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Changes in the content of total polyphenols and the antioxidant activity of different beverages obtained by *Kombucha* 'tea fungus'

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Abstract

Kombucha 'tea fungus' is a traditional refreshing drink obtained by fermentation of black tea with sugar as well as a strong symbiosis of acetic bacteria and yeasts. *Kombucha* tea has several health benefits such as antihyperglycemic, antilipidemic, antimicrobial, hepatoprotective, hypocholesterolemic and anticancer effects due to their antioxidant activity. In this study, six *kombucha* beverages were prepared by placing *Kombucha* 'tea fungus' in green, black and Echinacea teas, as well as goat, cow and soy's milk. The fermentation process was monitored by pH, total sugar amount, and titratable activity, as well as their antioxidant activities and total phenolic contents, were analyzed prior to the fermentation process and at the end of fermentation. The results showed that tea-based beverages were fermented for nine days and milk-based beverages were for 6 hours. Their sugar contents were significantly decreased ($p < 0.05$) as depending on their sugar contents. All fermented beverages displayed a statistically significant decrease ($p < 0.05$) in the DPPH and ABTS radical scavenging activity at the end of fermentation, while FRAP assays were displayed a statistically significant increase ($p < 0.05$). Further studies are necessary to the research of nutrients of tea and milk-based beverages on human organs the throughout fermentation.

Keywords: *Kombucha,* Antioxidant activities, Beverages, Functional food

Introduction

Since consumers have become increasingly aware of the role of diet in promoting health and preventing disease, new food products called functional food have been improved. Functional foods have a positive effect on health, physical and mental condition of the human body in addition to the nutritional value and the most famous examples of functional foods are fermented products, especially milk or dairy products (Siró, Kápolna, Kápolna, and Lugasi, 2008).

Kombucha, also named as tea fungus, has been consumed worldwide as a healthy drink for a very long time especially in China, Russia, and Germany (Vázquez-Cabral et al., 2017). Kombucha is a symbiosis of acetic acid bacteria (Acetobacter), including Acetobacter xylinum, A. xylinoides, Bacterium gluconicum, A. suboxydans, Gluconobacter liquefaciens, A. aceti and A. pasteurianus, various yeasts (the genera of Brettanomyces, Zygosaccharomyces, Saccharomyces, and Pichia depending on the source), including Saccharomyces cerevisiae, S. inconspicus, S. ludwigii, Schizosaccharomyces pombe, Candida tropicalis, C. krusei, Debaryomyces hansenii, Brettanomyces spp., Kloeckera spp., Zygosaccharomyces bailii and Z. Kombuchaensis, and lactic acid bacteria (Lactobacillus bulgaricus) (Battikh, Bakhrouf, and Ammar, 2012; Zubaidah, Yurista, and Rahmadani, 2018). Kombucha is composed of a floating cellulosic pellicle layer and the sour liquid broth (De Filippis, Troise, Vitaglione, and Ercolini, 2018) and converted the sugar which is its carbon source into organic acids and ethanol: sucrose into glucose and fructose and produce ethanol, while acetic acid bacteria convert glucose into gluconic acid and fructose to acetic acid (Malbaša, Lončar, Vitas, and

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Čanadanović-Brunet, 2011).

The metabolic activity of kombucha results in a refreshing beverage with the sour taste and many health beneficial compounds. The kombucha beverage can be used to help with headaches, gastric illnesses, diabetes, nervousness, and aging problems. Moreover, it possesses antibiotic properties, relieves rheumatism, gout, and hemorrhoids and has a positive influence on the cholesterol level, arteriosclerosis, toxin excretion, and blood cleansing. The most important effect is that it provides resistance to cancer and increases the immune system performance (Chakravorty et al., 2016).

Black and green teas are the usual and the best substrates known for the preparation of Kombucha drinks (Velićanski, Cvetković, Markov, Tumbas Šaponjac, and Vulić, 2014), but other substrates such as coca-cola, wine, beer, fruit drinks, milk, and herbal teas can be used (Malbaša et al., 2011). Furthermore, kombucha can be cultivated on dark beer, red wine, white wine, whey (Vukic et al., 2014), coffee, Jerusalem artichoke (Helianthus tuberosus L.) (Yang, He, Corscadden, and Udenigwe, 2015) and molasses (Lončar, Malbaša, and Kolarov, 2001). Kombucha constitutes several compounds that are known to be strong antioxidants, such as vitamins C and B2, as well as polyphenols, primarily catechins (Malbaša, Vitas, Lončar, Grahovac, and Milanović, 2014). Application of Kombucha in lactose fermentation is still under investigations and a few investigations have been reported and its technological and nutritional aspects in dairy products have been described recently (Malbaša et al., 2009).

In the present work, black, green and Echinacea teas, as well as goat, cow and soy's milk were used as a medium for Kombucha fermentation. To the author's knowledge, a few previous studies reported the antioxidant activity of other different fermented Kombucha beverages, however, there were no studies about the usage of different kind of milk as an alternative medium besides black and green tea. The aim of this work is to analyze fermentation of sucrose and lactose from three different types of teas, including black, green and Echinacea teas and milk, including goat, cow, and soy inoculated with plain Kombucha 'tea fungus' starter (which is not concentrated before application). Changes in the pH and total sugar of the beverages during fermentation were determined. This different tea and milk-based beverages compared to the traditional kombucha prepared from black tea with regards to colour, total phenolic content and antioxidant activities. In view of this, the recent study showed the antioxidant effect of kombucha application in the development of new fermented beverages.

Materials and Methods

Materials

The plain kombucha culture was cultivated on six different substrates: goat's, cow's, soy's milk as well as black, green, and Echinacea teas.

Milk: Pasteurised, homogenized goat, cow and soy's milk (approximate values of the milk fat content at the local market) were used for the production of fermented milk products. The used goat milk has contained, on average, 2.6-3.5 % protein, 4.5-5.5 % carbohydrate, and 10-13 % dry matter content; cow's milk has 3.1 % protein, 4.5 % carbohydrate, and 11 % dry matter content; soy's milk has 3.3 % protein, 0.2 % carbohydrate, and 6.5-7.0 % dry matter content.

Tea: The substrate from black tea was prepared using four grams of tea leaf and it was added to 1 l boiling water and kept boiling for 5 min. Sucrose (100 g) was added, the tea was then filtered through a stainless sieve and the decoction was boiled for another 5 min. The sugared tea was immediately distributed into several sterilized 250 ml-jars (each contained 150 ml) under aseptic conditions and allowed to cool the tea to room temperature. Each fermentation was repeated three times. The substrate from green tea, echinacea tea was prepared under the same conditions as the substrate from black tea.

Starter culture: Kombucha culture (3 g)

Fermentation Process

Kombucha starter was cultivated in different kinds of milk at room temperature. The inoculum was added to the milk in the amount of 10 % (v/v). The fermentation was performed until the pH 4.5. The obtained milk gel was centrifuged at 10000g for 30 min and stored at -20 ºC for further analysis.

The fermentation was also initiated by adding 3 % (w/v) starter to fresh medium and maintained at room temperature for 7 days for tea samples. Sampling was performed periodically at 0, 3, 7 days and the broth was centrifuged at 10000g for 20 min and stored at -20 ºC for further analyses. The fermentation was done in triplicate.

Methods

Determination of pH: pH of the beverages values was measured using a pH meter (WTW Inolab Level 1, Weilheim, Germany) during fermentation.

Total sugar content: The sugar content was assayed by the phenol-sulfuric acid method (Hall, 2013).

Determination of colour: The colour of the beverages were measured using the Hunter-Lab Colorflex (CFLX 45-2 Model Colorimeter; HunterLab, Reston, VA). The cylindrical quartz cell containing the sample was placed directly into the colourimeter, and L*, a*, and b* values were recorded. L* represented the lightness (L* = 0 yields blackness and L* = 100 indicates diffuse whiteness), a* represented the redness-greenness (negative values indicate greenness while positive values indicate redness/magenta) and b* represented the yellowness-blueness (negative values indicate blueness and positive values indicate yellowness) as index of the CIELAB.

Determination of total titratable acidity: Total titratable acidity was determined according to the potentiometric titration acidity of the beverages. The beverages were diluted 10 fold in deionized water and filtered. The filtrate was titrated up to a pH 8.1 with a solution of 0.1 N NaOH using phenolphthalein as indicator. The results were expressed as g 100 ml-1 with reference to anhydrous citric acid.

Determination of total phenolic content: The total phenolic contents of the beverages were measured according to Folin-Ciocalteu method (Singleton, Rossi, and Jr, 1965). 0.05 ml of the beverages were added to 2 ml of 2 % sodium carbonate. After 2 min, 0.1 ml Folin–Ciocalteu reagent was mixed with the above solution, the absorbance at 750 nm was then measured after 30 min. The total phenolic content was expressed as

gallic acid equivalents (GAE, mM) from the calibration curve.

Evaluation of the Antioxidant Capacity: To achieve a more realistic characterization of the antioxidant properties of the beverages, three different antioxidant capacity assays were applied: DPPH, ABTS, and FRAP assays as spectrophotometric assays. Modified versions of the original DPPH, ABTS and FRAP assays were performed to fit the antioxidant capacity analyses according to the procedures described by Brand-Williams et al., 1995 (Brand-Williams, Cuvelier, and Berset, 1995), Re et al., 1999 (Re et al., 1999) and Gou et al., 2003 (Guo et al., 2003), respectively. Trolox standard curves were correlated with the difference in absorbance between a final reading and the reagent blank reading for the spectrophotometric assays. The results were expressed as a mean of three determinations in milligrams of Trolox per gram of beverages.

Statistical Analysis: All experimental results were presented as mean values with their corresponding standard deviations. Significant differences between mean values ($P < 0.05$) were determined by ANOVA using SPSS 16 (SPSS Inc., Chicago, USA). Statistically significant differences were compared between treatment groups.

Results and Discussion

Development of fermentation

The six different kinds of beverages were produced: three milk-based beverages and the three tea-based beverages. The development of all fermentation processes was investigated by measuring pH values in time. Milk-based beverages were incubated until their pH 4.4 and tea-based beverages were incubated for 9 days. pH of these beverages throughout fermentation was presented in Figure 1.

Namely, since investigating the influence of fermentation duration on the milk and tea-based beverages, it has been statistically proven that fermentation duration was a significant variable. As shown in Figure 1, a statistically significant decrease in the pH was observed in all six beverages during the fermentation period ($P < 0.05$). The overall decrease in pH of the beverages would have been due to the increased concentration of organic acids produced during the fermentation process by Kombucha 'tea fungus' as inoculum (Battikh et al., 2012). In milk samples, fermentations were stopped when the pH reached to between 4.4 and 5.7, while these beverages had pH between 6.6 and 7.0 at the beginning of fermentation. Tea-based beverages had the pH between 5.2 and 6.4 prior to the fermentation process. At the end of day 9, the pH of the tea-based beverages varied between 3.4 and 5.6. pH for final product of Echinacea tea-based beverage was observed to be the most acidic, having a final pH of 3.4, while cow's milk-based beverage had the lowest pH of 4.43 at the end of fermentation duration. The pH exchange pattern during fermentation was as expected and it had the same shape for all substrates. Kanuric et al. (2018) investigated the effect of new starter culture on milk fermentation by kombucha and the pH value gradually decreased and after 9 h, fermentation was stopped (Kanurić et al., 2018). This difference between the pH values of the substrates could be the consequence of differences in chemical composition of substrates (Malbaša et al., 2011).

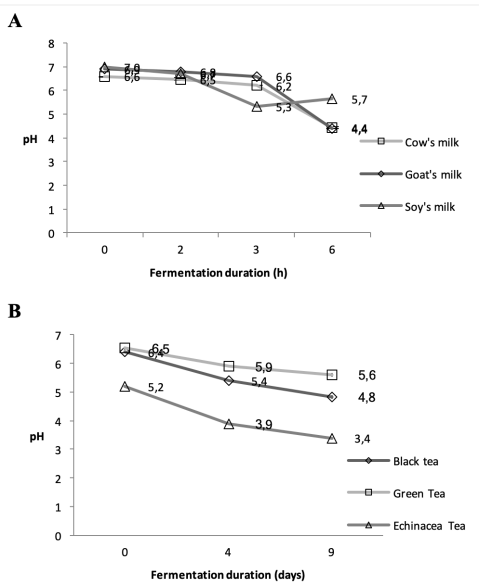

Figure 1. The pH values of milk (A) and tea-based beverages (B) throughout fermentation.

The total sugar content of the beverages is shown in Table 1. Considering these values, the total sugar content of milk and tea-based beverages were lower than unfermented sample (control). Total sugar content decreased Kombucha' tea fungus' consumed glucose in the beverages throughout the fermentation. Soy's milk-based and Echinacea tea-based beverages had the highest reduction in total sugar content while cow's milk-based and black tea-based beverages had the lowest reduction at the end of the fermentation period. Value of pH and amount of total sugar are standard parameters that indicate the success of the production process (Vitas, Cvetanović, Mašković, Švarc-Gajić, and Malbaša, 2018).

Table 1. Total sugar amount values of beverages throughout fermentation (mg l^{-1})

Milk based beverages	Fermentation time (hours)		Tea based beverages	Fermentation time (days)		
	0	6		0	3	9
Goat's milk	138.74±2.03$^{b, A}$	88.26±0.01$^{b, B}$	Black tea	155.59±0.81^{c}	155.47±0.93^{b}	154.48±0.023^{a}
Cow's milk	147.36±2.1$^{a, A}$	100.47±3.27$^{a, B}$	Green tea	159.62±0.031^{a,A}	155.63±0.031^{b,B}	155.064±0.041^{a,C}
Soy's milk	78.06±6.54$^{c, A}$	19.35±1.01$^{c, B}$	Echinachae tea	157.2±0.37^{b,A}	156.59±0.23^{a,B}	152.87±2.31^{a,C}

a-c Different letters in the same column indicate significant differences between means ($p < 0.05$).

A-C Different letters in the same row indicate significant differences between means ($p < 0.05$).

Colour and Titratable activity (TA)

As shown in Table 2, L*, a*, and b* values were measured throughout fermentation. Five beverages except for Echinacea tea-based beverages were observed to get lighter as the fermentation progressed in terms of L* values. Statistically significant changes ($p < 0.05$) in the L* values of all five beverages were observed from beginning point itself as compared with the final products. A sharp increase in the lightness was especially observed in Echinacea tea-based beverage at the end of the fermentation. However, as the L* values, statistically significant changes ($P < 0.05$) in the a* values of all six fermented beverages were observed between the beginning and the end of fermentation. As for the b* values, except for green tea-based beverages other five beverages were observed the increase in the yellowness at the end of the fermentation and statistically significant differences were found ($P < 0.05$) as compared with the beginning of fermentation. Overall, the L*, a* and b* values indicated that the colour value of the beverages changed during fermentation, despite their individual fluctuations. Previous studies showed that the colour changes were associated with the microbial transformation of polyphenols (Jayabalan, Marimuthu, and Swaminathan, 2007).

The statistically significant increase ($p < 0.05$) in the TA in milk and tea-based beverages was observed as compared with the beginning of fermentation. The TA of three milk-based beverages were found between 1.95 and 3.2 at the beginning of fermentation as well as between 8.4 and 12.75 at the end of fermentation. The TA of three tea based beverages varied between 0.45 and 0.65 prior to the fermentation process, while at the end of fermentation, these values varied between 0.65 and 1.55. The statistically significant increase ($P < 0.05$) in the TA in all beverages was observed as compared with the unfermented beverages. These observations were in agreement with the findings of other studies (Xu et al., 2012).

Table 2. The L*, a* and b* values of the milk (A) and tea-based beverages (B) throughout fermentation.

A

Milk based beverages	Fermentation duration (h)	Colour			TA
		L*	a*	b*	
Goat's milk	0	93.1±0.01	-0.88±0.02	8.5±0.01	2.05±0.05
	6	82.72±0.1	0.86±0.03	13.57±0.17	11.9±0.1
Cow's milk	0	91.97±0.02	0.25±0.01	10.64±0.03	1.95±0.05
	6	85.08±0.13	1.12±0.11	13.17±0.47	12.75±0.05
Soy's milk	0	85.77±0.04	1.03±0.11	20.21±0.03	3.2±0.1
	6	78.05±0.44	3.4±0.08	22.24±0.31	8.4±0.1

B

Tea based beverages	Fermentation duration (days)	Colour			TA
		L*	a*	b*	
Black Tea	0	4.62±0.56	2.35±0.16	3.53±0.38	0.45±0.05
	3	4.25±0.05	2.98±0.07	3.4±0.22	0.55±0.05
	9	4.29±0.11	3.61±0.39	4.3±0.37	0.65±0.05
Green Tea	0	6.01±0.2	1.41±0.19	5.25±0.16	0.45±0.05
	3	4.66±0.08	3.78±0.15	4.8±0.23	0.55±0.05
	9	3.12±0.37	2.16±0.8	2.79±0.47	0.65±0.05
Echinacea Tea	0	2.79±0.14	1.47±0.19	3.2±0.12	0.65±0.05
	3	8.09±0.13	4.12±0.1	7.79±0.4	1.05±0.05
	9	7.37±0.02	3.49±0.37	7.73±0.34	1.55±0.05

Determination of Total Phenolic Contents

The total phenolic contents are shown in Table 3. Total phenolic contents of beverages were progressively increased with fermentation duration. Goat's milk-based and green tea-based beverages had the highest total phenolic contents, while soy's milk-based and Echinacea tea-based beverages had the least amount of total phenolic prior to the fermentation. However, following the initiation of the fermentation process, all fermented beverages had a statistically significant increase ($P < 0.05$) in the total phenolics content. Goat's milk based and green tea-based beverages were observed to have the highest total phenolics content by the end of the fermentation since their initial total phenolic contents were high as well. The result of this study were found consistently with other reports (Chakravorty et al., 2016; Jayabalan et al., 2007). De Flippis et al. (2018) determined the total phenolic content of Kombucha tea fermented using ceylon black or bancha green tea. At the beginning of the fermentation, the total phenolic content was found the higher in green tea than black tea. During fermentation, the phenolic content were found stabile in green tea and increase in black tea (De Filippis et al., 2018). Literature data suggest that there were different extraction techniques were performed to extract the phenolic contents (Cvetanovic, Svarc-Gajic, Maskovic, Savic, and Nikolic, 2015). Vitas et al (2018) was found that their Kombucha fermented beverages had higher total phenolic contents as compared to corresponding initial beverages (Vitas et al., 2018). Ayed and Hamdi (2015) used cactus pear as substrate to produce Kombucha-fermented beverages and the results displayed that total phenol content increased with fermentation time (Ayed and Hamdi,

2015). This increase in phenolic components can be explained by the fact that acid hydrolysis and the bioconversion of condensed phenolic components takes place (Jayabalan, Subathradevi, Marimuthu, Sathishkumar, and Swaminathan, 2008).

Table 3. Total phenolic content of milk (A) and tea-based beverages (B) throughout fermentation (μgGAE ml^{-1})

A

Fermentation duration (h)	Milk-based beverages		
	Goat milk	Cow milk	Soy milk
0	540.65±38.6^{a,B}	345.15±4.12^{b,B}	334.8±24.46^{b,B}
6	1334.43±3.88^{a,A}	981.52±38.6^{b,A}	642.19±76^{c,A}

B

Fermentation duration (days)	Tea-based beverages		
	Green Tea	Black Tea	Echinacea Tea
0	326.5±52.68^{a,C}	227.78±0.88^{b,C}	209.65±8.87^{b,C}
3	380.87±28.1^{a,B}	261.81±0.93^{b,B}	256.52±9.05^{b,B}
9	472.09±4.94^{a,A}	312.26±18.55^{b,A}	279.29±21.55^{c,A}

a-c Different letters in the same row indicate significant differences between means ($p < 0.05$).
A-C Different letters in the same column indicate significant differences between means ($p < 0.05$)

Determination of Antioxidant Activity

Total phenolic contents of the beverages are expected to be directly related to their antioxidant capacity (Bravo, Goya, and Lecumberri, 2007). Since no single method can quantify the total antioxidant capacity of the samples, the usage of different antioxidant activity assays is possible. Therefore, three different methods were applied in the this research (DPPH, ABTS and FRAP) in order to achieve a general view of the antioxidant potential of the beverages during fermentation. The antioxidant capacity results of the beverages, measured by the three different methods, were presented in Table 4. Similar behavior patterns were commonly observed for the results of DPPH, ABTS and FRAP assays regardless of their action mechanism. The hypothesis of this study was to investigate the higher antioxidant activity of beverages at the end of fermentation compared to the beginning of fermentation as samples blank.

Results of DPPH assay showed different ability for milk and tea-based beverages (Table 4). Prior to fermentation, soy's milk-based beverage showed minimum DPPH value, while goat's milk based beverage showed minimum DPPH value at the end of fermentation. However, before and after fermentation, maximum DPPH value was observed in cow's milk based beverage. When the different kind of teas were applied as substrates, the highest DPPH value was observed in green tea-based beverages before fermentation while the lowest DPPH value was found in black tea-based beverages. However, at the end of fermentation, black tea-based beverage had the highest DPPH value and Echinacea tea-based beverage had the lowest DPPH value. Amarasinghe et al. (2018) traditionally carried out four kombucha beverages by placing the tea fungal mats in sugared Sri Lankan black tea at varying concentrations for a period of 8 weeks. A statistically significant decrease ($p < 0.05$) in the antioxidant activity was found in these four beverages after fermentation (Amarasinghe, Weerakkody, and Waisundara, 2018).

Table 4**.** The antioxidant capacity of the beverages measured by DPPH, ABTS, and FRAP (μmol Trolox g $extract^{-1}$).

A

Fermentation duration (h)	Milk based beverages								
	DPPH			ABTS			FRAP		
	Goat milk	Cow milk	Soy milk	Goat milk	Cow milk	Soy milk	Goat milk	Cow milk	Soy milk
0	2.8±0.14^{b,B}	3.61±0.79^{a,B}	0.28±0.076^{c,B}	1.03±0.08^{c,B}	1.16±0.02^{b,B}	1.09±0.096^{a,A}	1.46±0.039^{a,B}	0.75±0.28^{b,B}	0.65±0.20^{b,B}
6	0.061±0.006^{c,A}	0.28±0.003^{a,A}	0.2±0.014^{b,A}	0.81±0.06^{c,A}	0.96±0.03^{c,A}	1.03±0.02^{a,A}	2.06±0.031^{a,A}	2.02±0.02^{a,A}	1.39±0.05^{b,A}

a-c Different letters in the same row indicate significant differences between means ($p < 0.05$).
A-C Different letters in the same column indicate significant differences between means ($p < 0.05$).

B

Fermentation duration (days)	Tea based beverages								
	DPPH			ABTS			FRAP		
	Green Tea	Black Tea	Echinacea Tea	Green Tea	Black Tea	Echinacea Tea	Green Tea	Black Tea	Echinacea Tea
0	2.32±0.04^{b,A}	2.18±0.01^{c,C}	2.26±0.04^{b,B}	1.32±0.00^{c,A}	1.30±0.02^{c,B}	1.27±0.013^{b,C}	1.54±0.011^{b,A}	0.61±0.013^{c,C}	0.91±0.24^{b,B}
3	2.30±0.04^{b,C}	2.06±0.01^{b,A}	2.25±0.05^{b,B}	1.30±0.00^{b,A}	1.12±0.00^{b,B}	0.93±0.07^{a,C}	1.62±0.04^{a,A}	1.21±0.01^{b,B}	1.12±0.13^{b,B}
9	1.81±0.35^{a,AB}	2.02±0.02^{a,A}	1.59±0.11^{a,B}	1.29±0.00^{a,A}	1.06±0.00^{a,B}	0.87±0.01^{a,C}	1.66±0.01^{a,A}	1.38±0.002^{a,B}	1.37±0.12^{a,B}

a-c Different letters in the same column indicate significant differences between means ($p < 0.05$).
A-C Different letters in the same row indicate significant differences between means ($p < 0.05$).

Before and after fermentation, among the all tea substrates green tea was found to be the best substrate which showed higher ABTS value, whereas the least ABTS value was observed in Echinacea tea based beverage. Ayed and Hamdi (2015) performed to determinate the two antioxidant activity in the fermented juice: DPPH and ABTS. The DPPH and ABTS radical scavenging abilities of the fermented juice increased significantly (Ayed and Hamdi, 2015). However, the results in current studies displayed incompatible with that study. This situation may be explained to the utilization of phenolic compounds by the tea fungus. Because, scavenging effects were formed from many of the compounds present in the fruit and their synergistic effects (Amarasinghe et al., 2018).

Among the three tea substrates studied, green tea performed better ability which was similar to those observed in DPPH value. At the beginning of fermentation, the lowest FRAP value was observed in black tea and thisvalue was attained in Echinacea tea at the end of fermentation. Among Kombucha based beverages prepared from three different milk, fermented goat's milk was considered to be the best beverage which had the ability of FRAP reaction, while fermented soy's milk can have the lowest effect on the FRAP value both before fermentation and at the end of fermentation.

A review of the literature concerning antioxidant capacity of kombucha beverages reveals a quite difficult comparison among the reported data, mainly because of the utilization of different analytical methods (ABTS, CUPRAC, DPPH, FRAP, ORAC, or TRAP, among others), standards, reference units (in a wet or dry matter basis). Furthermore, substrates might influence significantly their antioxidant capacity (Ayed, Ben Abid, and Hamdi, 2017; Hrnjez et al., 2014; Malbaša et al., 2014, 2011; Villarreal-Soto, Beaufort, Bouajila, Souchard, and Taillandier, 2018).

Conclusion

The present study demonstrated that milk and tea-based beverages prepared from different substrates have excellent antioxidant activities. It has been interested to note and use kombucha-based beverages in preventing diseases caused by the overproduction of free radicals. Kombucha fermented beverage displayed an increase in antioxidant activities of beverages during fermentation. The extent of the activity depended upon the fermentation time and substrates. However, further investigations are required to investigate these results and the study which carried out with the different kind of substrates can be used as a platform to form more studies in the future based on different starter cultures, microbial compositions, fermentation duration etc. and their combined effects as well as on changing the health effect of kombucha beverages during various periods of fermentation.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

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Consent for publication

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