ENHANCEMENT OF THE FUNCTIONAL PROPERTIES OF COFFEE THROUGH FERMENTATION BY "TEA FUNGUS" (KOMBUCHA)

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ABSTRACT

The antioxidant and starch hydrolase inhibitory activities and the polyphenol contents of three roasted coffee powders (fine-ground coffee – FGC, coarse-ground coffee – CGC and instant coffee – IC) were determined following fermentation by the Kombucha "tea fungus" for 7 days. A statistically significant decrease ($P < 0.05$) in the pH was observed in FGC from 5.0 to 4.1. The chlorogenic acid content statistically significantly increases ($P < 0.05$) in CGC and FGC with final values of 12.9 and 13.2 mg/L, respectively. The caffeic acid content showed statistically significant increases ($P < 0.05$) only in FGC with a final value of 28.4 mg/L. Statistically significant increases ($P < 0.05$) in both starch hydrolases were observed from day 5 onward in all three beverages. In conclusion, this study provided preliminary evidence on the enhancement of the antioxidant and starch hydrolase inhibitory potential of coffee beverages through fermentation with the tea fungus.

PRACTICAL APPLICATIONS

Coffee is a crop and beverage that has a commercial value throughout the world, which is second only to tea in terms of consumption. It has been evaluated to contain many therapeutic properties mostly owing to the presence of phenolic compounds. Microbial fermentation has been known to result in the enhancement of therapeutic properties in beverages containing phenolics. Thus, the objective of this study was to increase the bioaccessibility of phenolic compounds of coffee, thereby enhancing its associated therapeutic properties. Kombucha "tea fungus"-based fermentation is well known to result in beverages that contain enhanced therapeutic properties without any additional ingredients required. The method is simple and can be applied in any domestic condition without a significant cost. Thus, the study highlights the product development of a fermented beverage, which can be prepared without any expenditure and contains significant therapeutic properties.

INTRODUCTION

Coffee belongs to the family Rubiaceae that contains more than 70 other species altogether. However, only two of them are of significant economic importance, viz., arabica (*Coffea arabica*) and robusta (*Coffea canephora*) (Clifford 1999). Coffee possesses a greater *in vitro* antioxidant activity as compared with other beverages. This may be due to the existence of intrinsic compounds such as chlorogenic acid and compounds formed during roasting such as melanoidins (Clifford and Kazi 1987). Overall, coffee contains several beneficial antioxidants and is one of the richest known sources of chlorogenic acid (Rice-Evans *et al.* 1996; Caemmerer and Kroh 2006). As a phytochemical of dietary importance, coffee is considered as one of the major dietary sources for obtaining and the incorporation of this chlorogenic acid into the diet (Rice-Evans *et al.* 1996). Despite the attention given toward chlorogenic acid, coffee also contains a significant number of bioactive molecules that characteristically exist in many other fruits and vegetables. Nevertheless, the principal health benefits of coffee have been associated with its caffeine content. Caffeine, in fact, induces

several pharmacological effects, mostly at the level of the central nervous system, where it has been recognized as a stimulant (Carlsson *et al.* 2004; Noordzji *et al.* 2005). Other dietary substances introduced by coffee include lipids, polysaccharides, phenolic compounds, melanoidins, soluble dietary fiber and minerals (Clifford and Kazi 1987).

It is of interest to determine whether the antioxidant potential of coffee can be enhanced through naturally occurring biochemical processes. The safest and most physiologically compatible process for enhancement of antioxidant and therapeutic potential is through fermentation. Thus, the objective of this study was to investigate whether the antioxidant potential of coffee could be enhanced through the addition of the Kombucha "tea fungus." The Kombucha culture is typically known as a symbiotic growth of acetic acid bacteria and osmophilic yeast strains in a thick jelly membrane (zoogleal mat), which has to be cultured in sugared tea (Jayabalan *et al.* 2008). Fermentation with tea fungus converts the added sugar into organic acids and ethanol. It utilizes sugar as its carbon source and forms a new jelly membrane during fermentation. Tea in the medium for cultivation provides necessary nitrogen sources (purine derivatives: caffeine and theophylline) for the tea fungus culture (Sreeramulu *et al.* 2000). Given this requirement, some studies have been able to successfully demonstrate the preparation of Kombucha beverage from the addition of the tea fungus to various types of plant-based powder, which are essentially not of *Camellia sinensis* origin (Liu *et al.* 1996). Thus, whether the tea fungus has a similar effect in a coffee medium is a worthy aspect of exploration. The starch hydrolase inhibitory ability of coffee has also been evaluated in this aspect. 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, which are present in the small intestinal brush border. Inhibitors of these enzymes can delay starch digestion, causing a reduction in the rate of glucose absorption into the bloodstream and consequently blunting postprandial plasma glucose rise in diabetic patients.

MATERIALS AND METHODS

Cultures and Chemicals

The bacterial strains present in the tea fungal mat used for this study were verified as *Acetobacter aceti* (MTCC 2945), while the yeast components were identified as *Zygosaccharomyces bailii* (MTCC 8177) and *Brettanomyces claussenii* (MTCC 7801). Roasted coffee in fine-ground (FGC) and coarse-ground (CGC) forms was generously provided by a local producer of coffee in Colombo, Sri Lanka. Instant coffee (IC) was purchased from a supermarket in Kandy, Sri Lanka. All three powders were not decaffeinated. Anhydrous sodium carbonate, Folin–Ciocalteu phenol reagent, KH_2PO_4 and K_2HPO_4 were obtained from Merck (Darmstadt, Germany). 4,6-Tripryridyl-s-triazine (TPTZ), gallic acid and trolox were purchased from Acros Organics (Morris Plains, NJ). All other reagents, chemicals and HPLC standards used for the study were purchased from Sigma Chemicals (St. Louis, MO).

Preparation of Fermented Coffee Beans and Determination of the pH and Titratable Acidity (TA)

One gram each of the coffee powder was added to 100 mL of boiling water and allowed to infuse for about 5 min, after which the infusions were filtered through a sterile sieve. Sucrose (10%) was dissolved in hot coffee and the preparation was left to cool to room temperature at 24 ± 3C. The infusions were then poured into sterile jars with lids. The cooled coffee was inoculated with 3% (w/v) of the freshly grown tea fungus for 7 days aseptically. The fermentation was carried out at 24 ± 3C. Sampling was performed periodically; each jar was sampled only once in order to avoid potential contamination. The inoculations of the coffees were carried out in triplicate samples. Unfermented coffee powders were also analyzed for 7 days continuously, along with the fermented counterparts. The fermented coffees were centrifuged at 7,240 × g for 10 min and prior to each of the assays and analyses. The color of the unfermented and fermented coffees was measured using a Minolta Spectrophotometer CM-3500d (Minolta Co., Ltd., Tokyo, Japan) using the method of Lee *et al.* (in press). The pH values of the samples were measured with a Thermo-Scientific, Orion Model 290A electronic pH meter (Waltham, MA), while the TA was measured according to the method of Chen and Liu (2000).

Determination of the Oxygen Radical Absorbance Capacity (ORAC) and Total Phenolic Content

The ORAC assay was carried out according to Prior *et al.* (2003) using a Thermo-Scientific Multiskan FC Microplate Reader and the values were expressed as micromoles of trolox equivalents (TE) per milliliter (μmol TE/mL). The method of Huang *et al.* (2002) was used for determining the total phenolic content using the Thermo-Scientific Multiskan FC Microplate Reader. The results were expressed as milligrams of gallic acid equivalents (GAE) per milliliter (mg GAE/mL).

Determination of the Di(Phenyl)-(2,4,6-Trinitrophenyl)Iminoazanium (DPPH) and Superoxide Radical-Scavenging Activities

The method of Lee *et al.* (in press) was used to determine the DPPH radical-scavenging activity. The value was calculated and expressed in EC_{50} (mg/kg). The scavenging ability of superoxide radical (O_2^-) was assessed by the method described by Lee *et al.* (2002) and expressed as % superoxide radical-scavenging activity.

Determination of the α-Amylase and α-Glucosidase Inhibitory Activities

The α-amylase inhibitory activity was evaluated according to the method of Liu *et al.* (2011), while the α-glucosidase inhibitory activity was carried out according to the method of Koh *et al.* (2009). Acarbose was used as the positive control for both assays and the data were expressed as IC_{50} (mg/mL).

Quantification of Chlorogenic Acid, Caffeine and Caffeic Acid Contents

Extract preparation and cleanup were carried out according to Bicchi *et al.* (1995). The compounds were analyzed by high-performance liquid chromatography (HPLC) following the method described by Farah *et al.* (2005), with some modifications. A Shimadzu LC2010 HPLC system (Kyoto, Japan) equipped with an SPD-M10AVP diode array detector (Kyoto, Japan) and a Phenomenex Luna C-18(2) column (Tokyo, Japan) (4.6 mm i.d. × 25 cm, 5 μm) were used for the analysis. Chromatograms were recorded at 325 nm for CGA and caffeic acid and 276 nm for caffeine. Identification of the three compounds was performed by comparing the retention time and the photodiode array spectra with their reference compounds. Quantification was made by comparing the peak areas with those of the standards. All three compounds were selected for analysis based on their dietary importance as coffee has been identified as the major contributor of these compounds to the human diet.

Statistical Analysis

IBM SPSS Statistics version 21.0 released in 2012 (IBM Corp., Armonk, NY) for Windows was used for the statistical analyses. The results were calculated and expressed as mean ± standard error mean (SEM) of ≥3 independent analyses. SEM was chosen instead of standard deviation (SD), given the significant number of samples being analyzed. P values of <0.05 were considered to be significant.

RESULTS AND DISCUSSION

The initial values of all the analyzed parameters prior to the fermentation process are indicated as day 0 in Figs. 1–5.

Color, pH and TA

According to Fig. 1, based on the L^* values alone, all three beverages were observed to get darker as the fermentation process progressed. Statistically significant ($P < 0.05$) changes in the L^* values were observed in all fermented beverages by day 7. In considering all L^*, a^* and b^* values, FGC

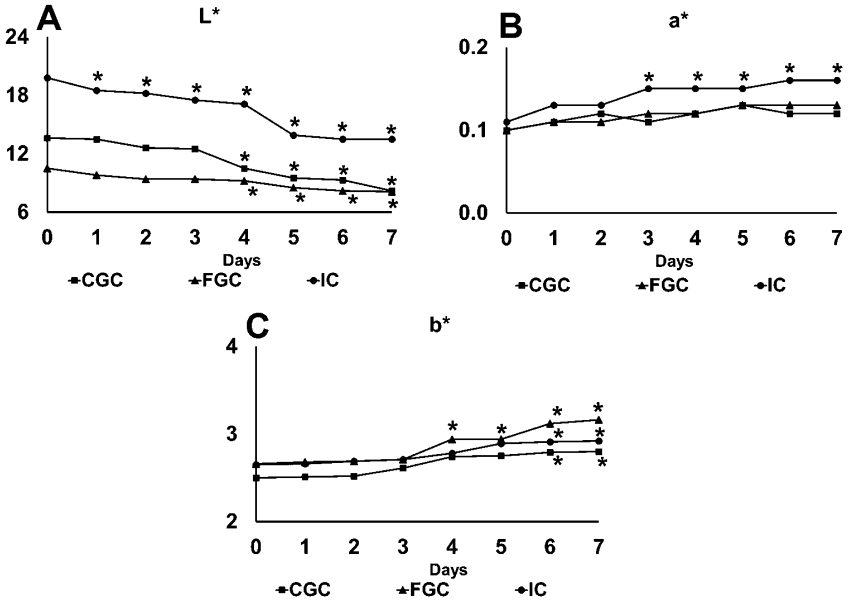

FIG. 1. (A) L^*, (B) A^* AND (B) B^* VALUES OF COARSE-GROUND COFFEE (CGC), FINE-GROUND COFFEE (FGC) AND INSTANT COFFEE (IC) THROUGHOUT THE 7-DAY PERIOD OF ANALYSIS
Error bars represent the SEM. *$P < 0.05$ versus the value of each coffee beverage at day 0.

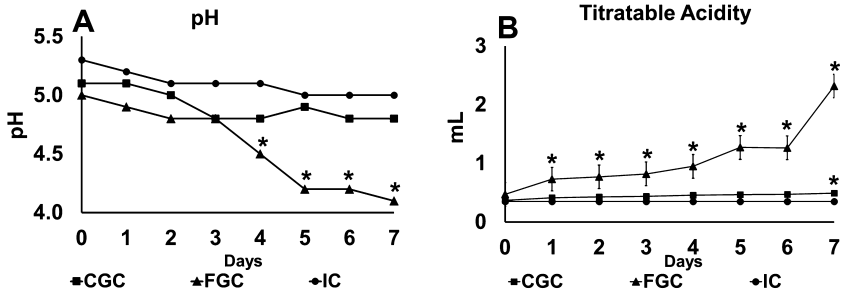

FIG. 2. (A) pH AND (B) TITRATABLE ACIDITY VALUES OF COARSE-GROUND COFFEE (CGC), FINE-GROUND COFFEE (FGC) AND INSTANT COFFEE (IC) THROUGHOUT THE 7-DAY PERIOD OF ANALYSIS
Error bars represent the SEM. $*P < 0.05$ versus the value of each coffee beverage at day 0.

was observed to become the darkest out of all three beverages, while IC was the lightest. This observation was in contrast with the color changes observed when the tea fungus was added to the tea, where a progressive lightening of color was observed and was associated with the microbial transformation of polyphenols (Chu and Chen 2006; Jayabalan *et al.* 2007). Nevertheless, as a whole, it is known that fermentation is able to promote a progressive polymerization of phenolic compounds to form brown-colored macromolecular products (Rice-Evans *et al.* 1996). Thus, it is possible that this mechanism of action took place during the fermentation of coffee. Another hypothesis for this increment in color can be explained using the explanation of Blanc (1996), where the enzymes liberated by bacteria and yeast in the tea fungus consortium were identified as capable of liberating polyphenolics from the cellulosic backbone (resulting in an increase in polyphenols in the soluble fraction). It is thus possible that the enzymes liberated by bacteria and yeast during Kombucha fermentation of coffee are the reasons for the degradation of complex polyphenols to small molecules, which, in turn, results in the increase in color.

All coffee samples had initial pH values between 5.0 and 5.5 prior to fermentation. As shown in Fig. 2, a statistically significant decrease ($P < 0.05$) in the pH was observed only in FGC as compared with the unfermented coffee, where the pH had decreased from 5.0 to 4.1. This was a contrasting observation as compared with the fermentation of tea using the same consortium of microbes (Chu and Chen 2006; Jayabalan *et al.* 2007). In tea samples, an overall decrease in pH is generally observed due to the increased concentration of organic acids produced during the fermentation process by bacteria and yeasts in the tea fungus consortium. The TA values corresponded with the pH values as well. A consistent statistically significant increase ($P < 0.05$) in the TA was observed only in FGC where the initial value of 0.469 mL had increased to 2.316 mL, although CGC had a statistically significant increase

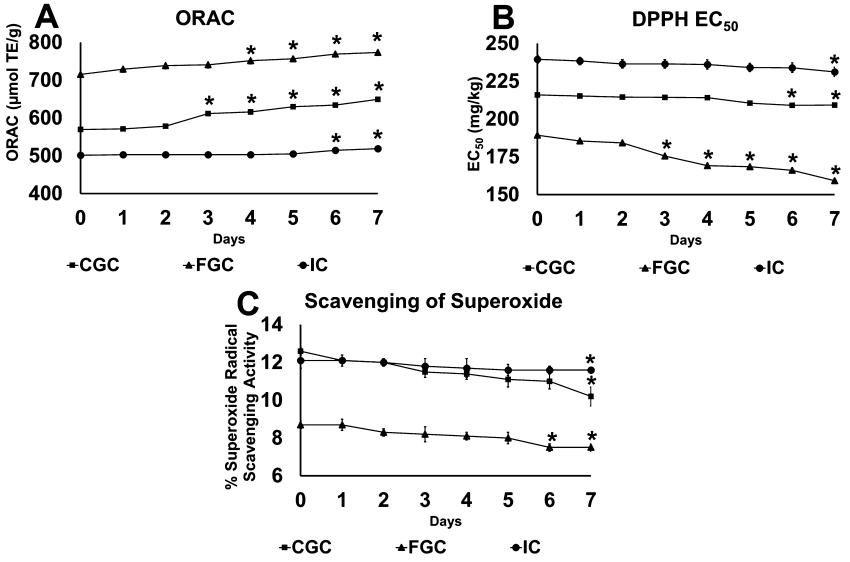

FIG. 3. THE (A) TOTAL PHENOLIC CONTENT, (B) CHLOROGENIC ACID, (C) CAFFEIC ACID AND (D) CAFFEINE CONTENTS OF COARSE-GROUND COFFEE (CGC), FINE-GROUND COFFEE (FGC) AND INSTANT COFFEE (IC) THROUGHOUT THE 7-DAY PERIOD OF ANALYSIS
Error bars represent the SEM. $*P < 0.05$ versus the value of each coffee beverage at day 0.

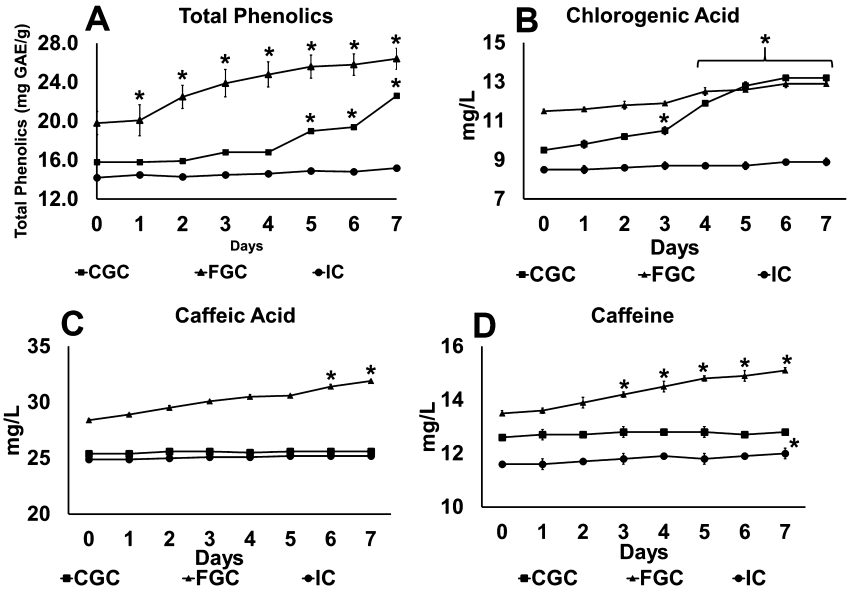

FIG. 4. THE (A) OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC), (B) DI(PHENYL)-(2,4,6-TRINITROPHENYL)IMINOAZANIUM (DPPH) EC_{50} AND (C) SUPEROXIDE-SCAVENGING ACTIVITIES OF COARSE-GROUND COFFEE (CGC), FINE-GROUND COFFEE (FGC) AND INSTANT COFFEE (IC) THROUGHOUT THE 7-DAY PERIOD OF ANALYSIS
Error bars represent the SEM. $^*P < 0.05$ versus the value of each coffee beverage at day 0.

($P < 0.05$) in the TA at the end of the fermentation process as well (from 0.369 to 0.489 mL). The observed changes, which were only limited to FGC, could be associated with the particle size. Out of the three types of coffee powder, FGC had the lowest particle size (2–3 μm) as compared with CGC (7–9 μm) and IC (5–6 μm). A higher surface area exposed to the reaction medium results in a higher amount of chemical changes. Thus, it is possible that the fungus consortium had a higher accessibility toward the reaction surface, thereby able to produce a higher content of organic acids.

Total Phenolic Content and Changes in the Chlorogenic Acid, Caffeic Acid and Caffeine Contents

The total phenolic contents are shown in Fig. 3A. FGC had the highest initial content of total phenolics (19.8 mg GAE/g) followed by CGC (15.8 mg GAE/g) and IC (14.2 mg GAE/g). Following the initiation of the fermentation process, FGC had the highest total phenolic content by day 7 with statistically significant increases ($P < 0.05$) observed from day 1 onward (26.4 mg GAE/g). Statistically significant

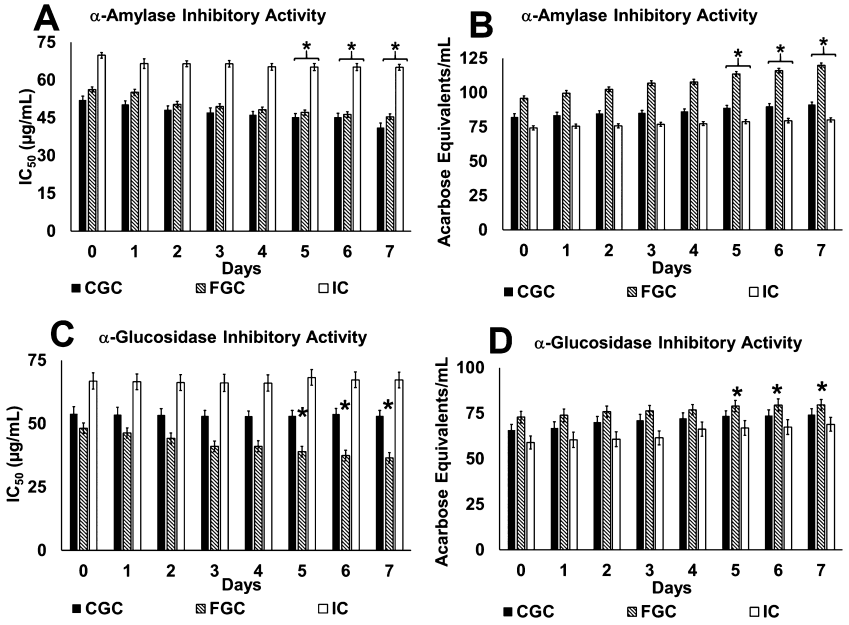

FIG. 5. THE A-AMYLASE INHIBITORY ACTIVITIES IN TERMS OF (A) IC_{50} VALUES AND (B) ACARBOSE EQUIVALENTS, AND THE A-GLUCOSIDASE INHIBITORY ACTIVITIES IN TERMS OF (C) IC_{50} VALUES AND (D) ACARBOSE EQUIVALENTS, OF COARSE-GROUND COFFEE (CGC), FINE-GROUND COFFEE (FGC) AND INSTANT COFFEE (IC) THROUGHOUT THE 7-DAY PERIOD OF ANALYSIS
Error bars represent the SEM. $^*P < 0.05$ versus the value of each coffee beverage at day 0.

increases ($P < 0.05$) in the total phenolic content were observed in CGC only from day 5 onward. There were no statistically significant changes in the total phenolic content ($P > 0.05$) in IC. As shown in Fig. 3B,C, the chlorogenic acid content had statistically significant increases ($P < 0.05$) from day 3 onward for CGC and day 4 onward for FGC. The final chlorogenic acid contents for CGC and FGC were 13.2 and 12.9 mg/L, respectively. The caffeic acid content showed statistically significant increases ($P < 0.05$) only in FGC from day 6 onward. The final caffeic acid content in FGC was 28.4 mg/L. Previous studies by Jayabalan *et al.* (2008) and Jayabalan *et al.* (2007) have reported an increase in the total phenolic content of Kombucha beverages following the initiation of the fermentation process. Tea catechins evaluated in the studies by Jayabalan *et al.* (2008), Chu and Chen (2006) and Chen and Liu (2000) were also observed to have increased by the end of the fermentation process. These three studies had also carried out the fermentation process for approximately 7 days. The hypothesis for this increment was previously explained in relation to the increase in color by Blanc (1996).

As shown in Fig. 3D, it was noteworthy that the caffeine content in all three beverages had overall increases as a result of the fermentation process as well. All three beverages were not decaffeinated and had initial caffeine contents ranging from 11.4 to 13.7 mg/L. FGC had the highest caffeine content (15.1 mg/L), followed by CGC (12.6 mg/L) and IC (12.0 mg/L). A statistically significant increase ($P < 0.05$) in the caffeine content was observed from day 3 onward of the fermentation process as compared with that prior to the fermentation process. Despite having the lowest caffeine content, IC was observed to contain a statistically significantly higher ($P < 0.05$) amount of caffeine by the end of the fermentation process on day 7, which was 11.6 mg/L. Given the associations of the positive health benefits of caffeine, the increase in its content could be considered as having a therapeutic significance as a result of the fermentation process.

ORAC, DPPH EC_{50} and Superoxide-Scavenging Activities

The ORAC, DPPH EC_{50} and superoxide-scavenging activities are shown in Fig. 4A–C, respectively. The antioxidant capacity of FGC was the highest before and after the fermentation process across all the antioxidant assays. It is also noteworthy that there was a better statistically significant correlation between the total phenolic content and the ORAC values than the DPPH EC_{50} and superoxide-scavenging values of all beverages on all days of analysis ($r^2 = 0.986$ for ORAC versus $r^2 = 0.753$ for DPPH EC_{50} and $r^2 = 0.657$ for superoxide-scavenging activity). Overall, when it came to the superoxide-scavenging activities, the trends were not as clear as the ORAC and DPPH EC_{50} values. Although three different antioxidant assays were carried out in this study, compounds exhibiting good antioxidant activity by one method have been known to demonstrate good antioxidant activity by the other methods, and likewise for compounds with low activity (Huang *et al.* 2005). However, the ORAC values may have had a better correlation with the total phenolic content as 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.

Starch Hydrolase Inhibitory Activities

The starch hydrolase inhibitory activities are shown in Fig. 5. Overall, the α-amylase inhibitory activities ranged between 32.5 and 71.5 μg/mL in terms of the IC_{50} values or 72.5 and 125.4 AE/mL prior to the fermentation process, while the α-glucosidase inhibitory activities ranged between 27.5 and 75.5 μg/mL in terms of the IC_{50} values or 51.5 and 75.8 AE/mL. Statistically significant increases in both α-amylase and α-glucosidase inhibitory activities ($P < 0.05$) were observed only from day 5 onward in all three beverages as compared with that prior to the fermentation process. The α-amylase and α-glucosidase inhibitory activities were the highest in FGC before and after the fermentation process. The values for all three fermented beverages were comparable with phytochemical products which have been known and established for their inhibitory potentials, indicating that all beverages have the ability to inhibit the breakdown of starch and the subsequent release of glucose into the physiological systems (Benzie 2003; Koh *et al.* 2009). It was additionally observed that all beverages were able to inhibit α-amylase better than α-glucosidase in comparing their IC_{50} and AE values. This is an important aspect as far as the mechanisms of action of the two enzymes are concerned. Because α-amylase is required for the cascading of reactions for α-glucosidase, the inhibition of α-amylase could be deemed as being more vital than α-glucosidase. Therefore, the trend displayed by all the tea fungus-fermented coffee drinks could be considered as therapeutically beneficial in terms of the prevention of starch breakdown and the subsequent curbing of glucose release into the physiological systems (Chu and Chen 2006).

CONCLUSIONS

This study was able to provide preliminary chemical evidence on the enhancement of the antioxidant and α-amylase inhibitory potential of coffee beverages through the addition of the tea fungus. Although this study was able

to confirm the antioxidant potential of three coffee products 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. From the results, it was observed that the α-amylase inhibitory activity in particular had increased with the fermentation process. This could have been due to the increase in the total phenolic content and the increase of the presence of compounds with α-amylase inhibitory activities as a result of the fermentation process (Koh *et al.* 2009). The starch hydrolase inhibitory properties of the coffee beverages evaluated in this study were also indicative of its usage to be consumed for the maintenance of health and wellness. It would also be a novelty to investigate the therapeutic potential of tea fungus-fermented beverages prepared from other types of brewed products not studied under this research work. A myriad of teas exist throughout the world, which are consumed either for pleasure or therapeutic purposes whose sensory properties and medicinal benefits might increase with a simple fermentation process such as the addition of the tea fungus.

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CONFLICTS OF INTEREST

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

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