



## DETERMINATION OF PHYSICOCHEMICAL AND SENSORY PROPERTIES OF KOMBUCHA BEVERAGE PREPARED WITH SAFFRON

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### ABSTRACT

This research aimed to explore the impacts of saffron extract (SE) on composition and sensorial features of kombucha beverage prepared with green tea (GT). For this point, SE was added to GT infusion then fermented at  $28 \pm 2$  °C (120 h). Total acidity of samples prepared with GT (control) and saffron extract added kombucha (SEK) reached to 3.96 and 4.02 g/L, respectively at the end of the fermentation. Total phenolic content (TPC) and total antioxidant capacity (TAC) of the beverages raised in proportion to uncultivated samples. The current findings demonstrated that SE addition to GT infusion resulted with an increment in TPC and TAC. At the end of the fermentation, increase of TPC in control and SEK were determined as 73.51% and 43.85%, respectively. The results revealed that SE addition to GT for kombucha fermentation provided enhanced nutritional properties as well as improving functional and sensorial attributes of the beverage.

**Keywords:** Kombucha, saffron, fermentation, antioxidant capacity

## SAFRAN KULLANILARAK HAZIRLANAN KOMBUCHA İÇECEĞİNİN FİZİKOKİMYASAL VE DUYUSAL ÖZELLİKLERİNİN BELİRLENMESİ

### ÖZ

Bu çalışmada safran ekstaktı (SE) kullanımının, yeşil çay (YÇ) ile hazırlanan kombucha içeceğinin bileşimi ve duyuşal özellikleri üzerine olan etkilerinin araştırılması amaçlanmıştır. Bu amaçla SE, YÇ infüzyonuna eklenmiş ve  $28 \pm 2$  °C'de (120 saat) fermente edilmiştir. YÇ ile hazırlanmış (kontrol) ve SE ilave edilmiş kombucha örneklerinin toplam asitlik değerleri fermentasyon sonunda sırasıyla 3.96 ve 4.02 g/L'ye ulaşmıştır. İçeceklerin toplam fenolik madde içeriği (TFM) ve toplam antioksidan kapasite değerlerinde (TAK), kültür içermeyen örneklere göre artış görülmüştür. Mevcut bulgular, YÇ infüzyonuna SE ilavesinin, TFM ve TAK değerlerinde artış meydana getirdiğini göstermiştir. Fermentasyon sonunda YÇ ile hazırlanan ve SE ilave edilen kombucha örneklerindeki TFM artışı sırasıyla %73.51 ve %43.85 olarak belirlenmiştir. Bu çalışmaya ait sonuçlar, kombucha fermentasyonunda kullanılan yeşil çaya safran ekstraktı eklenmesi ile, içeceğin fonksiyonel ve duyuşal özelliklerinin iyileştirilmesinin yanı sıra, besleyici özelliklerinin geliştirildiğini ortaya koymuştur.

**Anahtar kelimeler:** Kombucha, safran, fermentasyon, antioksidan kapasite

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## INTRODUCTION

Customers awareness to consume food products which support the maintenance of a good health and wellness and also prevent the risk of several illnesses through their bioactive components has been substantially increased. This demand had led to the development of functional foods as a major sector in the food market (Salmerón et al., 2015; Mousavi and Mousavi, 2019). Functional beverages are the most rapidly growing segment with strong consumer demand. Therefore, many studies have concentrated on the potential utilization of health promoting ingredients for functional beverages (Marete et al., 2011).

Kombucha is a very popular, non-alcoholic [ $<0.5\%$  (v/v)], fermented functional drink consumed worldwide due to many health benefits (i.e. antimicrobial, antioxidant, antidiabetic, and anticancer) (Marsh et al., 2014a; Chakravorty et al., 2019). The sweetened black or green tea and a mixed culture known as SCOBY (Symbiotic Culture of Bacteria and Yeast) including acetic acid bacteria and yeast species from the genus *Acetobacter*, *Gluconobacter*, *Saccharomyces*, *Torulopsis*, *Pichia*, *Brettanomyces*, and *Zygosaccharomyces* are used for kombucha production (Marsh et al., 2014b). Firstly, the yeasts convert the carbon sources to ethyl alcohol, which is subsequently converted to organic acids by the bacteria (Jayabalan et al., 2014). In traditional process, during fermentation, a layer of bio-cellulose pellicule segment is formed. Kombucha has high antioxidant capacity that have been associated with prevention of cancer, supporting of the immune system, improvement of joint rheumatism (Jayabalan et al., 2014, Srihari et al. 2013 a, Deghrigue et al. 2013; Shenoy et al. 2019). Jasmine tea, lemon balm tea, peppermint tea, mulberry tea, echinacea tea and winter savory tea can be used for kombucha production (Velićanski et al., 2007; Cvetković, 2008; Jayabalan et al., 2014). Moreover, these herbs can be used in combination with black or green tea for fermentation. All of these ingredients include many phytochemicals, provide the preferred antioxidant and antimicrobial activity and may prevent diseases related with oxidative stress

(Ćetković et al., 2007; Velićanski et al., 2014; Essawet et al., 2015).

The aim of this study was to reveal the possibility of using saffron as an ingredient with high phytochemical content for kombucha preparation to improve nutritional value, functional and sensory properties of the beverage.

Saffron (*Crocus sativus* L.) is one of the valuable herbs widely cultivated in India, Iran, Turkey, Morocco, Spain, Italy, France and Greece (Paşayeva and Tekiner, 2014; Azarabadi and Özdemir, 2018). It is widely used as a food colouring and flavouring spice, preservative and also utilized in cosmetics, textile, and medical applications (Rahaiee et al., 2015; Bathaie and Mousavi, 2010; Azarabadi and Özdemir, 2018).

The main bioactive compounds of saffron are crocin, picrocrocin and flavonoids (Termentzi and Kokkalou, 2008) presenting many health beneficial features into the food and beverages (Urbani et al., 2016). It also contains carbohydrates, mucilage, starch, gums, proteins, amino acids, fats, anthocyanins, fiber, vitamins (B1 and B2), minerals, alkaloids, saponins, safranal and picrocrocin together with other chemical compounds (Negbi, 1997; Melnyk et al., 2010; Shahi et al., 2016; Serrano-Díaz et al., 2013; Alavizadeh et al., 2014). Azarabadi and Özdemir (2018) analysed volatile compounds in saffron samples and found acetic acid, 2-(5H)-furanone, isophorone, 4-ketoisophorone, 2,6,6-trimethyl-1,4-cyclohexanedione, eucarvone and safranal as significant compounds.

Novel researches have focused on the antioxidant, antidepressant, anticancer, neuroprotective, and cardioprotective effects of bioactives of saffron (Hosseinzadeh et al., 2009; Ghasemi et al., 2015; Ashrafi et al., 2015; Aung et al., 2007; Mehri et al., 2012; Goyal et al., 2010). The research of Akbari- Fakhrebadi et al. (2019) indicated that saffron improved mitochondrial biogenesis, reduced oxidative stress, inflammation, and modulated metabolic biomarkers in exercised rats. Several researches have reported that saffron has also

antiinflammatory, cytotoxic, anticonvulsant, antihypertensive, antitussive, aphrodisiac and immunomodulating effects (Paşayeva and Tekiner, 2014). Melnyk et al. (2010) reported that gastric disorders, cardiovascular disease, insulin resistance, premenstrual syndrome, depression, insomnia and anxiety are alleviated or prevented by saffron constituents. To the best of our knowledge, this was the first study related to the use of saffron in kombucha production.

### MATERIALS AND METHODS

Green tea (*Camellia sinensis*) and sucrose were purchased from a local market in Bursa. Saffron stigmas harvested in 2018 was also purchased from Karataş Tic. in Safranbolu, Karabük, Turkey.

#### Preparation of kombucha

Preparation of sweetened green tea was shown in Figure 1. Firstly, 70 g of sucrose were dissolved in

900 mL of water and pasteurized at 98 °C for 15 min. Then, 8 g of green tea (ready to use tea bags) was infused with this water at 95 °C for 12 min and then filtered. Saffron was extracted according to the previous literature, in which a new saffron-based probiotic beverage was produced (Dabbagh Moghaddam et al., 2018). 1 g of dried saffron stigmas poured into 100 mL distilled water and stirred at 300 rpm overnight at room temperature. According to the results of preliminary tests, saffron extract was added as 10% (v/v) to cooled green tea infusion then inoculated with kombucha culture [10% (v/v)]. Kombucha culture used [10% (v/v)] in this study, was fermentation liquid of kombucha obtained after 2 weeks of fermentation (28±2 °C) on green tea infusion (10 g/L) sweetened with sucrose (70 g/L). Fermentation monitored at 28±2 °C. The green tea kombucha beverage was analyzed as the control sample.

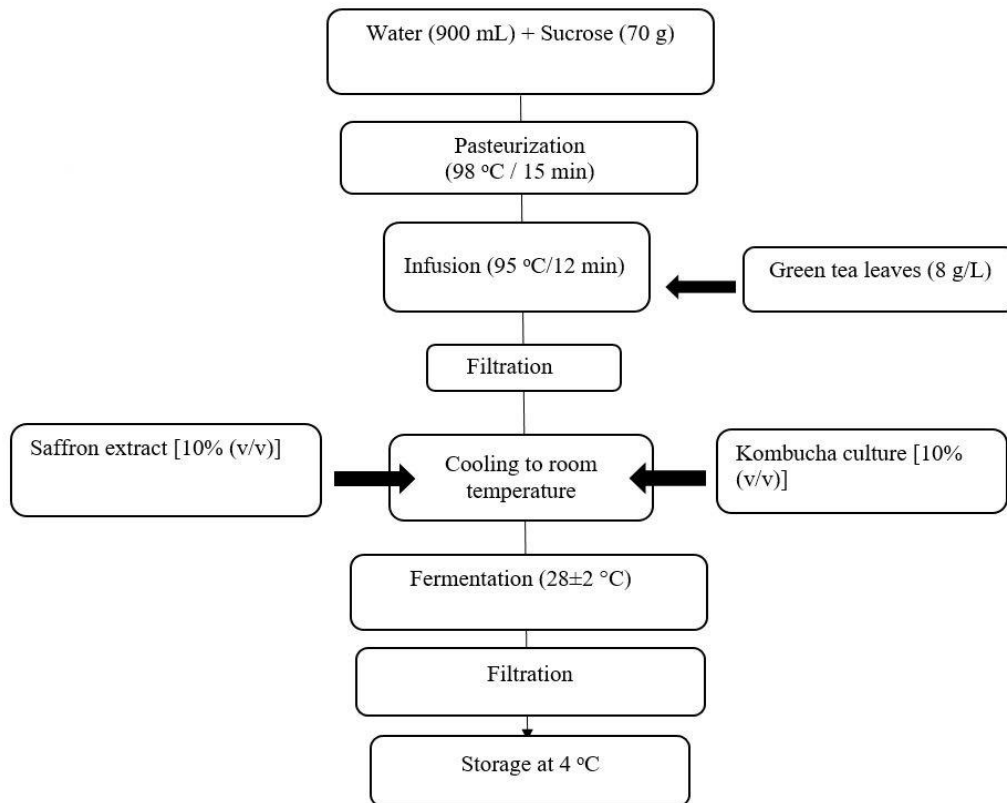


Figure 1. Kombucha beverage production

## ANALYSES

### Determination of some physical and chemical properties

Total acidity analysis were conducted with the potentiometric method, in which the samples were titrated with 0.1 N NaOH to pH 8.1 and the results were expressed as g/L acetic acid. (Cemeroglu, 2007). The pH was analyzed by a digital pH meter (Mettler Toledo Sevencompact pH/Ion pH meter, Canada). For colour analysis, Konica Minolta Chroma Meter, CR-5 (Japan) was used and L\*, a\*, b\*, chroma (C\*) and hue (h°) values were measured. L\*, a\*, b\* values were displayed as lightness/darkness, redness/greenness, and yellowness/blueness respectively. Chroma indicated the colour intensity and it changes from 0 (completely unsaturated) to 100 or more (pure colour) while hue value reflects the tone and is represented by the angles in red, yellow, green, and blue colour (Bakker et al., 1986).

### Extraction of Samples

Samples were extracted in accordance with the procedure of Vitali et al. (2009). 2 mL of sample was mixed with 20 mL extraction solution including HCl /methanol/water (1:80:10, v/v) and then shaken in a water bath at 250 rpm for 2 h at 20 °C. Right after, centrifugation was applied to the mixture at 3500 rpm for 10 min at 20 °C then they were stored at -20 °C until analyzed.

### Total Phenolic Content and Total Antioxidant Capacity Analyses

Previously prepared extracts (according to Vitali et al., 2009) were used for the determination of total phenolic content (TPC) and total antioxidant capacity (TAC). Shimadzu (UV 1208) spectrophotometer (Japan) was used for TPC and TAC analyses and all trials were performed in triplicate. TPC was determined according to Folin-Ciocalteu (FC) spectrophotometric method (Spanos and Wrolstad, 1990). Gallic acid was used for the calibration of the standard curve ( $R^2=0.9835$ ). 0.2/2.3/0.15 mL of extract/distilled water/ FC were added respectively and vortexed for 15 s. After the addition of 0.3 mL  $\text{Na}_2\text{CO}_3$  (35%) solution, the test tubes stayed at the dark for 2 h. Absorbance was measured at 725 nm and

the result was calculated as “mg gallic acid equivalent (GAE)/100 mL total soluble solid (tss). Total antioxidant capacity was determined with DPPH (2,2-diphenyl-1-picrylhydrazyl), CUPRAC (Cupric ion reducing antioxidant capacity) and FRAP (Ferric reducing antioxidant power) assays respectively. Trolox were used as the standard for the calibration curve in TAC analyses and determined as  $R^2 = 0.9929$ ,  $R^2 = 0.9987$  and  $R^2 = 0.9993$  for DPPH, CUPRAC and FRAP methods respectively. All of the TAC results were expressed as  $\mu\text{mol trolox/mL tss}$ . For the DPPH assay, 0.1 mL extract was mixed with 3.9 mL DPPH radical ( $6 \times 10^{-5}$  M) and vortexed for 30 s. Test tubes were stayed in dark at room temperature for 30 min to let the reaction occur and then the absorbance was measured at 515 nm (Katalinic et al. 2006). In CUPRAC method,  $\text{CuCl}_2$  ( $1 \times 10^{-2}$  M), neocuproine ( $7.5 \times 10^{-3}$  M) and ammonium acetate (1M) were mixed for the preparation of CUPRAC. 0.1 mL of extract, 0.9 mL distilled water and 3 mL CUPRAC were mixed and the final absorbance was measured at 450 nm after 30 min (Apak et al., 2008). In FRAP assay, 3 mL of daily prepared FRAP reagent was added to 0.3 mL of distilled water and 0.1 mL of the extract. FRAP was prepared with the mixture of 25 mL of 0.3 mol/L acetate buffer (pH 3.6), 2.5 mL 20 mmol/L  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  and 2.5 mL 10 mmol/L TPTZ solution in mmol/L HCl. The test samples and FRAP solution were incubated at 37 °C for 30 min. Afterwards, absorbance was measured at 595 nm (Benzie and Strain, 1996).

### Sensory analysis

Sensory analysis was completed at 0, 1 and 3 storage days. Colour, odour, appearance, sweetness, sourness and overall acceptability of the samples were evaluated. A nine-point hedonic scale test differing from “like extremely (9)” to “dislike extremely (1)” was used for these quality criterias (Altuğ and Elmacı, 2011). Ten panellists scored the beverages coded with three digit random numbers.

### Statistical analysis

Statistical evaluation was conducted using JMP software package version 8.0 (SAS Institute Inc. NC, 27513). As the significant differences were

determined ( $P < 0.05$ ), the least significant difference (LSD) test was used in defining the differences among means in three replications (Granato et al., 2014).

## RESULTS AND DISCUSSION

### pH and Total Acidity

pH and total acidity values of samples were given in Table 1. Samples were analyzed for their physicochemical properties during 120 hours of fermentation. The pH got a dramatic decline. Total acidity of kombuchas prepared with green tea (control) and saffron extract added kombucha

reached to 3.96 g/L and 4.02 g/L, respectively at the end of the 5th days of fermentation. All samples were accepted ready for consumption with these acidities and then stored at 4 °C for 3 days. Depending on the organic acid production, pH of kombucha beverages decreased. During storage, decrease in pH continued. Similar pH values were determined when kombucha beverages were prepared with thyme, lemon balm, peppermint and sage (Velićanski et al., 2013). The pH values of red and black goji berry kombucha beverages were monitored as 3.23 and 3.37, respectively (Abuduaibifu and Tamer, 2019).

Table 1. pH and total acidity of kombucha samples

Samples*	pH	Total acidity (g/L) (as acetic acid)
GTI	7.24±1.53 <sup>b</sup>	0.27±0.00 <sup>lm</sup>
GTSI	7.40±1.60 <sup>a</sup>	0.26±0.00 <sup>lm</sup>
G1	4.60±0.07 <sup>c</sup>	0.36±0.00 <sup>k</sup>
SK1	4.60±0.05 <sup>c</sup>	0.34±0.00 <sup>kl</sup>
G2	4.49±0.02 <sup>de</sup>	0.74±0.01 <sup>i</sup>
SK2	4.51±0.07 <sup>d</sup>	0.60±0.00 <sup>j</sup>
G3	4.45±0.22 <sup>ef</sup>	1.10±0.01 <sup>h</sup>
SK3	4.41±0.21 <sup>f</sup>	1.21±0.00 <sup>g</sup>
G4	4.07±0.16 <sup>g</sup>	2.30±0.00 <sup>f</sup>
SK4	4.04±0.17 <sup>g</sup>	2.64±0.00 <sup>e</sup>
G5	3.81±0.02 <sup>hi</sup>	3.96±0.01 <sup>a</sup>
SK5	3.74±0.04 <sup>j</sup>	4.02±0.01 <sup>a</sup>
GS1	3.84±0.03 <sup>h</sup>	3.60±0.00 <sup>c</sup>
SKS1	3.79±0.02 <sup>i</sup>	3.87±0.00 <sup>b</sup>
GS3	3.81±0.06 <sup>hi</sup>	3.48±0.00 <sup>d</sup>
SKS3	3.70±0.01 <sup>j</sup>	3.59±0.00 <sup>c</sup>

Different letters in the same column are significantly different ( $P < 0.05$ ).

Samples\*:

GTI: Sweetened green tea infusion

GTSI: Sweetened green tea and saffron extract added infusion

G1: 24 h fermented green tea kombucha

SK1: 24 h fermented saffron extract added kombucha

G2: 48 h fermented green tea kombucha

SK2: 48 h fermented saffron extract added kombucha

G3: 72 h fermented green tea kombucha

SK3: 72h fermented saffron extract added kombucha

G4: 96 h fermented green tea kombucha

SK4: 96 h fermented saffron extract added kombucha

G5: 120 h fermented green tea kombucha

SK5: 120 h fermented saffron extract added kombucha

GS1: 24 h stored green tea kombucha

SKS1: 24 h stored saffron extract added kombucha

GS3: 72 h stored green tea kombucha

SKS3: 72 h stored saffron extract added kombucha

**Colour analyses**

Table 2 represented the colour values of the samples. As visual attribute of beverages, colour is an important for quality and acceptability (Ulusoy and Tamer, 2019). As shown in Table 2, there were significantly different changes ( $P < 0.05$ ) in  $L^*$ ,  $a^*$ ,  $b^*$ , chroma and hue values of kombucha beverages. While saffron extract addition to green tea infusion decreased  $L^*$  and hue values, it caused an increase of  $a^*$ ,  $b^*$  and chroma values. Saffron is able to present a yellow to red range of colours, related with the utilized amount of saffron (Bathaie and Mousavi, 2010).

The effect of pH, temperature, light, and oxygen on the stability in water extracts of pigments of saffron was previously determined (Tsimidou and Tsatsaroni, 1993). During fermentation and storage,  $L^*$  values of both green tea kombucha and saffron extract added kombucha increased. Ayed et al. (2017) reported that the change of colour values due to chemical modifications of the phenolics and carotenoids during fermentation and storage. Watawana et al. (2018) indicated that microbial transformation of polyphenols causes the colour decrease in kombucha beverages.

Table 2. Colour values of the samples

Samples*	$L^*$	$a^*$	$b^*$	Chroma ( $C^*$ )	Hue ( $h^\circ$ )
GTI	85.03±0.01 <sup>f</sup>	2.80±0.16 <sup>i</sup>	37.15±0.04 <sup>b</sup>	37.30±0.04 <sup>e</sup>	87.36±3.04 <sup>ab</sup>
GTSI	75.27±0.01 <sup>m</sup>	20.04±1.91 <sup>d</sup>	91.64±0.36 <sup>a</sup>	91.77±1.28 <sup>d</sup>	82.07±2.71 <sup>c</sup>
G1	89.32±0.00 <sup>a</sup>	-0.38±0.22 <sup>n</sup>	28.68±7.39 <sup>d</sup>	24.42±0.01 <sup>l</sup>	89.71±2.04 <sup>a</sup>
SK1	78.27±0.01 <sup>h</sup>	17.07±1.30 <sup>h</sup>	94.86±0.22 <sup>a</sup>	95.92±0.04 <sup>c</sup>	81.87±0.58 <sup>c</sup>
G2	85.62±0.01 <sup>e</sup>	1.43±0.50 <sup>j</sup>	33.33±7.70 <sup>bcd</sup>	37.84±0.02 <sup>e</sup>	87.77±0.67 <sup>ab</sup>
SK2	77.30±0.02 <sup>ij</sup>	19.05±1.24 <sup>f</sup>	94.55±0.64 <sup>a</sup>	96.62±0.04 <sup>b</sup>	81.52±0.58 <sup>c</sup>
G3	86.39±1.13 <sup>d</sup>	0.84±0.25 <sup>k</sup>	35.16±2.32 <sup>bc</sup>	33.83±0.01 <sup>f</sup>	88.80±0.39 <sup>ab</sup>
SK3	78.79±0.02 <sup>g</sup>	17.26±1.47 <sup>g</sup>	95.36±0.36 <sup>a</sup>	96.74±0.01 <sup>b</sup>	81.78±0.64 <sup>c</sup>
G4	88.07±0.03 <sup>b</sup>	0.39±0.02 <sup>l</sup>	31.93±1.63 <sup>cd</sup>	30.99±0.01 <sup>g</sup>	89.28±0.13 <sup>ab</sup>
SK4	77.72±0.00 <sup>i</sup>	19.84±0.52 <sup>e</sup>	95.71±0.13 <sup>a</sup>	97.48±0.35 <sup>a</sup>	80.88±0.25 <sup>c</sup>
G5	87.95±0.00 <sup>b</sup>	0.36±0.08 <sup>l</sup>	30.62±0.03 <sup>cd</sup>	30.41±0.05 <sup>g</sup>	89.06±0.44 <sup>ab</sup>
SK5	77.02±0.02 <sup>jk</sup>	20.76±0.59 <sup>c</sup>	95.45±0.26 <sup>a</sup>	97.04±0.06 <sup>ab</sup>	80.43±0.28 <sup>c</sup>
GS1	86.92±0.06 <sup>c</sup>	0.86±0.39 <sup>k</sup>	32.69±2.03 <sup>bcd</sup>	33.87±0.01 <sup>f</sup>	88.90±0.63 <sup>ab</sup>
SKS1	76.66±0.05 <sup>k</sup>	21.83±0.37 <sup>a</sup>	95.19±0.92 <sup>a</sup>	96.98±0.07 <sup>ab</sup>	80.15±0.11 <sup>c</sup>
GS3	88.15±0.01 <sup>b</sup>	0.18±0.04 <sup>m</sup>	30.24±3.13 <sup>d</sup>	28.42±0.03 <sup>h</sup>	86.58±3.26 <sup>b</sup>
SKS3	76.00±0.04 <sup>l</sup>	21.22±0.01 <sup>b</sup>	94.74±0.79 <sup>a</sup>	95.73±0.02 <sup>c</sup>	80.28±0.01 <sup>c</sup>

Different letters in the same column are significantly different ( $P < 0.05$ ).

Samples\*:

GTI: Sweetened green tea infusion

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G1: 24 h fermented green tea kombucha

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G5: 120 h fermented green tea kombucha

SK5: 120 h fermented saffron extract added kombucha

GS1: 24 h stored green tea kombucha

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GS3: 72 h stored green tea kombucha

SKS3: 72 h stored saffron extract added kombucha

### Total Phenolic Content and Total Antioxidant Capacity

TPC and TAC results of kombucha samples were given in Table 3. The current findings indicated that saffron extract addition to green tea infusion caused a 33% increase of total phenolics. After 24 h fermentation increase of total phenolics in green tea kombucha and saffron extract added kombucha were determined as 37.96% and 20.57%, respectively. However, at the end of the 5 days fermentation, this increment was monitored as 73.51% and 43.85% for green tea kombucha and saffron extract added kombucha,

respectively. The highest total phenolic content was determined in the saffron extract added kombucha sample which was stored at 4 °C for 3 days. Gismondi et al. (2012) determined the total phenolics of Italian saffron as  $53.52 \pm 1.75$  µg GAE/mg dry weight (DW). Their result was higher than that reported by Rikabad et al. (2019) for Iran saffron ( $48.08 \pm 2.25$  mg GAE/g DW). Karimi et al. (2010) also reported the total phenolic content of methanolic extract of saffron as 6.5 mg GAE/g DW.

Table 3. Changes of total phenolic content and total antioxidant capacity of kombucha samples

Samples*	Total Phenolic Content (mg GAE** /100 mL tss***)	DPPH (µmol trolox/mL tss)	CUPRAC (µmol trolox/ mL tss)	FRAP (µmol trolox/ mL tss)
GTI	1923.81±89.52 <sub>g</sub>	171.94±11.37 <sub>fg</sub>	285.26±44.41 <sub>h</sub>	303.44±70.37 <sub>f</sub>
GTSI	2558.72±383.65 <sub>f</sub>	196.92±8.22 <sub>cde</sub>	382.02±51.33 <sub>g</sub>	369.78±40.11 <sub>f</sub>
G1	2654.05±588.27 <sub>ef</sub>	189.52±7.43 <sub>def</sub>	364.86±33.99 <sub>g</sub>	386.02±74.43 <sub>f</sub>
SK1	3085.03±630.14 <sub>de</sub>	234.25±4.96 <sub>a</sub>	528.88±50.59 <sub>bcd</sub>	591.81±13.30 <sub>de</sub>
G2	2960.41±138.36 <sub>def</sub>	157.76±6.83 <sub>gh</sub>	478.33±18.75 <sub>de</sub>	629.85±97.72 <sub>cde</sub>
SK2	3370.60±585.96 <sub>abcd</sub>	211.37±24.97 <sub>bc</sub>	531.09±58.61 <sub>bcd</sub>	737.69±93.23 <sub>bc</sub>
G3	3046.30±129.14 <sub>def</sub>	193.81±6.42 <sub>cde</sub>	416.52±17.73 <sub>fg</sub>	718.16±65.42 <sub>bc</sub>
SK3	3670.80±71.31 <sub>ab</sub>	227.73±9.94 <sub>ab</sub>	578.29±15.69 <sub>ab</sub>	738.51±69.37 <sub>bc</sub>
G4	3215.50±215.48 <sub>bcd</sub>	179.87±4.46 <sub>ef</sub>	462.23±19.79 <sub>ef</sub>	723.64±44.65 <sub>bc</sub>
SK4	3727.35±48.55 <sub>a</sub>	245.64±12.62 <sub>a</sub>	504.86±27.69 <sub>cde</sub>	867.82±46.92 <sub>a</sub>
G5	3338.09±110.99 <sub>abcd</sub>	202.25±9.66 <sub>cd</sub>	487.65±10.10 <sub>cde</sub>	696.78±52.98 <sub>bcd</sub>
SK5	3680.69±125.72 <sub>ab</sub>	237.38±3.10 <sub>a</sub>	632.12±37.01 <sub>a</sub>	740.56±90.37 <sub>bc</sub>
GS1	3156.19±251.31 <sub>cde</sub>	207.96±16.27 <sub>bcd</sub>	545.70±16.47 <sub>bc</sub>	774.52±27.01 <sub>ab</sub>
SKS1	3653.24±121.77 <sub>abc</sub>	188.13±13.92 <sub>def</sub>	545.51±31.97 <sub>bc</sub>	575.82±99.50 <sub>e</sub>
GS3	3243.80±28.62 <sub>abcd</sub>	149.01±24.20 <sub>h</sub>	490.69±50.49 <sub>cde</sub>	573.63±90.57 <sub>e</sub>
SKS3	3738.19±95.58 <sub>a</sub>	200.91±7.68 <sub>cd</sub>	521.69±25.32 <sub>bcd</sub>	544.39±30.62 <sub>e</sub>

Different letters in the same column are significantly different ( $P < 0.05$ ).

Samples\*:

GTI: Sweetened green tea infusion

GTSI: Sweetened green tea and saffron extract added infusion

G1: 24 h fermented green tea kombucha

SK1: 24 h fermented saffron extract added kombucha

G2: 48 h fermented green tea kombucha

SK2: 48 h fermented saffron extract added kombucha

G3: 72 h fermented green tea kombucha

SK3: 72h fermented saffron extract added kombucha

G4: 96 h fermented green tea kombucha

SK4: 96 h fermented saffron extract added kombucha

G5: 120 h fermented green tea kombucha

SK5: 120 h fermented saffron extract added kombucha

GS1: 24 h stored green tea kombucha

SKS1: 24 h stored saffron extract added kombucha

GS3: 72 h stored green tea kombucha

SKS3: 72 h stored saffron extract added kombucha

\*\* : GAE: gallic acid equivalent, \*\*\*:tss: total soluble solids

After 5 days of fermentation, TAC results determined by the DPPH (17.63%), CUPRAC (70.95%) and FRAP (129.63%) assays of green tea kombucha were significantly increased ( $P < 0.05$ ). Saffron extract addition significantly increased TAC determined by all assays. After 120 h fermentation of saffron extract added sample, TAC values (DPPH, CUPRAC and FRAP) were increased as 20.55%, 65.47% and 100.27%, respectively. Finally, at the end of the kombucha fermentation, DPPH (17.37%), CUPRAC (29.63%) and FRAP (6.28%) values of saffron extract added samples were found higher when compared to control. However, after 3 days of storage, except CUPRAC value of green tea kombucha, total antioxidant capacity of the samples reduced. In a previous study, it was also reported that saffron methanol extract solution

exhibited high antioxidant activity (DPPH) above 2000 ppm (Assimopoulou et al., 2005).

### Sensory evaluation

Table 4 displayed the sensory evaluation of kombucha samples. In light of colour and appearance, saffron extract added kombucha samples attained higher scores than green tea kombucha. When odour scores of green tea kombucha and saffron extract added kombucha compared, no statistically significant difference were found between each storage day ( $P > 0.05$ ). Sourness scores of both samples fell during storage. Although sweetness score of saffron extract added kombucha was lower than control after 5 days fermentation, it was higher than control during storage. Green tea kombucha samples attained higher scores by panelists in case of overall acceptability.

Table 4. Sensorial properties of kombucha beverages

Samples*	Colour	Odour	Appearance	Sweetness	Sourness	Overall acceptability
G5	8.00±0.70 <sup>b</sup>	7.80±0.44 <sup>a</sup>	8.40±0.54 <sup>ab</sup>	9.40±0.89 <sup>a</sup>	8.60±0.54 <sup>a</sup>	8.20±0.44 <sup>a</sup>
SK5	9.40±0.89 <sup>a</sup>	7.40±0.54 <sup>ab</sup>	9.00±0.70 <sup>a</sup>	8.20±0.83 <sup>bc</sup>	7.20±0.44 <sup>b</sup>	7.40±0.54 <sup>b</sup>
GS1	6.60±0.54 <sup>c</sup>	6.80±0.83 <sup>bc</sup>	7.60±0.54 <sup>bc</sup>	8.00±0.70 <sup>bc</sup>	7.00±0.70 <sup>b</sup>	7.40±0.54 <sup>b</sup>
SKS1	8.80±1.09 <sup>ab</sup>	6.40±0.54 <sup>c</sup>	8.60±0.89 <sup>a</sup>	8.40±0.89 <sup>ab</sup>	6.00±0.70 <sup>c</sup>	7.00±0.70 <sup>b</sup>
GS3	6.00±0.70 <sup>c</sup>	6.40±0.54 <sup>c</sup>	6.40±0.54 <sup>d</sup>	7.20±0.83 <sup>c</sup>	5.80±0.83 <sup>c</sup>	6.60±0.54 <sup>c</sup>
SKS3	8.80±0.83 <sup>ab</sup>	6.20±0.44 <sup>c</sup>	7.40±1.10 <sup>c</sup>	7.40±0.89 <sup>bc</sup>	5.20±0.44 <sup>c</sup>	6.40±0.54 <sup>c</sup>

Different letters in the same column are significantly different ( $P < 0.05$ ).

Samples\*:

G5: 120 h fermented green tea kombucha

GTSK5: 120 h fermented saffron extract added kombucha

GS1: 24 h stored green tea kombucha

SKS1: 24 h stored saffron extract added kombucha

GS3: 72 h stored green tea kombucha

SKS3: 72 h stored saffron extract added kombucha

### CONCLUSION

Generally, in the view of current results, it can be concluded that kombucha fermentation significantly increased the nutritional and functional characteristics of the beverages. TPC and TAC values of the kombucha beverages raised in comparison to their infusions. The results of several studies reflect the high nutritional value and antioxidant properties of saffron. Considering the health benefits, saffron is a promising ingredient for functional foods, drinks and kombucha analogs.

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