Anson et al. [31] in accordance with total phenolic content and antioxidant capacity analysis results.

Results and Discussion

The phenolic content of the Kombucha samples with sweetened beet sugar or honey, and also supplemented olive leaf are from 13.40-18.18 to 15.82-21.03 mg/100 g GAE (beet sugar) and from 10.40-15.40 to 12.09-17.09 mg/100 GAE (honey), respectively, at 12th day (Table 2). Extractable polyphenols have low-intermediate molecular mass, and can be extracted different solvents such as water, methanol, acetone etc., while hydrolysable polyphenols are bound to dietary fiber or protein, and they require after the hydrolysis during the extraction to make them soluble and bioavailable [32, 33]. In this study, it was determined that in extractable, hydrolysable,

and bioaccesible phenolics results have shown similar reaction for antioxidant capacity and total phenolic content (Table 2 and 3). The highest extractable and hydrolysable phenolic values (21.83 and 53.81 mg/100 mg GAE, respectively) was observed in the samples 5.0% olive leaf and beet sugar supplemented Kombucha samples in 12th day ($p \leq 0.05$), while the highest bioaccessible phenolic value was determined at the same samples in 2th day (37.07 mg/100 mg GAE), however in Kombucha samples with supplemented olive leaf (5.0%) and honey have consistently increased for fermentation days.

depends on climatic conditions, botanical origin, processing, etc. The addition of source of polyphenols, olive leaf and honey, significantly released of polyphenols into Kombucha liquid phase for 12 days and the antioxidant activity of the samples were influenced positively from this release.

The effects on human health of phenolics depended on their bioaccessibility, absorption in gastrointestinal tract, and their bioavailability *in vivo* [35]. Some food processing steps such as fermentation shows positive impact on the bioaccessibility of phenolics. It was reported that in previous

Table 2: Results of extractable, hydrolysable and bioaccessible total phenolics of Kombucha samples.

Sample	Extractable Phenolics			Hydrolysable Phenolics			Bioaccessible Phenolics		
	1st Day	2nd Day	12th Day	1st Day	2nd Day	12th Day	1st Day	2nd Day	12th Day
OSC	$13.40 \pm 0.32^{Egm*}$	14.56 ± 0.32^{Egl}	15.82 ± 0.32^{Dgk}	37.28 ± 0.53^{Fgr}	39.81 ± 0.53^{Fep}	41.71 ± 0.53^{Fgo}	29.19 ± 0.24^{Dgs}	28.38 ± 0.37^{Dgt}	20.64 ± 0.27^{Ghu}
OHC	10.40 ± 0.22^{Ghm}	11.35 ± 0.22^{Ghl}	12.09 ± 0.22^{Fhk}	39.60 ± 0.44^{Ffr}	41.08 ± 0.44^{Ddp}	42.13 ± 0.44^{Ego}	21.70 ± 0.22^{Hhu}	23.07 ± 0.22^{Ght}	24.02 ± 0.22^{Egs}
OS-1.0	14.70 ± 0.34^{Dem}	16.17 ± 0.34^{Cel}	17.44 ± 0.34^{Cck}	41.00 ± 0.32^{Eefr}	43.96 ± 0.32^{Ccp}	45.86 ± 0.32^{Deo}	30.88 ± 0.06^{Cet}	31.20 ± 1.80^{Ces}	22.72 ± 0.16^{Ffu}
OH-1.0	11.57 ± 0.16^{Ffm}	12.52 ± 0.16^{Ffl}	13.25 ± 0.16^{Efk}	41.92 ± 0.32^{Eer}	43.40 ± 0.32^{Ccp}	44.45 ± 0.32^{Dfo}	22.86 ± 0.16^{Gfu}	24.30 ± 0.06^{Fft}	25.25 ± 0.06^{Des}
OS-2.5	16.49 ± 0.22^{Bcm}	18.29 ± 0.22^{Bcl}	19.55 ± 0.22^{Bck}	45.37 ± 0.21^{Ddr}	48.75 ± 0.21^{Bbp}	50.65 ± 0.21^{Cco}	33.03 ± 0.38^{Bct}	34.82 ± 0.38^{Bcs}	25.11 ± 0.11^{Ddu}
OH-2.5	14.27 ± 0.06^{Ddl}	15.22 ± 0.06^{Ddk}	15.96 ± 0.06^{Ddk}	47.34 ± 0.12^{Ccr}	48.82 ± 0.12^{Bbp}	49.87 ± 0.12^{Cdo}	25.57 ± 0.06^{Fdu}	26.98 ± 0.12^{Edt}	27.93 ± 0.12^{Bcs}
OS-5.0	18.18 ± 0.22^{Aam}	19.76 ± 0.22^{Aal}	21.03 ± 0.22^{Aak}	48.96 ± 0.92^{Bbr}	51.91 ± 0.92^{Aap}	53.81 ± 0.92^{Aao}	35.39 ± 0.72^{Aat}	37.07 ± 0.72^{Aas}	26.70 ± 0.46^{Cbu}
OH-5.0	15.40 ± 0.21^{Cbm}	16.35 ± 0.21^{Cbl}	17.09 ± 0.21^{Cbk}	49.59 ± 0.42^{Aar}	51.07 ± 0.42^{Aap}	52.12 ± 0.42^{Bbo}	26.70 ± 0.21^{Ebu}	28.07 ± 0.21^{Dbt}	29.02 ± 0.21^{Aas}

* Data are mean ± SD (mg/100 mg GAE); Values with the different capital letters (A-H) within a column are between different olive leaf concentrations significantly different at a $p < 0.05$, with the different lowercase letters (a-h) within a column are between the beet sugar sources different significantly different at a $p < 0.05$, and with the different lowercase letters (k-u) within a row are between the fermentation days of significantly different at a $p < 0.05$.

Table 3: Antioxidant capacity results of extractable, hydrolysable and bioaccessible phenolics of Kombucha samples by ABTS methods.

Sample	Extractable Phenolics			Hydrolysable Phenolics			Bioaccessible Phenolics		
	1st Day	2nd Day	12th Day	1st Day	2nd Day	12th Day	1st Day	2nd Day	12th Day
OSC	$1.50 \pm 0.01^{Egm*}$	2.18 ± 0.02^{Egl}	2.49 ± 0.08^{Egk}	1.65 ± 0.08^{Fhr}	2.09 ± 0.03^{Fhp}	2.64 ± 0.02^{Gho}	2.47 ± 0.01^{Ghu}	2.84 ± 0.01^{Ght}	3.14 ± 0.02^{Ghs}
OHC	1.12 ± 0.01^{Ghm}	1.27 ± 0.01^{Ghl}	1.84 ± 0.05^{Fgk}	2.98 ± 0.02^{Dgr}	3.12 ± 0.01^{Egp}	3.32 ± 0.01^{Fgo}	3.55 ± 0.02^{Dgt}	3.65 ± 0.01^{Fgt}	4.36 ± 0.03^{Dgs}
OS-1.0	2.37 ± 0.01^{Cem}	2.43 ± 0.01^{Cel}	3.15 ± 0.03^{Dek}	2.66 ± 0.05^{Efr}	3.84 ± 0.02^{Ceo}	3.34 ± 0.03^{Ffp}	2.91 ± 0.01^{Ffu}	3.85 ± 0.01^{Efs}	3.48 ± 0.03^{Fft}
OH-1.0	1.33 ± 0.01^{Ffl}	1.38 ± 0.02^{Ffl}	2.46 ± 0.02^{Efk}	3.21 ± 0.02^{Cep}	3.45 ± 0.01^{Dfo}	3.51 ± 0.01^{Eeo}	3.84 ± 0.02^{Ceu}	4.09 ± 0.01^{Cet}	5.11 ± 0.06^{Ces}
OS-2.5	2.81 ± 0.01^{Bcl}	2.65 ± 0.01^{Bcm}	4.13 ± 0.11^{Cck}	3.21 ± 0.23^{Cdr}	4.49 ± 0.03^{Bcp}	5.85 ± 0.03^{Bco}	3.31 ± 0.02^{Edu}	3.96 ± 0.02^{DEds}	3.81 ± 0.06^{Eds}
OH-2.5	2.20 ± 0.01^{Ddm}	2.31 ± 0.01^{Ddl}	4.02 ± 0.57^{Cck}	3.39 ± 0.01^{Ccr}	3.72 ± 0.01^{Cdp}	4.05 ± 0.03^{Ddo}	4.05 ± 0.01^{Bcu}	4.28 ± 0.01^{Bct}	5.44 ± 0.08^{Bcs}
OS-5.0	3.26 ± 0.01^{Aam}	3.97 ± 0.01^{Aal}	5.02 ± 0.04^{Aak}	4.14 ± 0.03^{Aar}	5.22 ± 0.02^{Aap}	6.63 ± 0.05^{Aao}	3.63 ± 0.02^{Dbu}	4.07 ± 0.01^{CDbt}	4.37 ± 0.03^{Dbs}
OH-5.0	2.25 ± 0.01^{CDbm}	2.47 ± 0.01^{CDbl}	4.39 ± 0.07^{Bbk}	3.89 ± 0.02^{Bbr}	4.51 ± 0.01^{Bbp}	4.82 ± 0.02^{Cbo}	4.25 ± 0.01^{Aau}	4.82 ± 0.01^{Aat}	6.21 ± 0.03^{Aas}

* Data are mean ± SD (µmol trolox/g); Values with the different capital letters (A-H) within a column are between different olive leaf concentrations significantly different at a $p < 0.05$, with the different lowercase letters (a-h) within a column are between the beet sugar sources different significantly different at a $p < 0.05$, and with the different lowercase letters (k-u) within a row are between the fermentation days of significantly different at a $p < 0.05$.

Antioxidant capacity of samples has increased by time with fermentation according to $TEAC_{ABTS}$. The highest $TEAC_{ABTS}$ value (6.63 µmol trolox/g) was observed in the samples 5.0% olive leaf and beet sugar supplemented Kombucha samples in 12th day while % bioaccessibility was higher (6.21 µmol trolox/g) in 5.0% olive leaf and honey supplemented Kombucha samples ($p \leq 0.05$). Some authors stated that many minor components (phenolic acids, flavonoids, proteins, enzymes, organic acids, minerals etc.) are present in honey which are responsible for antioxidant activity [25, 34], and it

studies, the symbiotic consortium of acetic acid and some lactic acid bacteria can enhance the bioaccessibility of phenolics, and the antioxidant activity [36]. The presence of these microorganisms may contribute that the antioxidant activity of beverages due to metabolic activity of their [8, 37]. The bioaccessibility (%) antioxidant capacity of Kombucha samples was positively affected from the addition of olive leaf and honey compare beet sugar and olive leaf are supplemented (Figure 1). It was observed that the highest value was determined in the sample supplemented with 1.0% olive leaf

and honey at 12th day. The bioaccessibility (%) total phenols of samples are compared each other, in all samples supplemented with olive leaf and honey have been found to increase during fermentation while a decrease was observed in others (OSC, OS1.0, OS2.5, and OS5.0).

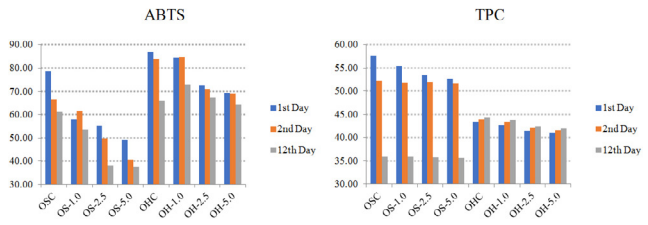

Figure 1: Bioaccesibility (%) of antioxidant capacity and total phenol results of Kombucha samples.

On the basis of these results; the total phenolic content and antioxidant capacity of Kombucha samples and their % bioaccessibilities have affected by C-source, chemical composition of olive leaf and honey, and the interaction of phenolic compounds with each other. Comparing the extractable to hydrolysable phenolics, hydrolysable are high, extractable is probably physically efficient. Furthermore, fermentation time assists in increasing phenolics for both beet sugar and honey supplementation. It may be recommended 12 day as fermentation time for maximum phenolic contents. Beet sugar is high calorie and more fermentable by SCOBY microbiota; honey is a good potential as natural ingredient but may show antimicrobial effect on the SCOBY microbiota. When evaluating of olive leaf ratio, results has increased with leaf amount 5.0% is the most effective ration in $TEAC_{ABTS}$.

Conclusion

Enrichment of kombucha tea with olive leaves and honey caused to increase in phenolic contents of extractable and hydrolysable extracts and the antioxidant activity of the fermented Kombucha beverages supplemented with beet sugar were higher following to the fermentation. The maximum phenolic contents were determined at the 12th day. However, the bioavailability of phenols in the beverages supplemented with honey and olive leaves was much higher than the samples supplemented with beet sugar only. It has been concluded that honey and olive can be good sources for the enrichment of kombucha tea due to satisfactory taste and flavor and nutritional characteristics.

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