Chapter

Usage of Kombucha 'Tea Fungus' for Enhancement of Functional Properties of Herbal Beverages

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Abstract

The following herbal teas were fermented with the Kombucha "tea fungus" for 7 days: *Acacia arabica*, *Aegle marmelos* root bark, *Aerva lanata*, *Asteracantha longifolia*, *Cassia auriculata*, *Hemidesmus indicus*, *Hordeum vulgare*, *Phyllanthus emblica*, *Tinospora cordifolia*. Microbial enumerations of the bacteria and fungi present in the broth and the tea fungal mats were carried out. At the end of the period of fermentation, the pH values ranged from 4.0 to 6.0, while the titratable acidity (TA) ranging from 2.5 to 5.0 g/mL. The TA was within the acceptable limits of consumption for all beverages. The Oxygen radical absorbance capacity (ORAC) assay indicated 5 of the fermented beverages to have statistically significant increases ($P < 0.05$) by the end of the period of fermentation. By day 7, the IC_{50} values of the α-amylase inhibitory activities ranged from 52.5 to 67.2 μg/mL, while the α-glucosidase inhibitory activity values ranged from 95.2 to 196.1 μg/mL by this time point. Overall, an enhancement of the antioxidant and starch hydrolase inhibitory potential of the seven herbal teas was observed as a result of the fermentation by addition of the tea fungus. Thus, this fermentation process could be highlighted as a novel and versatile methodology to obtain functional beverages.

Keywords: antioxidant activity, ORAC, Kombucha, starch hydrolase inhibitory activity, total phenolic content

1. Introduction

Kombucha or "tea fungus" is a fermented beverage which is believed to have its origins in northeast China, and has recently gained rapid popularity among the rest of the world [1]. The beverage is produced through the symbiotic growth of bacteria and osmophilic yeast strains in a thick jelly-like membrane which is cultured in sugared black tea (**Figure 1**) [2]. While the term "Kombucha" is the most commonly used name for the beverage on a commercial basis, it is also known by other names such as Chainii grib, Chainii kvass Champignon de longue vie, Ling zhi, kocha kinoko and red tea fungus [3]. For the production of the beverage, the substrate is incubated with the tea fungal mat statically under aerobic conditions, usually for a minimum of 7 days at 20–28°C [4]. However, to obtain a pleasantly sour beverage with palatability and acceptable sensory properties, the fermentation should terminate when the titratable acidity (TA) reaches 4.0–4.5 g/L—a level which has been confirmed as acceptable by longtime consumers of the Kombucha beverage and is known as the optimal consumable acidity [5].

Figure 1.
Fermented black tea using the Kombucha tea fungal mat after 7 days of fermentation.

There are several types of fermentation processes which take place in the production of the beverage and obtained by-products depending on the various metabolic pathways followed by the microorganisms [6]. Kombucha fermentation is a combination of three such pathways: Alcoholic, lactic, and acetic acid production [6]. These pathways take place primarily because of the presence of several yeasts and bacteria coexisting in the medium, where the fermentation is initiated by osmotolerant microorganisms and ultimately dominated by acid-tolerant species [6].

There are many yeast genus and species in the Kombucha culture, where a broad spectrum has been reported by many researchers including *Zygosaccharomyces*, *Candida*, *Kloeckera/Hanseniaspora*, *Torulaspora*, *Pichia*, *Brettanomyces/Dekkera*, *Saccharomyces*, *Lachancea*, *Saccharomycodes*, *Schizosaccharomyces*, and *Kluyveromyces* [7–10]. The dominant bacteria of the Kombucha tea culture are acetic acid bacteria, which are aerobic and thus, are able to use alcohol as a substrate to form acetic acid [6]. However, in contrast to yeast, these bacteria require large amounts of oxygen for their growth and activity [6]. Tea provides the necessary nitrogen sources for the bacteria and yeast cultures present in the tea fungal mat [11]. Black tea is the traditional and most dominant substrate used for the Kombucha fermentation, which is independent of its comparatively lower caffeine content (2%) as compared with green tea (5%) [12]. This could be because of the comparatively more acceptable sensory qualities and flavor characteristics generated in black tea as a result of the fermentation process.

Some studies have been able to successfully demonstrate the preparation of fermented beverages through the addition of the tea fungus to various types of plant-based products which are essentially not of *Camellia sinensis* origin [13, 14]. Additionally, Watawana et al. [15] has successfully demonstrated the substitution of sugar with other types of sweetening agents for carrying out the fermentation process of the Kombucha beverage. Considering the therapeutic properties of many of the other herbal teas available in the marketplace, whether their antioxidant potential can be enhanced by natural or artificial means is a reasonable query since value-addition to existing beverages for the purpose of novelty and meeting consumer demands is a matter requiring urgent attention in the functional beverage industry.

In this study, the following plant-based herbal teas were fermented by addition of a locally available tea fungal mat: *Acacia arabica* (AA), *Aegle marmelos* root bark (AM-RB), *Aerva lanata* (Ala), *Asteracantha longifolia* (Alo), *Cassia auriculata* (CA), *Hemidesmus indicus* (HI), *Hordeum vulgare* (HV), *Phyllanthus emblica* (PE), *Tinospora cordifolia* (TC). All these herbs which are commonly consumed in Sri Lanka for health and wellness purposes have almost similar taste and color as black tea. This somewhat reassures that the fermented product does not essentially result in any adverse sensory properties which may discourage consumer acceptability.

The selection of herbs was also based with the intention of widening the application of the tea fungus to other types of herbal teas which maybe more readily available depending on the vegetation of various regions and countries. Jayawardena et al. [16] has investigated the antioxidant and starch hydrolase inhibitory potential of these herbs, further justifying their selection as a fermentation medium for preparation of the novel beverages. Identification of the dominant bacteria and yeast species were carried out as well as the changes to the overall population of bacteria and yeast in the broth and pellicle. Other than pH and TA, the enhancement of the antioxidant potential was evaluated. Another aspect of this study was the investigation of the starch hydrolase inhibitory activities, namely α-amylase and α-glucosidase enzyme activities, of the fermented beverages. Starch hydrolase inhibitors retard the absorption of glucose by curbing the action of α-amylase and α-glucosidase [17]. Compounds which are able to impede these enzymes and thereby, delay starch digestion, resulting in a reduction in the rate of glucose absorption, which in turn blunts the postprandial plasma glucose increase in diabetic patients. The enhancement of the starch hydrolase inhibitory potential of the herbal teas through addition of the tea fungal mat was also investigated in this study.

2. Materials and methods

Acetobacter aceti was found to be the dominant bacterial strain present in the tea fungal mat which was used for the study. The dominant yeast components were identified as *Zygosaccharomyces bailii* and *Brettanomyces claussenii*. These aspects were confirmed through DNA sequencing as per the method by Marsh et al. [14]. Dried powders of the plants were obtained from the Ayurveda Medicinal Hall in Kandy, Sri Lanka during the months of June–July 2014.

2.1 Preparation of the fermented beverages and determination of pH, TA and overall population of bacteria and yeast in the broth and pellicle

One gram each of the plant powders were added to 100 mL of boiling water and infused for 5 min followed by filtration through a sterile sieve. Sucrose (10%) was dissolved in each beverage and the preparation was left to cool to room temperature at 24 ± 3°C. The cooled teas were aseptically inoculated with 10 mL of the freshly grown tea fungal broth which was originated from black tea for 7 days at 24 ± 3°C. Sampling was performed on a daily basis in order to avoid contamination. Broths of the fermented beverages were centrifuged at 7240 g for 10 min prior to the assays to remove any particulate matter present as a result of microbial action and coalescence of proteins, which might interfere with the measurements. An electronic pH meter (Orion model 290A) was used to measure the pH of the broths, while the TA was measured according to the method by Chen and Liu [12] using acid-based titration. Fermented broth (10 mL) was titrated with 0.1 M NaOH and the end-point was determined by measuring the pH using the electronic pH meter (Orion model 290A), where pH = 7.0 was taken as the end-point. The final TA of the fermented broths was expressed in g/mL.

Changes to the overall population of bacteria and yeast in the fermented broth and pellicle was determined according to the method by Chen and Liu [12]. Glucose-yeast extract-calcium carbonate agar (GYCA) and potato dextrose agar (PDA) media, were used for the growth of bacteria and yeast, respectively. As per the method by Chen and Liu [12], the GYCA medium was composed of 30 g of glucose, 5 g of yeast extract, 3 g of peptone, 10 g of calcium carbonate, 30 mL

of 95% ethanol, 20 g of agar, and distilled water added to make a final volume of 1 L. Aliquots of 1 mL were taken from both the broth and the tea fungal mat, while the upper pellicle portion was filtered with a sterile cheesecloth before sampling to remove the cellulose fibers. Both bacterial and yeast counts were expressed as colony-forming units per mL (cfu/mL).

2.2 Determination of the total phenolic content (TPC) and antioxidant activity

The method by Huang et al. [18] was used for determining the TPC. The results were expressed as milligrams of gallic acid equivalents (GAE) per milliliters (mg GAE/mL), using a gallic acid standard curve. The assay was carried out in 96-well format where the following constituent volumes were added in this particular order to a single well to add up to a total volume of 200 μL per well: Sample / blank (deionized water) / gallic acid standard—20 μL, Folin-Ciocalteu reagent—100 μL, Na_2CO_3 (30 g/L)—80 μL. The microplate was incubated at room temperature for 15 min following which the absorbance was read at 540 nm using a Thermo Scientific Multiskan GO Microplate Reader. The TPC value was back-calculated using the gallic acid standard curve.

The Oxygen Radical Absorbance Capacity (ORAC) assay was carried out according to the methodology by Prior et al. [19]. The final values for this assay were expressed as micromoles of trolox equivalents (TE) per milliliters (μmol TE/mL) using a trolox standard curve. Trolox is a vitamin E analogue and is ideal for the calculation of the ORAC value. This assay was carried out in 96-well format as well [19]. The following components were added to a single well in this particular order, adding up to a total volume of 200 μL per well: (1) Blank (phosphate buffered saline)/ trolox standard/sample—20 μL, (2) fluorescein working solution—160 μL, (3) 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH)—20 μL. AAPH was added in last since it is a radical initiator and for the reaction kinetics to occur in all wells at the same time point. The reaction kinetics were monitored for two hours as a fluorescence decay [485 nm (ex)/525 nm (em)], following which the area under the curve was used to calculate the ORAC value compared with those of the trolox standards.

As for the di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) radical scavenging activity assay, 100 μL of each of the broths of the fermented beverages were mixed with 1 mL of 0.1 mM DPPH in ethanol along with 450 L of 50 mM Tris-HCl buffer at pH 7.4. The solution was incubated at room temperature for 30 min and reduction of DPPH radicals was measured by reading the absorbance of the resulting solution at 517 nm. The antioxidant activity was calculated as % DPPH radical scavenging activity. Scavenging ability of superoxide radical (O_2^-) was assessed by the method described by Lee et al. [20], and was carried out in 96-well format using the Thermo Scientific Multiskan GO Microplate Reader similar to the TPC and ORAC assays.

2.3 Determination of the α-amylase and α-glucosidase inhibitory activities

The α-amylase inhibitory activity was evaluated according to the method by Liu et al., where acarbose was used as the positive control [17]. The reduction of turbidity at 660 nm was monitored over time using the Thermo Scientific Multiskan GO Microplate Reader, and the area under the curve was used to calculate the overall inhibitory activity. The α-glucosidase inhibitory activity was carried out according to the method by Koh et al., and the same positive control was used in this instance as well [21]. A reaction substrate consisting of 4-nitrophenyl R-D-glucopyranoside (PNPG), (30 mM) and α-glucosidase (16.65 mg/mL) were prepared in phosphate

buffer saline (PBS). A volume of 340 μL of inhibitor solutions of different concentrations was pipetted into separate reaction vials. Then, 20 μL of the α-glucosidase solution was added into each vial, and incubated at 37°C for 10 min. The PNPG solution (40 μL) was added to initiate the digestion. After 15 min, 200 μL of 1 M Na_2CO_3 solution was added to terminate the reaction. Aliquots of 300 μL from the reacted solutions were withdrawn and added each well of a 96-well microplate. Absorbance at 400 nm was read using the Thermo Scientific Multiskan GO Microplate Reader.

For both assays, experiments were performed in triplicate, and a curve of percentage inhibition against inhibitor concentration was plotted with the averaged values to back-calculate to the enzyme inhibitory values in the samples. Although for both assays, acarbose equivalence could also be used to express the final enzyme inhibitory data, in this instance, for ease of reference and comparison, the results were expressed as IC_{50} (mg/mL).

2.4 Statistical analysis

For the statistical evaluations, IBM SPSS Statistics version 21.0 released in 2012 (IBM Corp., Armonk, NY, USA) for Windows was used. Three or more independent analyses were utilized to calculate and express the results as mean ± standard error mean (SEM). *P* values of >0.05 were considered to be significant.

3. Results and discussion

As per **Figure 2**, all the beverages had a statistically significant decrease ($P < 0.05$) in the pH values by the end of the fermentation process on day 7. Out of the nine fermented beverages, HI and TC had statistically significant decreases ($P < 0.05$) in the pH from day 1 itself, whereas changes took place slightly later in the rest of the beverages. The decrease in pH of all the Kombucha samples as a whole would have been due to the increased concentration of organic acids (typically in the form of acetic acid) produced during the fermentation process. As per **Figure 1**, all unfermented teas had an initial pH value of between 6.0 and 8.0 prior to fermentation, while the pH values were between 4.0 and 6.0 on day 7 at the end of the fermentation period.

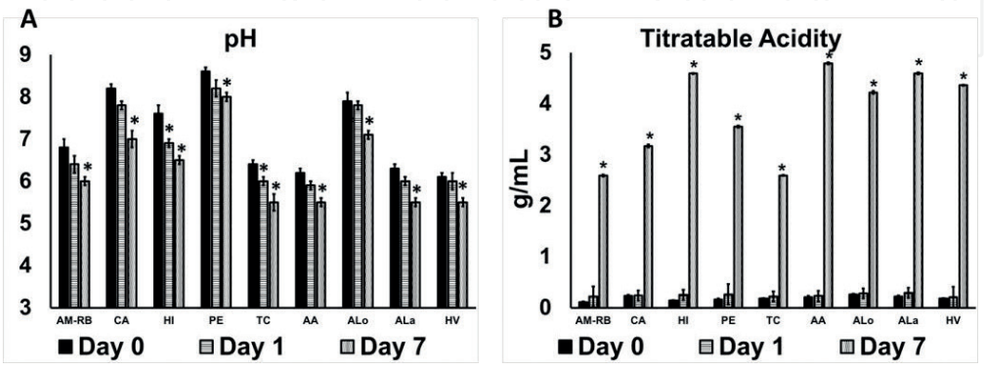

Figure 2.
*The (A) pH and (B) titratable acidity (TA) of the beverages prior to addition of the tea fungal mat (day 0), followed by day 1 and day 7 of fermentation. Values are expressed as mean ± SEM. * P < 0.05 versus the value of each tea at day 0. Abbreviations: Acacia arabica (AA), Aegle marmelos root bark (AM-RB), Aerva lanata (Ala), Asteracantha longifolia (Alo), Cassia auriculata (CA), Hemidesmus indicus (HI), Hordeum vulgare (HV), Phyllanthus emblica (PE), Tinospora cordifolia (TC).*

As per **Figure 2**, the TA of all the unfermented beverages had initial values ranging from 0.1 to 0.5 g/mL. Once the fermentation was complete by day 7, the TA values ranged between 2.5 and 5.0 g/mL, where all beverages had statistically significant increases in this particular parameter ($P < 0.05$). Given that the optimum consumable acidity level is 4.0–4.5 g/L for Kombucha beverages, only AA and HI did not have TA levels falling within this range by the end of the period of fermentation. Nevertheless, on a comparative basis, according to the TA reference levels as indicated by Reiss [5], it may be assumed that all of the Kombucha beverages were acceptable for consumption in terms of acidity.

The TPC and ORAC values are shown in **Figure 3**. AM-RB had the highest TPC prior to fermentation with HV being the lowest. By day 7, statistically significant increases ($P < 0.05$) in the TPC were observed in AM-RB, CA, HI and TC. Correlating with the TPC values, AM-RB had the highest ORAC values with HV being the lowest. The DPPH EC_{50} values appear to have complemented the ORAC values with the exceptions of AM-RB, TC, AA and ALa, where their values were observed to have statistically significant increases ($P < 0.05$) by day 7 (**Figure 3**).

As for the antioxidant assay of superoxide scavenging activity, the trends of increase or decrease whether it be statistically significant or not, were not as clear as the ORAC and DPPH EC_{50} values (**Figure 4**). A better correlation between the TPC and the ORAC values rather than the DPPH EC_{50} and superoxide scavenging values of all beverages on all days of analysis ($R^2 = 0.985$ for ORAC versus $R^2 = 0.745$ DPPH EC_{50} and $R^2 = 0.632$ for superoxide scavenging potential). This showed that the phenolic compounds present in the fermented beverages were better scavengers of the peroxyl radical which is generated through the AAPH.

The starch hydrolase inhibitory activities of α-amylase and α-glucosidase enzymes are shown in **Figure 5**. On the first day of fermentation, only CE, HI, PE and AA had statistically significant increases ($P < 0.05$) in the α-amylase inhibitory activities. However, by day 7 at the end of the fermentation period, all the beverages had statistically significant increases ($P < 0.05$) as compared with day 0. As for the α-glucosidase inhibitory activities, AM-RB, CA, HI, PE, AA, ALa and HV had statistically significant increases ($P < 0.05$) during both day 1 and 7 as compared with day 0. The fermentation process was able to enhance the

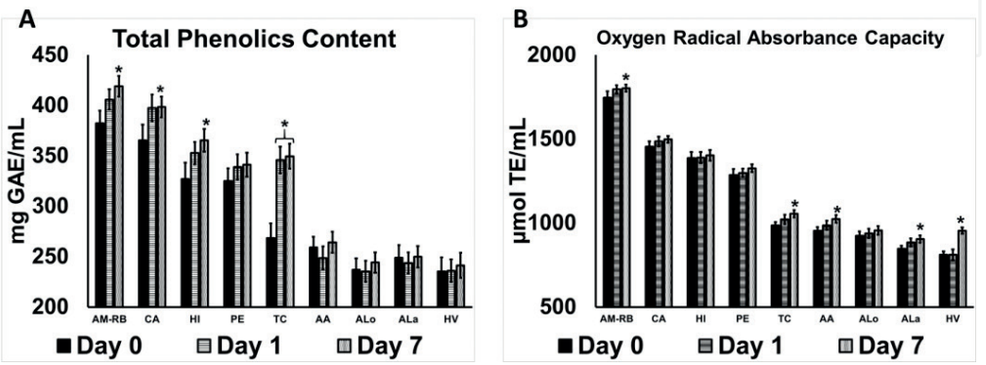

Figure 3.
*The (A) Total phenolic content (TPC) and (B) ORAC values of the herbs prior to addition of the tea fungal mat (day 0), followed by day 1 and day 7 of fermentation. Values are expressed as mean ± SEM. * $P < 0.05$ versus the value of each tea at day 0. Abbreviations: Acacia arabica (AA), Aegle marmelos root bark (AM-RB), Aerva lanata (Ala), Asteracantha longifolia (Alo), Cassia auriculata (CA), Hemidesmus indicus (HI), Hordeum vulgare (HV), Phyllanthus emblica (PE), Tinospora cordifolia (TC).*

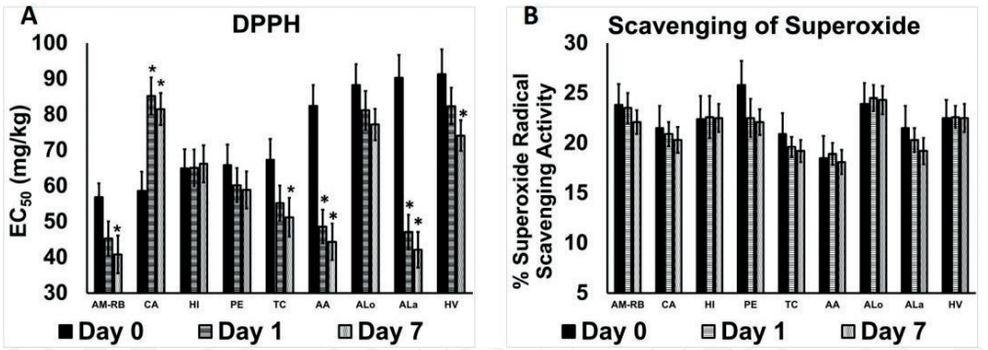

Figure 4.
*The (A) DPPH and (B) superoxide radical scavenging values of the herbs prior to addition of the tea fungal mat (day 0), followed by day 1 and day 7 of fermentation. Values are expressed as mean ± SEM. * P < 0.05 versus the value of each tea at day 0. Abbreviations: Acacia arabica (AA), Aegle marmelos root bark (AM-RB), Aerva lanata (Ala), Asteracantha longifolia (Alo), Cassia auriculata (CA), Hemidesmus indicus (HI), Hordeum vulgare (HV), Phyllanthus emblica (PE), Tinospora cordifolia (TC).*

α-amylase inhibitory activity better than the α-glucosidase inhibitory activity. This is an important aspect, given that α-amylase is required for the subsequent reactions of α-glucosidase, and therefore, curbing the reactivity of α-amylase is important from the perspective of reducing the release of simple sugars into physiological system [22].

Changes to the composition of the overall population of bacteria and yeast present in the broth and pellicle on days 1 and 7 are shown in **Tables 1** and **2**, respectively. As per the measurements from day 0, the inoculum of 10 mL which was added to initiate the fermentation process contained various numbers of bacteria and yeast. However, overall, an increase in the bacteria and yeast population in all of the fermented beverages was observed by the end of the period of fermentation. The increase in these numbers was indicative of the microbes' viability and successful livelihood in the beverages, thus, confirming their ability of thrive in the environments in similar fashion to black tea. An aspect which remains to be investigated concerning this parameter is whether some of the microbes have entered a viable but nonculturable (VBNC) state. Since the origins of the bacteria and yeast are from black tea itself, it is possible that some of the microbes have entered a state of low metabolic activity, and thus were not accounted for during the measurement of this parameter.

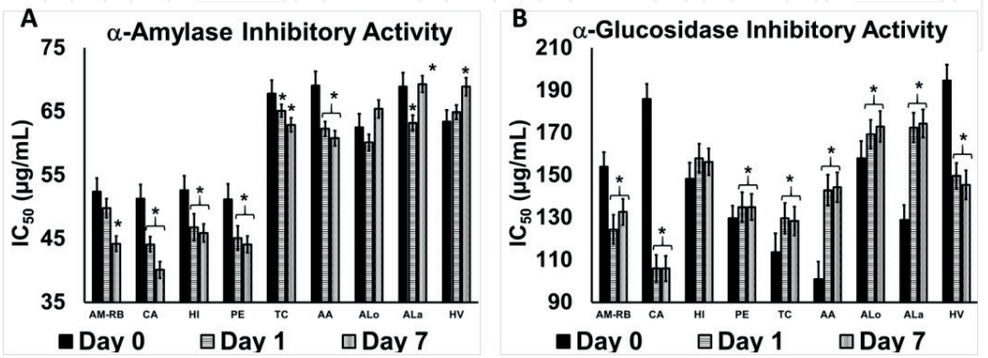

Figure 5.
*α-amylase and α-glucosidase inhibitory activity values of the herbs prior to addition of the tea fungal mat (day 0), followed by day 1 and day 7 of fermentation. Values are expressed as mean ± SEM. * P < 0.05 versus the value of each tea at day 0. Abbreviations: Acacia arabica (AA), Aegle marmelos root bark (AM-RB), Aerva lanata (Ala), Asteracantha longifolia (Alo), Cassia auriculata (CA), Hemidesmus indicus (HI), Hordeum vulgare (HV), Phyllanthus emblica (PE), Tinospora cordifolia (TC).*

Herb	Microbes	Days		
		0 (cfu/mL)	1 (cfu/mL)	7 (cfu/mL)
AA	Bacteria	$2.9 \pm 0.1 \times 10^6$	$8.6 \pm 0.2 \times 10^6$	$3.9 \pm 0.3 \times 10^9$
	Yeast	$1.5 \pm 0.2 \times 10^6$	$8.9 \pm 0.1 \times 10^6$	$9.2 \pm 0.1 \times 10^9$
AM-RB	Bacteria	$3.9 \pm 0.1 \times 10^6$	$6.5 \pm 0.3 \times 10^6$	$9.1 \pm 0.1 \times 10^{10}$
	Yeast	$6.9 \pm 0.2 \times 10^6$	$6.6 \pm 0.2 \times 10^7$	$9.5 \pm 0.1 \times 10^{10}$
ALa	Bacteria	$3.5 \pm 0.1 \times 10^6$	$6.6 \pm 0.2 \times 10^7$	$7.5 \pm 0.2 \times 10^9$
	Yeast	$5.3 \pm 0.1 \times 10^6$	$9.3 \pm 0.2 \times 10^5$	$9.6 \pm 0.1 \times 10^{10}$
ALo	Bacteria	$3.4 \pm 0.2 \times 10^6$	$6.6 \pm 0.2 \times 10^6$	$9.8 \pm 0.1 \times 10^{10}$
	Yeast	$5.1 \pm 0.2 \times 10^6$	$5.5 \pm 0.1 \times 10^7$	$1.8 \pm 0.1 \times 10^{10}$
CA	Bacteria	$1.9 \pm 0.2 \times 10^6$	$2.5 \pm 0.1 \times 10^6$	$3.6 \pm 0.1 \times 10^{10}$
	Yeast	$1.2 \pm 0.2 \times 10^6$	$8.8 \pm 0.2 \times 10^6$	$9.2 \pm 0.1 \times 10^{10}$
HI	Bacteria	$3.6 \pm 0.2 \times 10^6$	$5.9 \pm 0.1 \times 10^6$	$3.9 \pm 0.1 \times 10^{10}$
	Yeast	$5.3 \pm 0.1 \times 10^6$	$6.5 \pm 0.2 \times 10^7$	$9.0 \pm 0.1 \times 10^{10}$
HV	Bacteria	$4.1 \pm 0.1 \times 10^6$	$3.5 \pm 0.3 \times 10^6$	$4.8 \pm 0.1 \times 10^{10}$
	Yeast	$2.5 \pm 0.1 \times 10^6$	$4.4 \pm 0.3 \times 10^5$	$8.0 \pm 0.2 \times 10^{10}$
PE	Bacteria	$1.2 \pm 0.2 \times 10^6$	$3.9 \pm 0.2 \times 10^6$	$7.1 \pm 0.1 \times 10^9$
	Yeast	$3.2 \pm 0.1 \times 10^6$	$5.0 \pm 0.1 \times 10^7$	$8.0 \pm 0.2 \times 10^9$
TC	Bacteria	$1.9 \pm 0.2 \times 10^6$	$3.2 \pm 0.1 \times 10^6$	$2.3 \pm 0.2 \times 10^9$
	Yeast	$4.1 \pm 0.1 \times 10^6$	$8.3 \pm 0.2 \times 10^6$	$1.9 \pm 0.1 \times 10^{10}$

Abbreviations: AA, Acacia arabica; AM-RB, Aegle marmelos root bark; Ala, Aerva lanata; Alo, Asteracantha longifolia; CA, Cassia auriculata; HI, Hemidesmus indicus; HV, Hordeum vulgare; PE, Phyllanthus emblica; TC, Tinospora cordifolia.

Table 1.
Changes to the composition of the overall population of bacteria and yeast present in the broth prior to fermentation (day 0) as well as on day 1 and day 7.

The hypothesis for the increase in the TPC was explained by Blanc [23], where phytases liberated by bacteria and yeast in the tea fungus consortium were identified as capable of liberating polyphenol compounds from the cellulosic backbone of the fermentation medium. This metabolic reaction would have resulted in an increase in the polyphenols in the soluble fraction of the fermented beverages produced in this study. Phytases liberated by bacteria and yeast during Kombucha fermentation are also able of causing degradation of complex polyphenols to smaller molecules which would also result in the increase of TPC. The therapeutic effect of the Kombucha fermentation process has been associated with the increased presence of polyphenols, compounds produced during the fermentation period, and synergistic action between different compounds which are liberated at various stages of the fermentation process [24–26].

The opportunities and challenges for the food and beverage industry in the area of evidence-based functional foods with a low glycemic index which are able to curb the starch digestion rates are on the rise, given the increasing incidence of diabetes throughout the world [27]. Given the noteworthy starch hydrolase inhibitory activities of the fermented herbal beverages generated in this study, their importance in

Herb	Microbes	Days	
		1 (cfu/mL)	7 (cfu/mL)
AA	Bacteria	$3.7 \pm 0.2 \times 10^6$	$2.2 \pm 0.1 \times 10^8$
	Yeast	$1.1 \pm 0.2 \times 10^5$	$1.6 \pm 0.2 \times 10^7$
AM-RB	Bacteria	$1.1 \pm 0.1 \times 10^6$	$1.5 \pm 0.1 \times 10^8$
	Yeast	$1.5 \pm 0.2 \times 10^5$	$5.5 \pm 0.2 \times 10^7$
ALa	Bacteria	$6.6 \pm 0.2 \times 10^6$	$7.5 \pm 0.1 \times 10^8$
	Yeast	$1.5 \pm 0.1 \times 10^5$	$9.2 \pm 0.1 \times 10^7$
ALo	Bacteria	$2.1 \pm 0.2 \times 10^6$	$9.5 \pm 0.1 \times 10^8$
	Yeast	$1.6 \pm 0.2 \times 10^5$	$1.9 \pm 0.1 \times 10^7$
CA	Bacteria	$2.8 \pm 0.1 \times 10^6$	$2.8 \pm 0.1 \times 10^8$
	Yeast	$5.5 \pm 0.1 \times 10^5$	$4.8 \pm 0.2 \times 10^7$
HI	Bacteria	$3.9 \pm 0.1 \times 10^6$	$3.9 \pm 0.1 \times 10^8$
	Yeast	$4.9 \pm 0.1 \times 10^5$	$5.9 \pm 0.3 \times 10^7$
HV	Bacteria	$4.4 \pm 0.2 \times 10^6$	$7.5 \pm 0.1 \times 10^8$
	Yeast	$4.7 \pm 0.1 \times 10^5$	$5.5 \pm 0.1 \times 10^7$
PE	Bacteria	$3.5 \pm 0.1 \times 10^6$	$6.6 \pm 0.2 \times 10^8$
	Yeast	$2.2 \pm 0.1 \times 10^5$	$2.9 \pm 0.1 \times 10^7$
TC	Bacteria	$3.1 \pm 0.1 \times 10^6$	$2.5 \pm 0.1 \times 10^8$
	Yeast	$6.6 \pm 0.1 \times 10^5$	$1.9 \pm 0.2 \times 10^7$

Abbreviations: AA, Acacia arabica; AM-RB, Aegle marmelos root bark; Ala, Aerva lanata; Alo, Asteracantha longifolia; CA, Cassia auriculata; HI, Hemidesmus indicus; HV, Hordeum vulgare; PE, Phyllanthus emblica; TC, Tinospora cordifolia.

Table 2.
Changes to the composition of the overall population of bacteria and yeast present in the pellicle on day 1 and day 7.

support of preventing metabolic diseases such as diabetes could be highlighted in this aspect. Recently, there have been many warnings on the side effects of anti-diabetic drugs such as Rosiglitazone and Pioglitazone which highlight the urgent need of alternative and safer means of blood glucose control—a prospect which could be ideally achieved through functional foods which contain bioactive ingredients with the ability to regulate blood glucose concentration toward the normal range [28].

Last but not least, the selection of a particular fermentation period for this study requires justification. The beverages and their analytical parameters were monitored for 7 days. This was the minimum duration by which the fermented beverages could be consumed without the presence of metabolic artifacts resulting from prolonged fermentation according to literature [25, 29, 30]. In particular, according to the study by Amarasinghe et al. [30] where the study was conducted for monitoring the fermentation for a period of 8 weeks, the Kombucha samples displayed a decrease in the antioxidant activity during the 2 months of fermentation. This was suggestive that suggestive the functional properties of the beverage had decreased. It was also implied in this study that it is possible through prolonged fermentation to result in the accumulation of organic acids, which might reach harmful levels for direct consumption [30].

4. Conclusions

Given the ease of preparation of the beverages which were investigated in this study, as well as the economic viability, the products could be promoted as functional beverages which could be consumed as means of supportive therapy. In particular, they could be used for the prevention and containment of disease conditions in association with their demonstrated antioxidant and starch hydrolase inhibitory properties. Although the therapeutic mechanisms of action of these beverages in the human physiology is yet to be elucidated. However, this study serves as a platform for the identification and promotion of some novel beverages as functional food which can be easily prepared in households using edible plants. Nevertheless, having mentioned as such, during the selection of plant material for fermentation with the Kombucha tea fungal mats, care needs to be taken to identify plant-based material which carry similar sensory perceptions as black tea, thus, reducing the chances of the fermented products possessing adverse sensory perceptions.

During this study, the sensory evaluations of the novel fermented beverages was not carried out. This is one aspect which needs to be explored in future studies. It is only hypothesized in this instance that the organoleptic properties would be acceptable, since the teas are similar in physical properties to *Camellia sinensis*-based tea. However, for a proper verification, sensory evaluation needs to be carried out.

The microbial interactions taking place during the fermentation needs to be investigated further as well. For instance, as mentioned previously, whether the microbes undergo a state of VBNC needs to be elucidated. Additionally, whether microorganisms which have probiotic effects such as *Lactobacillus* spp., and *Bifidobacterium* are able to thrive in the fermented beverage and thus be incorporated, is an aspect worth exploring as well. Should this be successful, the consumer value of the beverages would increase and have a superior marketability due to its combined functional properties of comprising of phenolic compounds as well as probiotics.

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Conflict of interest

The author has no conflicts of interest to declare, financial or otherwise.

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