

# Production and Analysis of *Kombucha* : A Black Tea based Functional Beverage

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*Kombucha* is a sweet, slightly acidic, low-alcoholic functional beverage produced by fermentation of tea using Symbiotic culture of bacteria and yeast (SCOBY). It can be considered as an alternative of carbonated soft drinks because it contains lesser calories and sugar content and is having other health promoting properties. Though tea is widely used as a beverage but its fermentation to produce a functional beverage is not well exploited in India. Home-brewing of the beverage has become more common in developed countries because of the growing recognition of kombucha as a "healthy" product. Therefore, a standard protocol for the production of a safe fermentation process with significant health value is desirable. In the present study, Black tea based *Kombucha* is prepared using different concentrations of tea (5–15 g/L) and sugar (40–80 g/L). Optimum concentration of tea and sugar was obtained by applying Central Composite Rotatable Design (CCRD) under Response Surface Methodology (RSM) using 'Design Expert 13' for getting the product with maximum antioxidant activity. Fermentation was carried out by varying inoculum level from 5–15% and it was observed that 5% inoculum level was optimum for characteristic fermentation. Periodic changes in pH, TSS, titrable acidity, total sugars, reducing sugars, antioxidant activity, total phenolic content, vitamin C content and alcohol content was recorded for all the thirteen formulations suggested by RSM during fermentation period of 7 days. Microbiological and sensory evaluation of all the combinations was done and selected two combinations (Beverage 6 with Tea @ 5 g/L and Beverage 13 with Tea @ 15 g/L, both having Sugar @ 80 g/L) received a score of 8.14 and 7.90, respectively.

**Keywords:** Black tea, Carbonated soft drinks, Fermentation, Microbiological and sensory evaluation, Symbiotic culture of bacteria and yeast

## Introduction

The carbonated soft drinks were the largest beverage industry by volume being used in the U.S.A. before 2016. But after publication of various reports highlighting the correlation of soft drinks intake with metabolic disorders leading to obesity and health related issues, the use of these beverages were banned in the schools.<sup>1</sup> Same type of health risks have been reported not only with the beverages containing natural-sugar but also with beverages containing artificial sweeteners that lead to mass public health campaigns for limiting soft drink consumption.<sup>2-3</sup> After publications regarding serious health effects of soft drink, the interest of people diverted more towards the production and consumption of functional beverage because the natural product present in these beverages makes them more acceptable.<sup>4-5</sup>

The year 2018 has been named after the name of "Fancy water or *Kombucha*" by The Wall Street Journal.<sup>6</sup> The market of functional beverages is considered to be spreading rapidly in the market.<sup>7-8</sup>

Functional beverages are the drinks either non-alcoholic or have alcohol content below 0.5% containing non-traditional ingredients. These ingredients can be minerals, vitamins, amino acids, dietary fibers (DFs), probiotics, added raw fruits, etc.<sup>9</sup> According to Kim and Adhikari<sup>10</sup> functional beverages are considered to be most effective active sector among functional foods because of their ease of product formulations by incorporating desirable nutrients and bioactive compounds, ease of distribution and refrigeration for better storage and shelf life of product and possibility to meet consumer demands by varying components, container size, shape and appearance.

Tea (*Camellia sinensis*) is the most commonly used and well accepted beverage throughout the world because of its claimed health benefits. It was estimated that black tea constitutes about 80% of the 2.5 million metric tons of the total dry tea production.<sup>11</sup> It is a rich source of biologically active compounds such as polyphenols and polysachharides. Natural polyphenols found in green tea and black tea is epicatechin (EC), epigallocatechin (EGC), EC gallate (ECG), and EGC gallate (EGCG).<sup>12</sup> Tea

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polysaccharides have shown glycosidal inhibitory, antibacterial, antioxidants and immune-modulatory properties.<sup>13</sup> Though tea is widely used as a beverage but its fermentation to produce a functional beverage is not well exploited in India. Moreover, some of the studies draw attention to the need for regulatory guidelines and the potential of kombucha in the market and peoples' health.<sup>14</sup> It has been highlighted that lack of regulation and the low level of literacy about this product can limit its development, marketing, and impact on health. Thus, it is essential to highlight the potential value of this product and invest in its development and marketing. Keeping this in mind, the present study was proposed to produce black tea based functional beverage using Symbiotic Culture of Bacteria and Yeast (SCOBY) and to carry out the physicochemical, microbial and sensorial evaluation of developed functional beverage.

## Materials and Methods

### Standardization of Starter Culture Concentration

As a standard method, *Kombucha* beverage is prepared using 5 g/L of black tea and 80 g/L of sugar with different inoculum concentration (5–15%) to select an appropriate inoculum level that will be used for standardization of production protocol for *Kombucha*.<sup>15</sup> As the pH and titrable acidity is a characteristic feature of kombucha and has to fall between standard value of pH 2.5–3.5 and acidity upto 1%, so different set of flasks inoculated with varying inoculums level (5–15%) was set up to find the optimum inoculums level percent that can produce the product with desired specifications.

### Optimization of Substrate Concentration for the Development of Black Tea Based Functional Beverage

Tea concentration (5–15 g/L) and Sugar concentration (40–80 g/L) were statistically optimized by Central Composite Rotatable Design (CCRD) in Response surface methodology (RSM) using Design Expert 13.0. The objective of using this software was that it is influenced by multiple variables and suggests a response that gives the maximum value of desired parameter. The experimental design matrix in actual form of variables is given in Table 1.

Samples were periodically drawn and analysis of °Brix, total sugars,<sup>16</sup> and antioxidant activity<sup>17</sup> was done till the completion of fermentation. Different concentration of tea and sugar was added to 500 ml of distilled water and then the mixture was heated for boiling (10 minutes). The infusion was then filtered

using a sterile sieve. After waiting for these samples to reach room temperature, inoculation was done with previously fermented *Kombucha* broth and SCOBY at pre-optimized inoculum concentration. Jars were covered with muslin cloth and were fermented at 25°C for 7 days. Process for preparation of fermented tea beverage is given in Fig. 1.

### Analysis of Black Tea Beverage during Fermentation

#### Physico-chemical Analysis

Samples of the beverage were taken at 0, 1<sup>st</sup>, 3<sup>rd</sup> and 7<sup>th</sup> day of fermentation for physicochemical and microbiological analysis. Digital pH meter equipped with a glass electrode was used to measure pH of samples. pH meter was first calibrated at pH 4.0 and 7.0 using standard buffer solutions. Concentration of total soluble solids in fermented beverage was analyzed with the aid of 0–32°Brix Erma Hand Refractometer, previously calibrated with distilled water. The reading was normalized against a standard temperature of 20°C.<sup>18</sup> The results were expressed as °Brix (1 degree brix is defined as 1 g of TSS in 100 grams of solution). Titrable acidity was measured in

Table 1 — Experimental design of process variables and values of experimental data for optimization of substrate concentration

Runs	A: Tea (g/L)	B: Sugar (g/L)
1	2.928	60
2	10	60
3	10	60
4	10	88.284
5	10	60
6	5	80
7	10	31.715
8	10	60
9	5	40
10	15	40
11	10	60
12	17.071	60
13	15	80

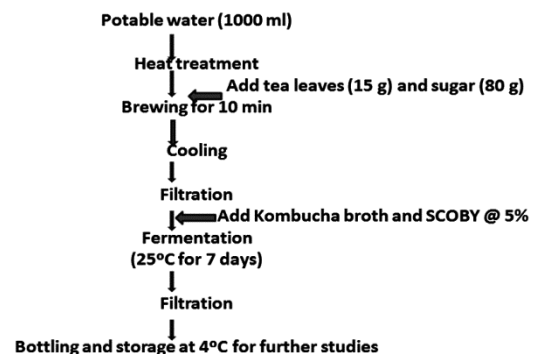


Fig. 1 —Flow chart of Process for Production of fermented tea beverage

percentage of acetic acid anhydrous as per AOAC<sup>18</sup> and expressed as acetic acid content (g/100 ml). Total sugars were estimated using phenol sulphuric acid method given by Du Bois *et al.*<sup>16</sup>, using standard glucose curve and recorded in terms of total sugar (%). Estimation of reducing sugar was carried out by Dinitro Salicylic (DNS) Acid (Miller)<sup>19</sup> in terms of reducing sugar (%). Estimation of ethanol content (% v/v) in beverage sample was done by the method of Caputiet *al.*<sup>20</sup> The hydrogen or radical scavenging property was determined using the stable radical DPPH (2, 2 diphenyl-1 picrylhydrazyl hydrate) as suggested by Blois<sup>17</sup> and expressed as antioxidant activity (%). Total polyphenolic content was determined according to the Folin-Ciocalteu method (Singleton *et al.*)<sup>21</sup> and expressed as mg Gallic Acid Equivalent (GAE)/ml. Vitamin C content was estimated using 2,6 dichlorophenolindophenol visual titration method given by Ranganna<sup>22</sup> and expressed as mg/100 ml.

#### Microbiological Analysis

At each sampling day, 10 ml of sample was collected and then blended with 90 ml of 0.1% sterile peptone water and subjected to serial dilutions. For Yeast count in sample, spread plating technique was adopted using Glucose Yeast Chloramphenicol (GYEC) agar. For Acetic acid bacteria count in sample, spread plate technique was adopted using Tryptone agar (TA).

#### Sensory Analysis

The developed fermented beverage was evaluated for sensory parameters by ten semi-trained panel of judges on a nine point hedonic scale.<sup>23</sup> Sensory properties were assessed on the basis of appearance/color, flavor, mouth feel and overall acceptability.

#### Statistical Analysis

All the parameters were tested in triplicate and data was analyzed statistically by using One-way ANOVA IBM SPSS Statistics version 22.0.

## Results and Discussion

#### Standardization of Starter Culture Concentration

Appropriate level of starter culture is necessary to produce a beverage with good taste, colour and aroma. As a standard method *Kombucha* beverage is prepared using 5 g/L of black tea and 80 g/L of sugar with different inoculum concentration (5–15%) to select an appropriate inoculum level. It was observed that beverage produced using 5% starter culture was

considered to be overall acceptable as compared to other two beverages. High amount of inoculum in other beverages resulted in production of high acidity, making the beverage sour in taste (Fig. 2). As depicted in Fig. 2 (b), the value of titrable acidity on day 3 was 0.23 at 5% inoculum level, while the corresponding value at 10% and 15% inoculum level was 0.39 and 0.42, respectively. Similarly, It further increased to 0.66 and 0.85 on day 7<sup>th</sup> with 10% and 15% inoculum level. Though course of change in pH was almost the same irrespective of the inoculums level. As per literature, inoculums concentration in the range of 5–10% is used for fermentation of *Kombucha* beverage.<sup>24</sup> Earlier, Muhialdin *et al.*<sup>25</sup> used 10% inoculum level to analyse the effect of sugar source and fermentation time on kombucha. Similarly, Aye *et al.*<sup>26</sup> reported that *Kombucha* beverage produced using 5% inoculum level with a tea concentration of 1% and sugar concentration of 10% was optimally fermented.

#### Optimization of Substrates Concentration for the Development of Black Tea Based Functional Beverage

Statistical optimization of tea (5–15 g/L) and sugar (40–80 g/L) concentration was carried out using RSM plan under Design Expert 13.0 software. *Kombucha* beverages were produced with different concentrations of tea and sugar according to RSM plan and responses were studied in terms of TSS (°Brix), total sugars (g/100 ml) and antioxidant

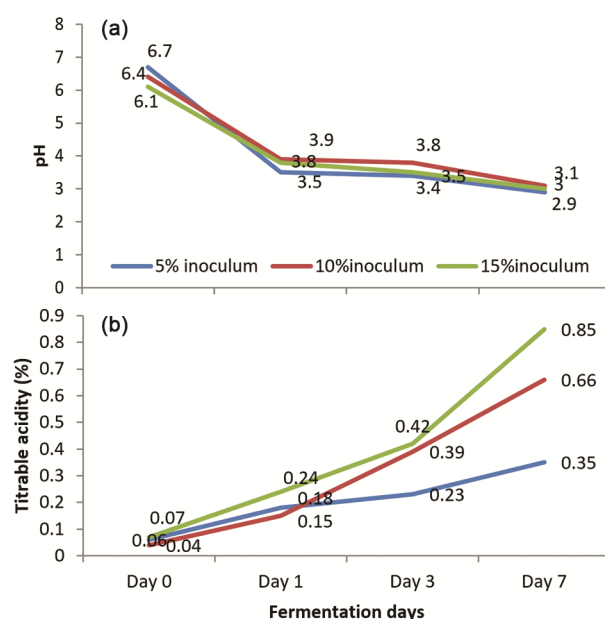


Fig. 2 — Periodic change of (a) pH and (b) titrable acidity during fermentation with different inoculum level

activity (%). Thirteen combinations of two fermentative factors along with their responses are presented in Table 2. It represents that maximum antioxidant activity (72.34%) was achieved at a temperature of 25°C with tea concentration of 15 g/L and sugar concentration of 80g/L. Further, total sugar concentration of 5.40 g/100ml and TSS of 6°Brix was recorded, which is desirable for producing sensory acceptable *Kombucha* beverage. By keeping constrains at ‘in range’ for TSS and total sugars, and ‘maximum’ for antioxidant activity, a solution was provided by RSM as shown in Table 2. Results indicated that beverage produced with tea concentration of 15 g/L, sugar concentration of 80 g/L and fermented at 25°C resulted in highest antioxidant activity, with a desirability of 0.938% for beverage production. On the basis of highest antioxidant activity, this concentration of tea and sugar was selected for further studies. Jakubczyk *et al.*<sup>27</sup> prepared *Kombucha* beverage using 10% of sugar concentration and 0.8% of different types of teas (black tea, white tea, green tea and red tea). After analyzing their antioxidant activity, it was noted that

antioxidant activity was found to be highest in green tea as compared to other types of tea. In a study by Amarasinghe *et al.*<sup>28</sup>, *Kombucha* was prepared with different concentration of teas in the range of 1.66–8.0 g/100 ml and with constant sugar concentration of 1.5 g/100 ml. It was observed that beverage produced using high tea concentration (4 g/100 ml and 8 g/100 ml) displayed higher antioxidant activity, as compared to other beverages having low tea concentration.

In Table 3, a model F-value of 130.19 indicated that the model is significant. There is only 0.01% chance that an F value this large could occur due to noise. The predicted R<sup>2</sup> of 0.9243 is in reasonable agreement with the Adjusted R<sup>2</sup> of 0.9818. A ratio greater than 4 is desirable. Hence, our ‘Adequate Precision’ ratio of 32.618 indicates an adequate signal. The Multiple regression for antioxidant activity response is given as Eq. 1:

Equation in terms of Coded Factors

$$\text{Antioxidant activity} = + 70.54 + 0.352457 \times A + 0.678072 \times B + 0.14 \times AB + 0.134375 \times A^2 + 0.356875 \times B^2 \dots (1)$$

Table 2 — Experimental response profile for optimization of substrate concentration for obtaining maximum antioxidant activity

Tea (g/L)	Sugar (g/L)	Response 1 TSS (°Brix)	Response 2 Total sugars (g/100ml)	Response 3 Antioxidant activity (%)
2.928	60	5.2	4.90	70.34
10	60	3.9	3.51	70.54
10	60	3.9	3.51	70.54
10	88.284	6.1	5.13	72.13
10	60	3.9	3.51	70.54
5	80	6.0	5.22	71.23
10	31.71	1.7	1.37	70.26
10	60	3.9	3.51	70.54
5	40	2.7	2.13	70.12
15	40	2.0	1.91	70.67
10	60	3.9	3.51	70.54
17.071	60	4.7	4.01	71.16
15	80	6	5.40	72.34

Table 3 — Analysis of variance for antioxidant activity response

Source	Sum of Squares	df	Mean Square	F-value	p-value	significant
Model	5.69	5	1.14	130.19	< 0.0001	significant
A-Tea Concentration	0.9938	1	0.9938	113.67	< 0.0001	—
B-Sugar Concentration	3.68	1	3.68	420.73	< 0.0001	—
AB	0.0784	1	0.0784	8.97	0.0201	—
A <sup>2</sup>	0.1256	1	0.1256	14.37	0.0068	—
B <sup>2</sup>	0.8860	1	0.8860	101.34	< 0.0001	—
Residual	0.0612	7	0.0087	—	—	—
Lack of Fit	0.0612	3	0.0204	—	—	—
Pure Error	0.0000	4	0.0000	—	—	—
Cor Total	5.75	12	—	—	—	—
R <sup>2</sup>	0.9894	—	—	—	—	—
Adjusted R <sup>2</sup>	0.9818	—	—	—	—	—
Predicted R <sup>2</sup>	0.9243	—	—	—	—	—
Adeq Precision	32.6182	—	—	—	—	—

\*P-values<0.05 indicate good fitness of model

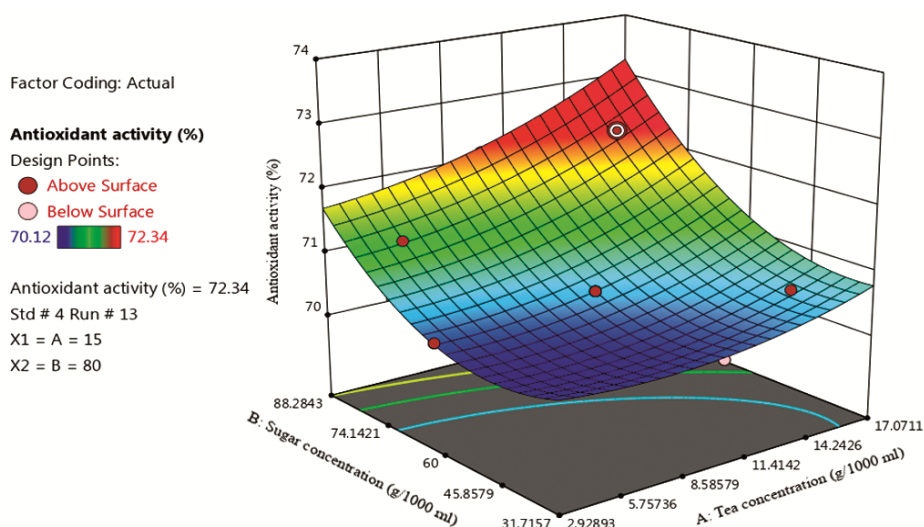


Fig. 3 — 3D plot for interaction of tea concentration and sugar concentration vs. antioxidant activity

3D surface plots as shown in Fig. 3, represented the inter relation between tea and sugar concentration for antioxidant activity response. It depicted that with increase in tea and sugar concentration, antioxidant activity also increased.

#### Physicochemical and Microbiological Analysis of Fermented Beverage

*Kombucha* beverage production was carried out as per the mentioned protocol. Different concentrations of black tea and sugar was taken and boiled in 500 ml of water. After about 10 minutes of boiling, tea decoction was sterilized and cooled down to room temperature, 5% of starter culture and SCOBY was inoculated in the tea decoction and it was then fermented at 25°C for 7 days. Within 7 days a daughter SCOBY formed on the top of broth was removed and the drink was filtered and stored in bottles. All the 13 formulations as mentioned in Table 1 were evaluated for change in physico-chemical and microbiological parameters. To have a better depiction of each fermentation separately the comparative change in particular parameter (pH, TSS, titrable acidity, total sugars, reducing sugars, antioxidant activity, total phenolic content, vitamin C, alcohol content, yeast count and acetobacter count) is presented in Fig. 4 and discussed.

**pH**— Organic acids such as acetic acid, gluconic acid which are produced during fermentation by acetic acid bacteria decreases the pH of *kombucha*. pH between 2.5 and 3.5 is considered acceptable for these beverages. Fig. 4a revealed that in each case there is a periodic fall of pH from 6 to near about 4

after one day of fermentation, that further decreases to about 3.5 within 3<sup>rd</sup> day and finally at the end of fermentation, that is 7<sup>th</sup> day, pH range of all the formulations were in quite accordance with the acceptable limits (2.5–3.5). Average pH of unfermented tea beverages was observed to be 6.09, which dropped to 2.84 after 7 days of fermentation. It has been reported by Liu *et al.*<sup>29</sup> and Greenwalt *et al.*<sup>30</sup> that pH of kombucha beverage decreases from 5.0–2.5 during the fermentation of 6 days. Similar observations were recorded in reports where pH of black tea *Kombucha* beverage dropped to 3.0 when fermentation was carried out for 7 days at 28°C.<sup>31</sup>

**Total Soluble Solids** – Sucrose is the basic carbon source and it plays an important role on metabolism of yeast and acetic acid bacteria. Starter culture consumes the added sucrose and influences the concentration of total soluble solids.<sup>32</sup> It was observed that total soluble solids decreased with increase in fermentation time. TSS (°Brix) at the start of fermentation was different for all the formulations. Fig. 4b depicts that TSS was in the range of 3.1–8.9 at the start and was in the range of 7.0–2.2 on 1<sup>st</sup> day of fermentation which reduced subsequently till the last day of fermentation. TSS at the 7<sup>th</sup> day of fermentation was in the range of 1.7–6.0.

**Titrable Acidity**—Yeast invertase hydrolyzes the sucrose into fructose and glucose. These are further metabolized by acetic acid bacteria into gluconic and acetic acid.<sup>33</sup> An increase in titrable acidity with increase in fermentation time has been observed in the present study also. Titrable acidity of unfermented

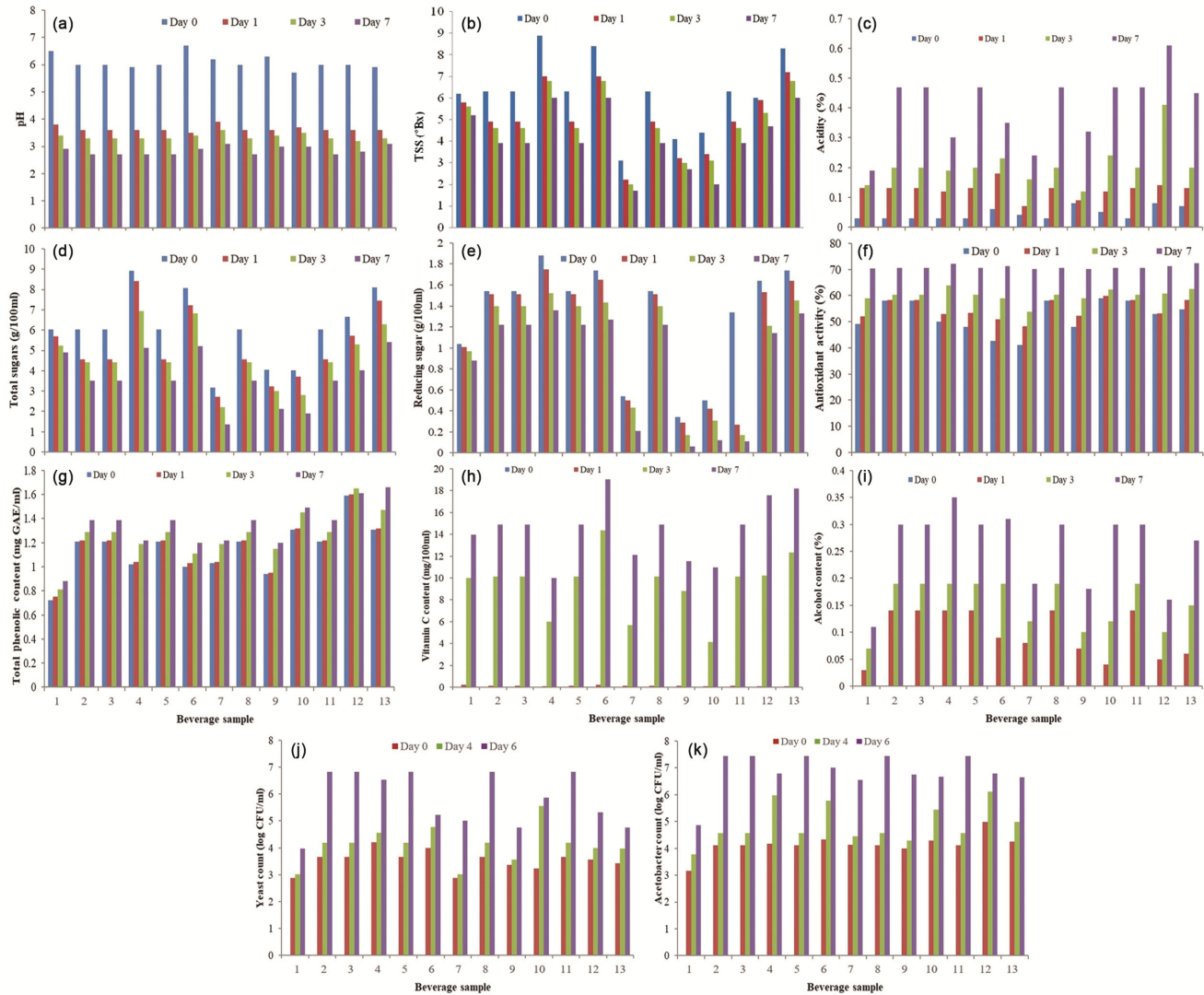


Fig. 4(a-k) — Periodic analysis of change (a) pH; (b) Total soluble solids; (c) Titrable acidity; (d) Total sugar content; (e) Reducing sugar content; (f) Antioxidant activity; (g) Total phenolic content; (h) Vitamin C content; (i) Alcohol content; (j) Yeast count and (k) *Acetobacter* count during fermentation

beverages was about 0.03%, which increased to about 0.1% at 1<sup>st</sup> day of fermentation and then after 7days, acidity of all the formulations were in quite accordance with the acceptable limits (<1%) (Fig. 4c). Similar changes in acidity during fermentation is also reported in fermented beverage produced using black tea and fermented at temperature of 22°C.<sup>34</sup> It has been reported by Chen and Liu,<sup>35</sup> that acidity of *Kombucha* beverage increases to 11 g/L during 30 days of fermentation and then it decreases. According to Tu *et al.*<sup>36</sup>, acetic acid concentration in *Kombucha* prepared using Soy whey as a substrate after 8 days of fermentation was found to be 5.77 g/L. In another study, acidity value of 1.067% has been reported that

was higher than acidity observed in the present study.<sup>37</sup> Highest acidity (0.61%) was observed in Beverage 12 and lowest acidity (0.19%) was recorded in Beverage 1.

**Total Sugars and Reducing Sugars** – Sugar content in *kombucha* changes with fermentation time. It was analyzed that total sugar and reducing sugar content was high in the starting of fermentation, then it started decreasing because of the metabolism of sugar into alcohol and further to acids. For getting a sweet *Kombucha*, usually the fermentation time of one week is sufficient, while one who likes vinegary taste would prefer long fermentation. Different

concentration of sugar was added in all the formulations hence, sugar concentration in all the unfermented beverages were different in the range of about 3–8 g/100ml which decreased to 2.8–6 g/100ml at 3<sup>rd</sup> day of fermentation and later fall in the range of 1.91–5 g/100ml at last day of fermentation. Similarly, reducing sugar content at the start of fermentation was in the range of 1–2 g/100 ml, which fall to 0.1–1 g/100ml on 7<sup>th</sup> day of fermentation (Fig. 4d & 4e). Functionally different strains are responsible for conversion of sugars to ethanol (present in water used for steeping tea leaves) to ethanol.<sup>38</sup> The contribution of acetic acid bacteria that is *Gluconobacter* as well as *Acetobacter* is that they result in formation of acetic acid by utilizing ethanol, additionally they form gluconic acid from glucose.<sup>39</sup> Due to uninterrupted production of acetic acid, more and more alcohol is produced because acetic acid results in more stimulation of yeast cells and in turn ethanol facilitates more and more production of acetic acid by increasing the growth of acetic acid bacteria.<sup>29</sup> Other organic acids such as gluconic acid, tartaric acid, malic acid, citric acid are also produced during the process of kombucha fermentation.

**Antioxidant Activity** – It has been reported by Malbasa *et al.*<sup>40</sup>, that starter culture affects the antioxidant activity of beverage. The inhibition percentage of samples depicted the antioxidant activity of beverage. Higher the percentage of inhibition, higher is the antioxidant activity of sample. It was analyzed that antioxidant activity of beverage increased with increase in fermentation time but the change was very slight. Highest antioxidant activity was observed in Beverage 13 while lowest was observed in Beverage 9. Antioxidant activity in different formulations was dependent on the amount of tea added. Beverage prepared using high concentration of tea were observed to have high antioxidant activity than other beverages. In the reports of Ahmed *et al.*<sup>41</sup>, antioxidant activities of *Kombucha* made from tea, barley and rice were studied, in which antioxidant activity of Black tea *Kombucha* was found to be 86.69% and it increased with increase in fermentation time. Same pattern of increase in antioxidant activity was observed in the present study where, antioxidant activity of different formulations was found to be about 40–50% at 0<sup>th</sup> day, which increased to about 55–60% at 3<sup>rd</sup> day and then increased to near about 70% at last day of

fermentation (Fig. 4f). Antioxidant activity of green tea *Kombucha* was analysed by Jayabalan *et al.*<sup>42</sup>, in which DPPH scavenging abilities increased with increase in fermentation time and was found to be 84% at 12<sup>th</sup> day of fermentation. According to Fu *et al.*<sup>43</sup>, DPPH radical scavenging activity of green tea *kombucha* was found to be 95.30% after fermentation of 90 days at 30°C, while radical scavenging activity of black tea kombucha was found to be 38.7%. This difference can be due to higher level of polyphenols in green tea than other teas.<sup>43</sup>

**Total Phenolic Content** – The present study revealed that there was not much change in phenolic content during fermentation. Total polyphenols content increased during fermentation time but the change was very slight. Total phenolic content in different formulations was dependent on tea concentration. Beverage prepared using high concentration of tea were observed to have more total phenolic content than other beverages. In our study, it can be deduced from Fig. 4g, that total phenolic content of unfermented beverage formulations was found to be in range of 0.7–1.5 mg/ml, which increased to 0.8–1.6 mg/ml at 3<sup>rd</sup> day, followed by 0.8–1.7 mg/ml at 7<sup>th</sup> day of fermentation. Similarly, there was an increase in total phenolics in black and green tea *Kombucha* after 2 weeks of fermentation in reports of Kallel *et al.*<sup>44</sup> In their report, Unfermented tea has total phenolics of about 0.78g GAE/L in green tea *Kombucha* and 1.01 g GAE/L in black tea *Kombucha*. After 15 days of fermentation, total phenolic content increased to 1.08 g GAE/L in green tea *Kombucha* and 1.12 g GAE/L in black tea *kombucha*. As per our study, average total phenolic content observed at last day of fermentation in beverages were found to be 1.34 mg GAE/ml. Similar observations were also recorded in studies of Jafari *et al.*<sup>45</sup>, in which there was an increase in polyphenols content when *Kombucha* beverage was fermented for 14 days in room temperature. Increase in total phenolic content was also observed in kombucha developed using prickly pear juice (*O. ficus-indica*), having highest concentration of total phenolics on 9<sup>th</sup> day of fermentation.<sup>46</sup> Maximum phenolic content was observed in Beverage 13 (1.66 mg GA/ml) and minimum content (0.88 mg GAE/ml) was observed in Beverage 1.

**Vitamin C** – In the present study, it was observed that there was no Vitamin C content present in unfermented tea, which increased to about

0.1mg/100ml at day 1 and then further increased in the range of 9–19 mg/100ml at the end of fermentation (Fig. 4h). Vitamin C content of 1.51 mg/ml was reported by Bauer–Petrovska and Petrushevaska–Tozi<sup>47</sup> which was much high as compared to vitamin C content observed in the present study. In reports of Vitas *et al.*<sup>48</sup>, Kombucha beverage was produced with *A. millefolium* flowers, and observed that Vitamin C increased with increase in fermentation time and it ranged from 0.48–1.14 mg/L at last day of fermentation. Highest amount of Vitamin C content (19.01 mg/100 ml) was observed in Beverage 6 and lowest content (10.98 mg/100 ml) was observed in Beverage 10. Low value of vitamin C content can be due to differences in the microbial culture and its differential ability to produce Vitamin C.

**Alcohol Content** – All the non alcoholic drinks including Kombucha should have alcohol content less than 0.5% (Food Standards Australian New Zealand).<sup>49</sup> In the present study it was estimated that there was no alcohol present in unfermented beverage prior to addition of SCOBY. It can be revealed from Fig. 4i that alcohol content at 1<sup>st</sup> day of fermentation was found to be less than 0.14% and then increased in the range of 0.1–0.35% at 7<sup>th</sup> day of fermentation. Alcohol content increased with increase in fermentation time which is similar to that of studies by Chen and Liu<sup>50</sup>, in which alcohol content increased with fermentation time and reached maximum value of 0.5% on 10<sup>th</sup> day of fermentation. In our experiment, alcohol content at last day of fermentation was in the range of 0.11–0.35%. Concentration of alcohol in our samples were much lower than that of concentration reported by Chakravorty *et al.*<sup>51</sup>, where alcohol increased from 0.46% to 2.8% after 7 days of fermentation. Kallel *et al.*<sup>44</sup> produced kombucha beverage using black tea as substrate and observed alcohol content of 0.3% at 15<sup>th</sup> day of fermentation. Kombucha beverage produced using black and green tea as substrates produced ethanol content in the range of 0.69–1.11% during fermentation of 10 days.<sup>52</sup> All the beverages had alcohol content less than 0.5%, highest alcohol content (0.35%) was observed in beverage 4 and lowest content was observed in beverage 1.

**Determination of Yeast and Acetic Acid Bacteria Count** - It is very well known that yeast converts sucrose into fructose and glucose, which is then converted into acids such as gluconic acid and

acetic acid by *Acetobacter*. After inoculating SCOBY it was observed that both Yeast and Acetic acid bacteria increased with increase in fermentation time. In all the beverages it was observed that at 1<sup>st</sup> day of fermentation, average Yeast count was log 3.52 CFU/ml which increased to log 5.77 at 7<sup>th</sup> day of fermentation (Fig. 4j). Similarly, at the 1<sup>st</sup> day of fermentation average count of Acetic acid bacteria was found to be log 4.14 and it increased to log 6.87 at 7<sup>th</sup> day of fermentation (Fig. 4k). Hence in our experiment it was observed that Yeast and Acetobacter count increased with increase in fermentation time and also Acetobacter count was found to be more than Yeast count, which specifies the dominancy of Acetobacter. According to reports of Chen and Liu<sup>50</sup>, similar findings were observed in which, yeast cell count increased from  $0.21 - 2.74 \times 10^6$  CFU/ml at day 0 to  $12-78.5 \times 10^6$  CFU/ml at day 6<sup>th</sup>. In the same study, change in acetobacter count was also observed in which it increased from  $1.50 - 18.8 \times 10^3$  CFU/ml at day 0 to  $9.30 - 91.50 \times 10^3$  CFU/ml at day 6<sup>th</sup>. It was also observed in their study that yeast and acetobacter count gradually decreased with prolong fermentation. Sreeramulu *et al.*<sup>53</sup>, reported findings in which Yeast and Acetic acid bacteria count in fermented black tea beverage increased rapidly till 4<sup>th</sup> day of fermentation, but after that it declined by 6 days of fermentation, further it continued to decline. Yeast and acetobacter count at 0<sup>th</sup> day of fermentation was found to be  $10^2$  CFU/ml which increased to  $10^7$  during 4<sup>th</sup> day and then declined to  $10^4$  CFU/ml at 6<sup>th</sup> day of fermentation.

#### Sensory Evaluation of Developed Fermented Beverages

In sensory evaluation, beverages produced were evaluated on basis of their flavour, mouth–feel and overall acceptability. In this experiment, different black tea based beverages were produced with different concentration of tea and sugar. Beverages with Code 1–13 were evaluated by 10 panelist and the beverage with code 6 was found to be most preferred beverage on the basis of colour, aroma, mouthfeel and overall acceptability with the highest score of 8.15 (Table 4). There are several scientific reviews covering topics of Kombucha processing methods, chemical composition, health benefits and toxicity related to it, but there are not many publications covering sensory and consumer reports on Kombucha beverage.

Table 4 — Sensorial evaluation of mean values of Beverages by panelists on hedonic scale

Sr. No.	Beverage Sample	Sensory Parameters					Mean Score
		Appearance/ Colour	Flavour	Mouth–feel	Overall acceptability		
1	1	7.97	7.95	7.52	7.95	7.84	
2	2	6.44	5.11	5.22	5.22	5.50	
3	3	6.44	5.11	5.22	5.22	5.50	
4	4	6.16	6.11	5.88	5.88	6.01	
5	5	6.44	5.11	5.22	5.22	5.50	
6	6	7.83	8.20	8.30	8.18	8.15	
7	7	7.22	6.16	6.16	6.05	6.39	
8	8	6.44	5.11	5.22	5.22	5.50	
9	9	5.88	5.38	5.55	5.55	5.54	
10	10	5.16	4.55	4.55	4.55	4.70	
11	11	6.44	5.11	5.22	5.22	5.50	
12	12	5.55	4.77	4.44	4.88	4.88	
13	13	7.70	8.21	8	7.98	7.9	
CD @ 5%		0.167	0.172	0.160	0.160	0.172	

### Conclusions

Keeping in view, the observations of the present study, it can be inferred that black tea based fermented beverage *Kombucha* is a nutrient rich alternate to the carbonated beverages. Fermentation of black tea with symbiotic culture of bacteria and yeast (SCOBY) leads to increase in antioxidant activity and total phenolic content. At the same time, the vitamin C content of the fermented beverage was in the range of 9–19 mg/100 ml, while it was absent in tea extract. Though tea is already one of the most widely consumed beverage not only in India but worldwide, but its fermentation at the pre-optimized conditions can further lead to the production of a functional beverage. After the increased popularity of “Functional Foods”, market of functional beverages like kombucha can spread rapidly in the market. Functional beverages are considered to be most effective active sector among functional foods because of their ease of product formulations by incorporating desirable nutrients and bioactive compounds, ease of distribution and refrigeration for better storage and shelf life of product. Further, metabolome analysis of fermented traditional kombucha and its variants prepared by blending with fruits or vegetables juice can be an important area of future research in India. Similarly, further studies can be inclined towards establishing regulatory guidelines for the final product and towards determining the potential of this product in the market and peoples’ health

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