Chiang Mai J. Sci. 2018; 45(1) : 136-146
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

# Value-added Tea (*Camellia sinesis*) as a Functional Food using the Kombucha 'Tea Fungus'

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Received: 3 June 2016
Accepted: 6 December 2016

## ABSTRACT

The objective of this study was to determine the antioxidant and starch hydrolase inhibitory activities of Chinese black tea, oolong tea, green tea and Sri Lankan black tea through fermentation with the 'tea fungus' (Kombucha). The fermentation was carried out for 7 days and the functional properties were evaluated along with the pH, titratable acidity (TA) and color. The pH decreased significantly ($P$ <0.05) from day 1 throughout the fermentation as compared with the unfermented teas. A statistically significant increase ($P$ <0.05) of the TA was observed from day 5 onwards. The color of all four teas were observed to get lighter. Sri Lankan black tea and green tea had statistically significant increases ($P$ <0.05) in the total phenolics content and antioxidant activities from day 1 along with the fermentation. This was further supported by the increases in the polyphenol contents in all fermented teas. All four fermented teas inhibited a-amylase better than α-glucosidase. Overall, Sri Lankan black tea was observed to be a better substrate than other examined in this paper for the preparation of Kombucha tea in view of its comparatively superior functional properties.

**Keywords:** α-amylase, α-glucosidase, black tea, green tea, Oolong tea, oxygen radical absorbance capacity

## 1. INTRODUCTION

Tea (*Camellia sinensis*) is one of the most popular beverages in the world and is consumed by more than two-thirds of the world's population typically in the forms of black (78%), green (20%), or oolong tea (2%) [1]. Consumption of tea has been associated with decreasing the risk of contracting many non-communicable diseases due to its inherently high flavanol content. The biological benefits of consuming this beverage is associated with its strong antioxidant and anti-angiogenic activity as well as its potential to inhibit cell proliferation and modulate carcinogen metabolism [2]. A value-added beverage based on tea is commonly known as "Kombucha", which is produced by the fermentation of tea and sugar by a symbiotic association of bacteria and yeasts forming a "tea fungus". This beverage tastes like apple cider and is often home-made by fermentation using a tea fungal mat passed from one home to another. While the composition and

properties of tea are well documented, scientific information on the tea fungus, its composition and health effects are scarce. Nevertheless, benefits have been reported by a testimony of users [3-5]. A few reviews have also appeared recently, documenting the scientific literature available to date [6-8].

The objective of this study was to investigate the enhancement of antioxidant properties of four varieties of tea (Chinese black tea, oolong tea, green tea and Sri Lankan black tea) when fermented with the tea fungus. Another aspect of this study was to investigate the starch hydrolase inhibitory activities of these four fermented teas in comparison with their unfermented counterparts. The incorporation of starch hydrolase inhibitors into the diet has been known to retard the absorption of glucose through inhibition of α-amylase and α-glucosidase. These two enzymes are important in the breakdown of carbohydrates, which are present in the small intestinal brush border. Inhibition of the activity of these enzymes will reduce the rate of glucose absorption into the blood, and thereby, reduce the physiological glucose levels. The starch hydrolase inhibitory potential of the Kombucha beverages as well as the enhancement of this potential through addition of the tea fungus was also investigated in this study. Microbiological enumeration was also carried out on all fermented teas as well, in addition to several quality parameters such as colour and pH. The fermented beverages and their analytical parameters were monitored for a period of 7 days, which was the least duration by which the beverage could be consumed following the fermentation process according to published literature [8-9]. Although the process will last up to 2 months, it was imperative to investigate whether the therapeutic potential had already set in by the least possible duration of the fermentation process, without the production of unwanted metabolites resulting from the fermentation process.

## 2. MATERIALS AND METHODS

Lapsang Souchong Chinese black tea, oolong tea and green tea were obtained from Yunnan Province, China, while the Sri Lankan black tea dust was obtained from Watawala Plantations, Sri Lanka. Selection of these tea samples was based on literature which highlighted these teas to contain very high amounts of antioxidant compounds [10-11]. All chemicals used for this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck-Millipore (Temecula, CA, USA) unless otherwise specified.

### 2.1 Starter Culture

A starter culture or the tea fungal mat was obtained from the residents of Yunnan province, China. The major bacterial strains present in the tea fungal mat used for this study were verified and authenticated as *Acetobacter* spp., while the yeast were identified as *Zygosaccharomyces bailii* and *Dekkera* spp, where the method by Jayabalan et al. [4] was utilized for the process. The identification process was carried out at the Temasek School of Applied Sciences, Temasek Polytechnic, Singapore.

### 2.2 Preparation of Kombucha Teas and Determination of the Color, pH and TA

One gram of Chinese black tea (CBT), Chinese green tea (CGT), Chinese oolong tea (COT) and Sri Lankan black tea (SLBT) were added to 100 mL of boiling water and allowed to infuse for about 5 min before the infusions were filtered through a sterile sieve. Sucrose (10%) was dissolved in hot tea and the mixture was cooled down to room

temperature at 24 ± 3 °C. The infusions were then poured into sterile jars covered with cheesecloth. The cooled sweetened tea was inoculated with 3% (w/v) of the freshly grown tea fungus for 7 days aseptically. The fermentation was carried out at 24 ± 3 °C. Sampling was performed periodically during the 7 days of fermentation; each jar was sampled only once in order to avoid any possible contamination. The color of the unfermented and fermented teas was measured using a Minolta Spectrophotometer CM - 3500d (Minolta Co. Ltd., Tokyo, Japan) according to the method by Watawana et al. [12]. The pH values of the samples were measured with an electronic pH meter (Orion model 290A) according to Watawana et al. [12], while the TA was measured in mL NaOH/100 mL according to the method by Chen and Liu [13].

### 2.3 Enumeration of Acetic Acid Bacteria and Yeasts

The number of counts of acetic acid bacteria and yeast in the fermented broth in 7 days of fermentation were performed based on the method reported by Chen and Liu [13] for the 7 days of fermentation. Cell counts were expressed as colony-forming units per milliliter (cfu/mL).

### 2.4 Determination of the Total Phenolics Content and Antioxidant Capacity Assays

The Oxygen Radical Absorbance Capacity (ORAC) assay was carried out according to the method as detailed by Prior et al. [14] and expressed in trolox equivalents (TE) per gram (fresh weight). The method as described by Singleton and Rossi [15] was used for determining the total phenolics content and expressed as mg gallic acid equivalents (GAE) per gram (fresh weight). Scavenging activity on DPPH was assessed according to the method by Blois [16], although in 96-well format according to Watawana et al. [12]. Scavenging ability on superoxide radical ($O_2^{\bullet-}$) was assessed by the method described by Watawana et al. [12] and the values were expressed as % inhibition.

### 2.5 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. [17] while the α-glucosidase inhibitory activity was carried out according to the method by Koh et al. [18]. Acarbose was used as the positive control for both assays and the results were expressed as both $IC_{50}$ (µg/mL) and µmol acarbose equivalents per mL (µmol AE/mL).

### 2.6 Quantification of Tea Polyphenols

Epicatechin isomers [(-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG)], catechin and gallic acid (GA) were quantified in the unfermented and fermented teas according to the method described by Jayabalan et al. [4]. A Shimadzu (Kyoto, Japan) HPLC system equipped with a diode array detector (SPDM10Avp) and a phenomenex Luna C-18(2) column (4.6 mm i.d. × 25 cm, 5 µm) was used for the analysis. The mobile phase consisted of 0.1% aqueous orthophosphoric acid (A) and 100 % acetonitrile (B). The gradient used was as follows: 0-12 min, 15 % B; 12-22 min, 25 % B; and 22-30 min, 15 % B. The flow rate and column temperature were maintained as 1.0 mL $min^{-1}$ and 35 °C, respectively. Tea polyphenols were detected at 280 nm while the concentrations of the compounds were quantified using standard curves. The theaflavin (TF) content was determined according to the method by

Thanaraj and Seshadri [19] using the same HPLC set-up which was used to quantify the epicatechin isomers. Other than usage of HPLC standards, LC-MS was carried out for identification and verification of the respective peaks.

### 2.7 Statistical Analysis

Results were calculated and expressed as mean ± standard error mean (SEM) of ≥ 3 independent analyses. *P* values of > 0.05 were considered to be significant as calculated using one-way ANOVA.

## 3. RESULTS AND DISCUSSION

The initial values of all the analyzed parameters prior to the fermentation process (day 0) are listed in Figures 1-4.

### 3.1 Color, pH and TA

As shown in Figure 1, based on the L* values alone, the colours of all four fermented beverages were observed to become lighter as the fermentation progressed. Statistically significant changes ($P$ <0.05) in the L* values of all four fermented teas were observed from day 1 as compared with the unfermented teas. Especially, a significant increase in the lightness was observed in COT from day 5 onwards of the fermentation. In terms of the a* values, SLBT and CBT were observed to move towards the green end of the spectrum, while a clear trend was not observed in the remaining two fermented teas. However, as per the L* values, statistically significant changes ($P$ <0.05) in the a* values of all four fermented teas were observed from day 1 of the fermentation as well. As for the b* values, all four fermented teas were observed to move towards the yellow end of the spectrum statistically significantly ($P$ <0.05) as compared with the unfermented teas, from day 4 onwards. Overall, the L*, a* and b* values indicated the color of the teas moving from the dark ends towards the lighter ends of the CIELAB color space, despite their individual fluctuations. The progressive lightning of color of the fermented teas had been observed in previously conducted studies as well, where the changes were associated with the microbial transformation of polyphenols [3, 9]. This trend also appeared to be different from one substrate to another, where Watawana et al. [20] observed an increase in 'yellowness' when king coconut water was fermented with the tea fungus.

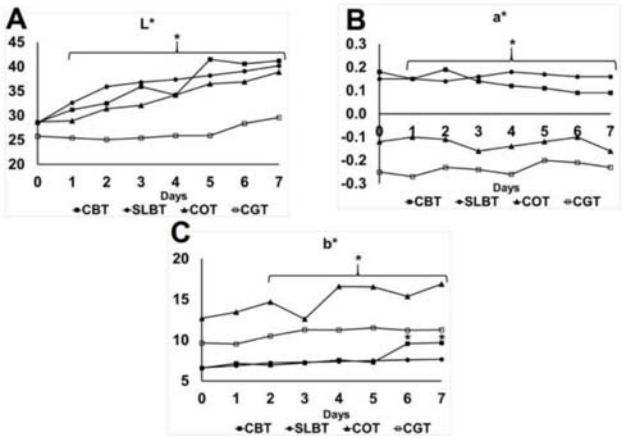


**Figure 1.** The (A) L* (B) a* and (B) b* values of CBT, CGT, COT and SLBT throughout the 7-day period of analysis. Results were calculated and expressed as mean ± standard error mean (SEM) of ≥ 3 independent analyses. * $P$ < 0.05 versus the value of each tea at day 0.

As shown in Figure 2A, all teas had starting pH values between 6.4 and 6.7, which were measured prior to the fermentation process. A statistically significant decrease ($P < 0.05$) in the pH was observed in all four Kombucha beverages from day 1 throughout the fermentation period as compared with the unfermented teas. At the end of day 7, the pH of the teas varied between 3.1 and 4.3. SLBT was observed to be the least acidic at the end of day 7, having a final pH of 4.3, while CBT had the lowest pH of 3.1. The statistically significant increase ($P<0.05$) in the TA (Figure 2B) set in from day 4, for COT, and from day 5, for other Kombucha samples as compared with the unfermented teas. The TA of all teas varied between 0.23 mL NaOH/100 mL and 0.42 mL NaOH/100 mL prior to the fermentation process, while at day 7, the values varied between 4.86 mL NaOH/100 mL and 5.99 mL NaOH/100 mL.

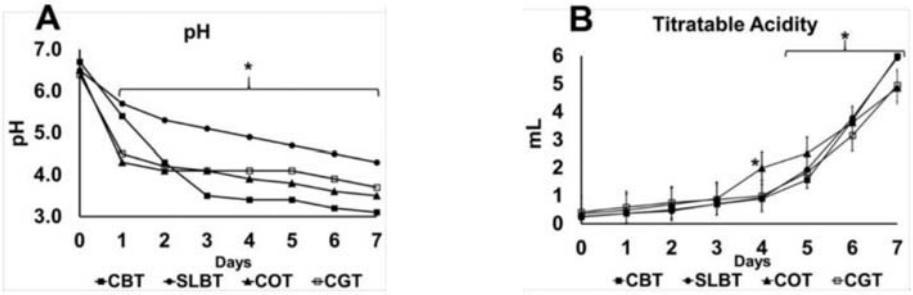


**Figure 2.** The (A) pH and (B) titratable acidity values of CBT, CGT, COT and SLBT throughout the 7-day period of analysis. Results were calculated and expressed as mean ± standard error mean (SEM) of ≥ 3 independent analyses. * $P < 0.05$ versus the value of each tea at day 0.

### 3.2 Changes in the Total Acetic Acid Bacteria and Yeast Cell Counts

The acetic acid bacterial and yeast cell counts are shown in Table 1. The viable bacterial and yeast counts in the fermented broths of all four teas appeared to increase over the 7 day period. The increase was indicative of the ability of the teas to act as a suitable substrate to sustain the livelihood of the microorganisms. The acetic acid bacterial and yeast cell counts are shown in Table 1. The yeast cell counts outnumbered the acetic acid bacterial cell counts for all teas on all days of the fermentation period. This was in concurrence with the findings from Chen and Liu [13] as well as Chu and Chen [9].

### 3.3 Changes to the Phenolic Compounds

The total phenolic contents are shown in Figure 3A. SLBT had the highest total phenolic content prior to the fermentation process. However, following the initiation of the fermentation process, SLBT and CGT had a statistically significant increase ($P < 0.05$) in the total phenolic contents from day 1 onwards. The values of all Kombucha samples at day 1 were maintained throughout the 7-day duration with no statistically significant decrease or increase compared with those in day 1. SLBT was observed to have the highest total phenolic content by the end of the period of analysis since their initial total phenolic content was high as well. Despite the overall increase in the total

phenolic content in SLBT and CGT following the initiation of the fermentation process, the individual polyphenol contents had variations in terms of the quantities in all the teas. The changes in the values of EGCG, EGC, ECG, EC, TF and GA are shown in Table 2. The EGCG contents - which are known to be typically higher than the EGC contents, had a statistically significant increase ($P < 0.05$) in all the fermented teas from day 1 onwards, with the exception of COT. A similar trend was observed in the instances of EC and TF. The ECG content in all the teas had statistically significant increases ($P < 0.05$) from day 1 onwards of the fermentation, whereas the EGC content had statistically significant increases ($P < 0.05$) only in COT. Statistically significant increases ($P < 0.05$) of the GA contents was observed only in SLBT. The increment in the phenolic compounds was previously explained by Blanc [21], where the degradation of complex phenolic compounds in the teas subjected to the increased acidic environment of the fermentation process, and by the enzymes liberated by bacteria and yeast in the tea fungus consortium were identified as being responsible. According to Haslam [22], this biotransformation process is typically known to result in a decrease in color of the Kombucha beverages, which was observed from the changes in color as highlighted in this study.

**Table 1.** Changes in the acetic acid bacteria and yeast cell counts in CBT, CGT, COT and SLBT throughout the 7-day period of analysis expressed as mean ± SEM (cfu/mL). * $P < 0.05$ versus the value of each tea at day 0.

| Tea | Microorganism | Days | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| CBT | Acetic acid bacteria ($\times 10^3$ cfu/mL) | 9.2±0.7 | 11.4±0.9 | 11.7±1.0 | 13.6±0.5 | 13.7±0.6 | 14.5±0.4 | 15.5±0.2 |
| | Yeast ($\times 10^6$ cfu/mL) | 8.1±0.6 | 9.4±0.4 | 11.4±0.7 | 12.3±0.6 | 12.5±0.4 | 13.1±0.5 | 13.4±0.6 |
| CGT | Acetic acid bacteria ($\times 10^3$ cfu/mL) | 9.4±0.2 | 10.2±0.5 | 11.1±0.6 | 11.6±0.7 | 12.1±0.3 | 12.2±0.4 | 12.5±0.3 |
| | Yeast ($\times 10^6$ cfu/mL) | 8.5±0.5 | 9.2±0.1 | 10.2±0.7 | 11.4±0.2 | 11.7±0.3 | 12.1±0.4 | 12.2±0.3 |
| COT | Acetic acid bacteria ($\times 10^3$ cfu/mL) | 9.5±0.3 | 10.5±0.1 | 11.2±0.4 | 11.6±0.3 | 11.7±0.8 | 12.2±0.9 | 12.5±1.1 |
| | Yeast ($\times 10^6$ cfu/mL) | 8.6±0.3 | 8.7±0.1 | 9.0±0.2 | 9.1±0.5 | 9.2±0.1 | 9.6±0.4 | 9.8±0.2 |
| SLBT | Acetic acid bacteria ($\times 10^3$ cfu/mL) | 9.5±0.2 | 10.9±0.3 | 11.3±0.3 | 11.8±0.5 | 12.3±0.4 | 12.5±0.6 | 12.6±0.2 |
| | Yeast ($\times 10^6$ cfu/mL) | 8.3±0.4 | 8.5±0.2 | 8.6±0.4 | 9.0±0.5 | 9.2±0.4 | 9.5±0.1 | 9.6±0.4 |

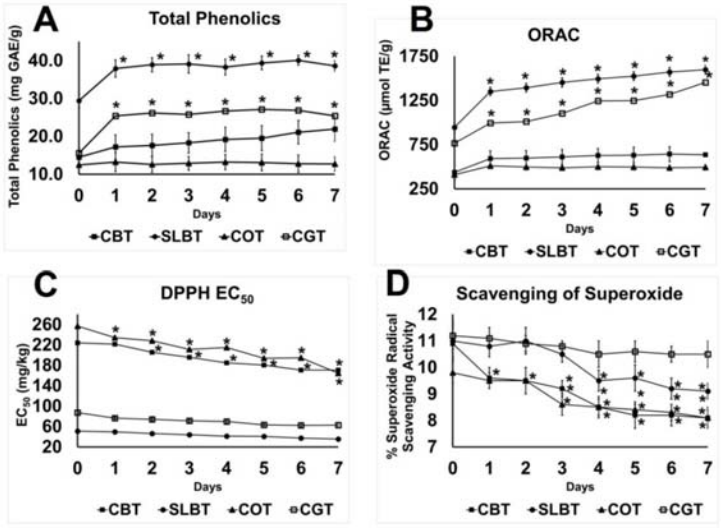


**Figure 3.** The (A) total phenolic content, (B) ORAC, (C) DPPH $EC_{50}$ and (D) superoxide scavenging activities of CBT, CGT, COT and SLBT throughout the 7-day period of analysis. Results were calculated and expressed as mean ± standard error mean (SEM) of ≥ 3 independent analyses. * $P < 0.05$ versus the value of each tea at day 0.

**Table 2.** Changes in the polyphenol contents (EGCG, EGC, ECG, EC, TF and GA) in CBT, CGT, COT and SLBT throughout the 7-day period of analysis expressed as mean ± SEM. * $P < 0.05$ versus the value of each tea at day 0.

| Tea | Polyphenol | Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| CBT | EGC (mg/g) | 11.6±1.1 | 16.9±1.2* | 17.2±1.3* | 17.3±1.2* | 16.9±1.1* | 18.5±1.5* | 17.2±1.2* | 16.9±1.3* |
| | EGCG (mg/g) | 23.5±1.6 | 28.4±1.5 | 28.5±1.9 | 27.9±1.7 | 26.8±1.4 | 28.4±1.6 | 28.3±1.8 | 27.9±1.5 |
| | ECG (mg/g) | 9.8±1.6 | 15.8±1.5* | 14.9±1.7* | 15.2±1.5* | 14.8±1.6* | 15.4±1.7* | 15.4±1.5* | 15.8±1.6* |
| | EC (mg/g) | 3.5±0.7 | 5.6±0.5* | 5.7±0.6* | 5.8±0.4* | 5.6±0.6* | 5.7±0.5* | 5.8±0.4* | 5.6±0.6* |
| | TF (mg/g) | 4.8±0.2 | 7.9±0.3* | 8.1±0.5* | 8.0±0.4* | 7.9±0.6* | 7.5±0.1* | 7.8±0.5* | 7.9±0.4* |
| | GA (mg/g) | 6.9±1.1 | 7.5±1.1 | 7.6±1.3 | 7.7±1.2 | 7.9±1.1 | 8.1±1.0 | 8.1±1.2 | 8.2±1.3 |
| CGT | EGC (mg/g) | 15.6±1.5 | 20.8±1.6* | 21.1±1.5* | 21.5±1.4* | 21.6±1.5* | 21.5±1.6* | 21.4±1.5* | 21.5±1.4* |
| | EGCG (mg/g) | 26.9±1.9 | 30.8±1.8 | 31.2±1.7 | 31.1±1.9 | 32.4±1.8 | 32.1±1.7 | 32.5±1.9 | 32.4±1.8 |
| | ECG (mg/g) | 11.6±1.8 | 18.9±1.5* | 17.8±1.8* | 18.4±1.9* | 18.5±1.7* | 18.4±1.8* | 18.2±1.4* | 18.5±1.8* |
| | EC (mg/g) | 4.5±1.6 | 8.9±1.5* | 9.1±1.8* | 9.2±1.7* | 9.5±1.9* | 9.2±1.8* | 9.5±1.7* | 9.2±1.5* |
| | TF (mg/g) | 5.9±1.1 | 9.8±1.2* | 9.4±1.0* | 9.8±0.9* | 9.8±1.1* | 9.4±1.2* | 9.8±1.1* | 10.5±1.2* |
| | GA (mg/g) | 8.9±1.3 | 10.7±1.2 | 11.9±1.4 | 12.4±1.2 | 12.0±1.3 | 12.4±1.5 | 12.1±1.1 | 12.3±1.0 |
| COT | EGC (mg/g) | 6.5±0.9 | 7.5±1.0 | 8.2±1.1 | 8.1±0.9 | 8.2±0.9 | 8.1±1.1 | 8.0±1.2 | 8.1±1.1 |
| | EGCG (mg/g) | 15.4±1.5 | 21.6±1.6* | 21.5±1.4* | 22.1±1.4* | 22.4±1.5* | 23.5±1.6* | 22.1±1.4* | 22.4±1.2* |
| | ECG (mg/g) | 6.5±1.5 | 8.4±1.6* | 8.5±1.4* | 8.4±1.5* | 8.6±1.6* | 8.6±1.5* | 8.7±1.4* | 8.8±1.5* |
| | EC (mg/g) | 2.9±0.5 | 3.2±0.6 | 3.5±0.4 | 3.4±0.5 | 3.5±0.7 | 3.4±0.9 | 3.5±0.8 | 3.5±0.4 |
| | TF (mg/g) | 2.5±0.5 | 2.6±0.6 | 2.5±0.5 | 2.6±0.4 | 2.4±0.6 | 2.7±0.5 | 2.8±0.6 | 2.6±0.5 |
| | GA (mg/g) | 5.9±0.8 | 6.4±0.7 | 6.5±0.8 | 6.5±0.7 | 6.4±0.6 | 6.5±0.8 | 6.4±0.7 | 6.5±0.6 |
| SLBT | EGC (mg/g) | 22.5±1.6 | 29.8±1.7* | 30.1±1.5* | 30.0±1.8* | 30.1±1.6* | 30.2±1.8* | 30.1±1.4* | 30.5±1.8* |
| | EGCG (mg/g) | 41.8±1.9 | 42.5±1.8 | 43.1±1.7 | 42.9±1.9 | 43.8±1.5 | 43.7±1.9 | 43.9±1.8 | 43.6±1.9 |
| | ECG (mg/g) | 16.9±1.1 | 20.7±1.3* | 20.5±1.4* | 20.4±1.8* | 20.4±1.2* | 20.9±1.4* | 20.1±1.3* | 20.8±1.5* |
| | EC (mg/g) | 6.8±1.6 | 15.4±1.4* | 15.9±1.5* | 15.4±1.6* | 15.8±1.8* | 15.4±1.4* | 15.9±1.6* | 15.9±1.4* |
| | TF (mg/g) | 8.7±1.2 | 16.4±1.3* | 16.8±1.1* | 16.7±1.2* | 16.4±1.3* | 16.8±1.4* | 16.8±1.1* | 16.9±1.3* |
| | GA (mg/g) | 15.4±1.8 | 25.9±1.7* | 25.8±1.6* | 25.9±1.5* | 26.4±1.4* | 26.1±1.3* | 26.5±1.6* | 26.1±1.5* |

**3.4 Antioxidant Activity**

The ORAC, DPPH $EC_{50}$ and superoxide scavenging activities are shown in Figures 3B, 3C and 3D, respectively. Similar to the total phenolics content, the ORAC values of SLBT was the highest prior to the fermentation process, followed by the values of CBT, CGT and COT. Following the initiation of the fermentation process, SLBT and CGT had statistically significant increases ($P < 0.05$) in the ORAC values from day 1 onwards and these contents were maintained throughout the 7-day fermentation duration with no statistically significant decrease or increase. The DPPH $EC_{50}$ values complemented the ORAC results prior to the fermentation process, where CBT, CGT and COT had the highest values and SLBT the lowest. However, following the initiation of the fermentation process, only SLBT had a statistically significant decrease ($P < 0.05$) in the DPPH $EC_{50}$ values from day 1 onwards. It is also noteworthy that there was a better statistically significant correlation between the total phenolic content and the ORAC values rather than the DPPH $EC_{50}$ values of all teas on all days of analysis ($r = 0.986$ for ORAC vs $r = 0.753$ DPPH $EC_{50}$, $P < 0.05$). As for the superoxide scavenging activities, the trends were not as clear as the ORAC and DPPH $EC_{50}$ values. However, SLBT and COT was also shown to have a statistically significant increase in the superoxide scavenging potential ($P < 0.05$). A weak correlation existed between the superoxide scavenging potential and the total phenolic content ($r = 0.541$, $P < 0.05$). Out of the three different antioxidant assays were carried out in this study, the ORAC values may have had a better correlation with the total phenolic content since the phenolic compounds present in the unfermented and fermented beverages may have been better scavengers of peroxyl radicals which are generated during the ORAC assay. These observations were also stated by Watawana et al. [12].

**3.5 Starch Hydrolase Inhibitory Activities**

The starch hydrolase inhibitory activities are shown in Figure 4. Overall, the α-amylase inhibitory activities in terms of the $IC_{50}$ values ranged between 18.5 and 138.4 μg/mL or 72.5 and 216.8 μmol AE/mL prior to the fermentation process, while the α-glucosidase inhibitory activities in terms of the $IC_{50}$ values ranged between 69.3 and 158.7 μg/mL or 72.5 and 216.8 μmol AE/mL. Only the $IC_{50}$ and AE values of CBT, CGT and SLBT exhibited statistically significant increases or decreases ($P < 0.05$) in the α-amylase inhibitory activities after the initiation of the fermentation process. The statistically significant difference in the COT sample of the α-amylase inhibitory activity was observed on day 7. The α-amylase and α-glucosidase inhibitory activities were observed to be the highest in SLBT either prior to the fermentation process or at the end of the fermentation period. The values for fermented SLBT were comparable with phytochemical products which are known and established for their inhibitory potentials, indicating that both teas have the ability to inhibit the breakdown of starch and the subsequent release of glucose into the physiological systems [23]. It was additionally observed that all teas were able to inhibit α-amylase better than α-glucosidase when comparing their $IC_{50}$ and AE values. Similar observations were also noted by Watawana et al. [12] and Watawana et al. [30]. It may be concluded that the beverages resulting from the tea fungus-based fermentation is able to enhance the α-amylase inhibitory activity better than the α-glucosidase inhibitory activity of the unfermented tea samples.

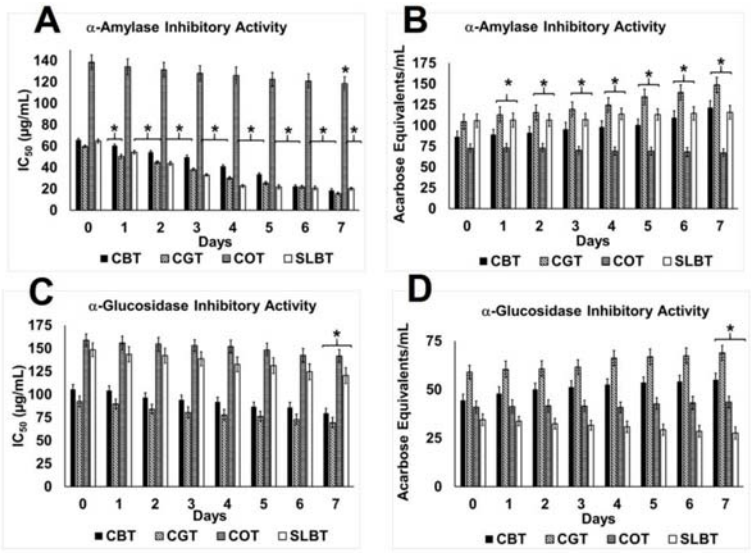


**Figure 4.**The α-amylase inhibitory activities in terms of (A) $IC_{50}$ values & (B) acarbose equivalents, and the α-glucosidase inhibitory activities in terms of (C) $IC_{50}$ values & (D) acarbose equivalents, of CBT, CGT, COT and SLBT throughout the 7-day period of analysis. Results were calculated and expressed as mean ± standard error mean (SEM) of ≥ 3 independent analyses. * $P < 0.05$ versus the value of each tea at day 0.

### 3.6 Therapeutic Potential of the Kombucha Teas and Limitations of This Study

Although this study was able to confirm the antioxidant potential of four teas fermented with the tea fungus, further *in vivo* and/or clinical studies are warranted for the demonstration of their therapeutic potential to be relevant to human health. While green, oolong and black tea of Chinese origin has been thoroughly investigated in this aspect, SLBT - especially that which has been fermented with the tea fungus, has not been systematically evaluated through *in vitro*, *in vivo* and/or clinical means. The difference in the antioxidant and starch hydrolase inhibitory potential between CBT and SLBT needs to be commented on as well. China and Sri Lanka have been known for their cultivation, production and export of tea all around the world. However, given the differences in terms of geography and climate, it is quite likely that the phytochemicals present in the teas would differ significantly from each other as shown in previous studies. SLBT was shown to contain more functional properties than CBT. This could be due to the more tropical location of Sri Lanka. Countries located in the tropics have been gifted with a wide range of plant species where they have been consumed as regular items of the diet and occasionally as cures for ailments, disease prevention or simply for the purpose of maintaining health and wellness. Due to the ample exposure to sunlight, the leafy herbs of the tropics develop more defenses to withstand the naturally harsh weather and environmental conditions in the form of phytochemicals (i.e. antioxidants) - especially against solar radiation, which leads to them be more concentrated with bioactive compounds [24].

## 4. CONCLUSIONS

The study successfully demonstrated the enhancement of the antioxidant and starch hydrolase inhibitory potential of four types of teas when fermented with the tea fungus. Fermented SLBT was discovered to be the best product of the four Kombucha beverages, in the light of its highest antioxidant and starch hydrolase inhibitory activities at the end of the fermentation period. The ease of preparation of the fermented beverages adds further value to their functional properties, since the fermentation process can be carried out easily in domestic environments with no significant expenses. The starch hydrolase inhibitory property of the beverages is also an indication of its potential to be promoted as a beverage to be consumed for the maintenance of health and wellness. While the fermentation process has been carried out in other types of substrates such as coffee and coconut water, it would be of interest to see whether other types of herbal beverages can also be fermented through addition of the tea fungus, thereby, resulting in novel functional health drinks.

## ACKNOWLEDGEMENTS

The authors wish to extend their gratitude to the National Institute of Fundamental Studies, Hantane Road, Kandy, Sri Lanka for the financial and analytical support and Temasek School of Applied Sciences, Temasek Polytechnic, Singapore for the analytical support provided for this study.

## CONFLICTS OF INTEREST

The authors report no conflicts of interest, financial or otherwise.

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