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ARTICLE

Evaluation of Different Teas against Starch Digestibility by Mammalian Glycosidases

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Current work investigated the ability of different tea (green, oolong and black teas) in inhibiting human salivary α-amylase (HSA) and mammalian α-glucosidase (AGH). The inhibitory profiles were correlated to their major polyphenol content (theaflavins and catechins). The fully fermented black tea was demonstrated to be most potent in inhibiting HSA and AGH (IC_{50} of 0.42 to 0.67 and 0.56 to 0.58 mg of tea leaves/mL respectively). Its capability in retarding the digestion of a real food system (rice noodle) was further elucidated with an *in vitro* digestion study. Results indicated that black tea was able to retard starch digestion moderately, thereby allowing a gradual reduction of sugar liberation. Polyphenolic profile analysis suggested that the oxidized catechins, theaflavins, may be responsible for its activity. We have found that refractive index (RI) measurement is a rapid, direct, and highly convenient method for quantifying the degree of enzymatic starch digestion and kinetics. The RI method has good linearity range, limit of detection (0.1596 mg/mL, maltose equivalent) and limit of quantitation (0.6312 mg/mL) and was successfully applied in our study.

KEYWORDS: Tea; theaflavins; catechins; α-amylase; α-glucosidase; starch digestion retardation

INTRODUCTION

Dietary carbohydrates form the key energy supply in the human diet. Among them, polysaccharides which are represented almost exclusively by starch play the major role. Starch is composed of two types of molecules, namely, the linear amylose (polymer of α-1,4-linked glucose molecules) and the highly branched amylopectin (polymer of α-1,4-linked glucose molecules at the linear region and α-1,6-linked glucose molecules at the branching sites). The key enzymes involved in starch digestion are α-amylase and α-glucosidase. α-Amylase hydrolyzes the α-1,4-linkage of starches, but its action is sterically hindered in the vicinity of α-1,6 branching sites. The three principal products of α-amylase digestion are maltose, maltotriose and α-dextrins. (*1*). Further digestion of these molecules takes place in the small intestine by α-glucosidase, which is a class of brush-border bound enzymes, comprising primarily malto-glucoamylase (acts on α-1,4 linkages in oligosaccharides of 5−9 glucose molecules long) and sucrose-isomaltase (exhibits the ability to cleave α-1,6 linkages in α-dextrins) (*2*). The main product of α-glucosidase digestion is glucose which will be actively transported into the bloodstream, hence resulting in the rise of plasma glucose.

Physiological effects of carbohydrates vary substantially, depending on their ease of digestion, and the subsequent blood glucose response. Ingestion of rapidly digestible carbohydrates will lead to a sharp rise of postprandial blood glucose. Frequent consumption of such foods, or high glycemic index foods, is suggested to play a causative role in development of various chronic diseases such as type 2 diabetes, obesity and cardiovascular diseases (CVD) (*3*−*5*). Introduction of α-amylase and α-glucosidase inhibitor into the diet has been proposed to be effective in retarding carbohydrate digestion (*6*, *7*). The use of enzyme inhibitor produces carbohydrate malabsorption and, hence, moderates blood glucose and insulin elevation. This is shown to be beneficial in the treatment of type 2 diabetes patients (*8*, *9*). Hence, enzyme inhibitors such as acarbose are accepted diabetic drugs in some countries. However, these drugs cause some adverse health effects such as promoting hypoglycemia at higher dose, as well as diarrhea and flatulence due to the gut microflora fermentation on the undigested carbohydrates. Natural α-amylase and α-glucosidase inhibitor has been identified in various plants such as cumin seeds, mulberry leaves and mangosteen pericarp (*10*−*12*). Many of them are traditional medicinal plants, and bioactive compounds present in them possess various health benefits. It would be ideal if a beverage we often drink, such as tea or coffee, can retard starch digestion and reduce the glycemic index.

Tea is a water extract of the leaves of the plant *Camellia sinensis*. Numerous publications reported that tea has great potential in preventing various chronic diseases such cancer, diabetes, CVD, obesity and osteoporosis (*13*, *14*). This is largely contributed by its rich flavonoids such as catechins and theaflavins. The type of processing methods applied to fresh tea leaves determines the profile of flavonoid present in green (nonfermented), oolong (semifermented) and black (fully fermented) teas. Processing of black tea typically involves withering (softens the tea

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leaves and reduces its moisture content), rolling (crushes the leaves and releases the enzymes), fermentation (enzymatic oxidation of polyphenols) and frying (enzyme inactivation to halt the reaction) (*15*). Manufacturing of oolong tea has a similar processing sequence, but the duration of oxidation is significantly shortened. Meanwhile, in green tea production, tea leaves are withered and fried for enzyme inactivation prior to rolling and drying. Catechin and its derivatives are a major group of flavonoid found in green tea. Enzymatic oxidation (by polyphenol oxidase) in black tea manufacturing converts catechins into dimers and polymers such as theaflavins and thearubigins (*16*). Apart from their antioxidant bioactivity, tea flavonoids are also shown to be inhibitors of α-amylase and α-glucosidase (*17*−*19*). Most of these studies concentrated only on the pure polyphenols; it remains unknown whether the tea, in the form we consume, has any inhibitory activity on starchy food digestion.

In this study, inhibitory activity of the individual catechins and theaflavins against human salivary α-amylase (HSA) and mammalian α-glucosidase (AGH) was first determined. Next, inhibitory activity of tea infusions from green, oolong and black teas against these enzymes was evaluated and correlated to their polyphenolic profile. Results obtained would justify if the extent of tea fermentation will influence their inhibition potency. Effects of tea against a real food system (rice noodle) digestion were then elucidated using a simple *in vitro* digestion experimental setup. This experiment was aimed to investigate the efficiency of tea in retarding starch digestion when consumed along with a carbohydrate rich food. The experimental steps were specially designed to allow a simple and rapid *in vitro* digestion analysis.

MATERIALS AND METHODS

Materials. Six tea samples in the form of tea bag were purchased from local grocery stores. Among them, LB and DB were black teas. LO and DO were samples representing oolong teas. Green tea samples were denoted as LG and DG. Samples coded with the prefix of L or D were products from two different manufacturers. Samples were ground into a homogeneous mass and brewed at 80 °C for 30 min in phosphate buffer at the desired concentration. The phosphate buffer utilized had the following conditions: pH 6.9; 50 mM; 6.85 mM NaCl. Phosphate buffer mentioned throughout this paper had the same conditions (unless stated otherwise). Tea mixtures were filtered by vacuum filtration and centrifuged (15 min and 5000*g* at 25 °C) for removal of insoluble tea leaves prior to the subsequent analyses.

The positive control acarbose was obtained by dissolving a Glucobay 50 tablet from Bayer Healthcare (Leverkusen, Germany) in phosphate buffer at the desired concentration. Each tablet consisted of 50 mg of acarbose. The mixture was centrifuged (15 min and 10000*g* at 25 °C) for insoluble filler removal prior to the subsequent analyses.

Pure catechin standards, rice starch (S7260), HSA (A0521; 104 units/mg solid), and 3,5-dinitrosalicyclic acid, 4-nitrophenyl α-D-glucopyranoside (PNPG), and AGH (I1630) were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO). One unit of HSA liberates 1.0 mg of maltose from starch in 3 min at pH 6.9 and at 20 °C. A stock HSA solution of 104 U/mL in phosphate buffer was prepared and diluted to the final concentration of 4.59 U/mL. The flavanols used were (−)-epigallocatechin gallate ((−)-EGCg, E4268), (−)-epigallocatechin ((−)-EGC, E3768), (−)-epicatechin gallate ((−)-ECg, E3893), (−)-epicatechin ((−)-EC, E1753) and (+)-catechin hydrate ((+)-C, C1251). Pure theaflavin standards (theaflavin (TF1, 00020252); theaflavin monogallate (TF2, 00020253); theaflavin digallate (TF3, 00020254)) were all obtained from ChromaDex, Inc. (Irvine, CA). Catechin and theaflavin were dissolved in phosphate buffer (pH 6.9, 50 mM, containing 6.85 mM NaCl) at desired concentration prior to the subsequent analyses.

Human Salivary α-Amylase Inhibition Assay. Rice starch stock solution (25 mg/mL) in phosphate buffer was heated at 100 °C for one min and then cooled to room temperature before being diluted with phosphate buffer to the final concentration of 2.0 mg/mL. The acarbose, catechins, theaflavins and tea were prepared in phosphate buffer and diluted into a range of desired concentrations. First, 82 μL samples of inhibitor of different concentrations were pipetted into separate reaction vials. Next, 8 μL of rice starch solution was pipetted into each reaction vial. This was followed by the addition of 10 μL of diluted HSA to start the digestion in a 37 °C water bath. After 12 min, 100 μL of 3,5-dinitrosalicyclic acid (DNSA) reagent was added into each vial for reaction termination. DNSA solution was prepared as described by Bernfeld (*20*). Reaction vials were immersed in a 100 °C water bath for 5 min for color development. Upon the addition of 500 μL of deionized water, an aliquot of 200 μL from each reaction vial was pipetted into a separate well of a microplate. Absorbance at 540 nm was measured by Multiskan Spectrum microplate reader (Thermo Electron Corporation, Waltham, MA). A control vial was prepared by replacing the inhibitor solution with phosphate buffer. The entire experiment was repeated by substituting the live HSA with denatured HSA solution treated at 100 °C for 10 min. Percentage of HSA inhibition was calculated with eq 1. The assay was performed in triplicate, and a curve of percentage inhibition against inhibitor concentration was plotted with the averaged values. IC_{50} (concentration of inhibitor required to produce a 50% inhibition of the initial rate of reaction) of each inhibitor was determined by interpolation from such a curve. Examples of HSA inhibition curves were as illustrated in **Figure 1a**.

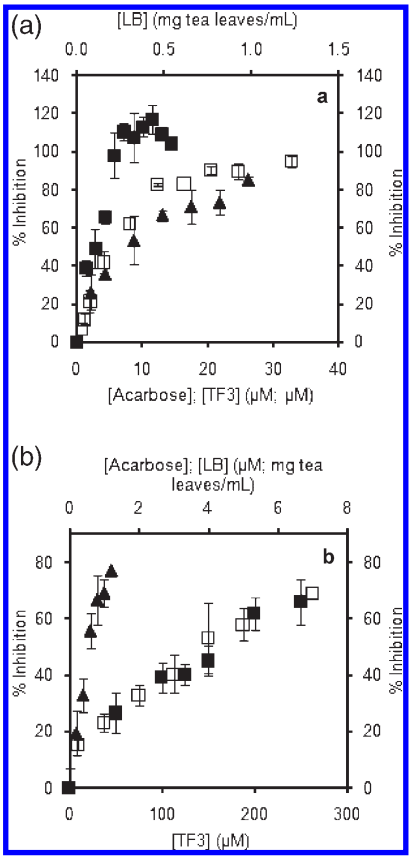

Figure 1. (**a**) Degree of HSA inhibition at different inhibitor concentration. (**b**) Degree of AGH inhibition at different concentration for (□) acarbose, (■) TF3, and (▲) LB.

$$\%\ \text{inhibition} = \frac{(A_{\text{control}} - A_{\textit{controlblank}}) - (A_{\text{sample}} - A_{\text{sampleblank}})}{(A_{\text{control}} - A_{\text{controlblank}})} \times 100 \quad (1)$$

where A_{control}, $A_{\text{controlblank}}$, A_{sample} and $A_{\text{sampleblank}}$ refer to the absorbance value of reaction vial containing live enzyme and buffer, dead enzyme and buffer, live enzyme and inhibitor and dead enzyme and inhibitor respectively. Substrate was present in all these vials.

α-Glucosidase Inhibition Assay. Reaction substrate PNPG (30 mM) and AGH (16.65 mg/mL) solutions were prepared in phosphate buffer saline (PBS) respectively. After vortexing for 5 min, the AGH mixture was centrifuged at 4 °C and 10000*g* for 30 min. The top clear supernatant was subsequently utilized in the assay.

Table 1. HSA and AGH Inhibition Activity (Expressed as IC_{50}) of Acarbose, Selected Polyphenols and Different Teas as Well as the Total Phenolic Content of These Teas[a]

	IC_{50}		
inhibitor	against HSA	against AGH	total phenolic content (g of GAE/g tea leaves)
acarbose	5.7 μM	3.8 μM	–
theaflavin digallate	2.9 μM	165 μM	–
theaflavin monogallate	5.5 μM	310 μM	–
theaflavin	67 μM	>400 μM	–
epicatechin gallate	1.5 mM	330 μM	–
epigallocatechin gallate	1.4 mM	220 μM	–
catechin hydrate	>20 mM	540 μM	–
black tea L (LB)	0.42 mg/mL	0.56 mg/mL	0.115 ± 0.004
oolong tea L (LO)	3 mg/mL	0.98 mg/mL	0.134 ± 0.005
green tea L (LG)	4.2 mg/mL	2.8 mg/mL	0.106 ± 0.002
black tea D (DB)	0.67 mg/mL	0.58 mg/mL	0.101 ± 0.003
oolong tea D (DO)	4.4 mg/mL	1.05 mg/mL	0.113 ± 0.004
green tea D (DG)	4.4 mg/mL	3.4 mg/mL	0.125 ± 0.004

[a] IC_{50} refers to the concentration of inhibitor required to produce a 50% inhibition of the initial rate of reaction, as determined by interpolation from **Figure 1a**,**b** as an example. GAE is the total phenolic content of the teas expressed as g gallic acid and it is an average of triplicates ± SD. A dash represents that the assay was not carried out.

First, 340 μL of an inhibitor of different concentrations was pipetted into separate reaction vials. Next, 20 μL of AGH solution was added into each vial, and incubation at 37 °C for 10 min followed. Then, 40 μL of PNPG solution was added to initiate the digestion. After 15 min, 200 μL of 1 M Na_2CO_3 was added for reaction termination. An aliquot of 300 μL was withdrawn from each vial and added into a separate well of a microplate. Absorbance at 400 nm was measured by a BioTek Synergy HT multidetection microplate reader (Winooski, VT). A control vial was prepared by replacing the inhibitor solution with phosphate buffer. The entire experiment was repeated by substituting the live AGH with dead AGH treated at 100 °C for 10 min. Percentage of AGH inhibition was calculated with eq 1. The assay was performed in triplicate, and a curve of percentage inhibition against inhibitor concentration was plotted with the averaged values. IC_{50} of each inhibitor was determined by interpolation from such a curve. Examples of AGH inhibition curve were as illustrated in **Figure 1b**.

Determination of Total Phenolic Contents. The total phenolic content (TPC) of tea samples was determined according to a modified Folin−Ciocalteu assay (*21*). In brief, 20 μL of a tea or gallic acid of different concentration was pipetted into a separate well of a microplate. Next, 100 μL of the 10-times diluted Folin−Ciocalteu reagent and 80 μL of 75 mg/mL Na_2CO_3 were pipetted into each well. Incubation at 37 °C for 30 min followed prior to the final absorbance at 765 nm, which was recorded on a BioTek Synergy HT multidetection microplate reader. Phenolic content of the tea was expressed as gram gallic acid equivalents (GAE). Recorded values (**Table 1**) were averages of triplicate measurements.

Quantification of Polyphenolic Compounds. Tea solutions (32 mg of dried tea leaves/mL) of deionized water were prepared according to the steps mentioned in the earlier part of this manuscript. Each catechin standard (EGCg, EGC, ECg, EC and C) was dissolved in water to six standard solutions with the concentration ranging from 50 to 500 μM. Meanwhile, each theaflavin standard was dissolved in water to six standard solutions with the concentration range of 25 to 250 μM for TF1 and 25 to 150 μM for both TF2 and TF3. All tea and standard solutions were filtered through a 0.45 μm regenerated cellulose membrane filter prior to injection into the high performance liquid chromatography (HPLC) system, which consisted of a HPLC-PDA with UV detector (Waters, 2695/2696) and a C18 column (250 × 4.6 mm/5 μm, Atlantis). It was equipped with an autoinjector. Mobile phases consisted of 0.1% (v/v) formic acid in water (eluent A) and 0.1% (v/v) formic acid in acetonitrile (eluent B). A gradient elution was adopted as follows: 0−28 min, 8% B; 28−80 min, linear gradient 8−15% B; 80−85 min, linear gradient of 15−31% B; 85−88 min, linear gradient of 31−8% B. The flow rate and column temperature were maintained at 1.0 mL/min and 25 °C ± 5 °C respectively. Sample injection volume was 20 μL. Catechins and theaflavins were detected at 280 nm. Identification of each catechin and theaflavin in teas was made by comparing the retention time and spectrum with that of the standards. HPLC−MS was performed for peak affirmation (data not shown). Quantification of catechins and theaflavins was done according to their respective standard curve.

Pancreatin *in Vitro* Digestion Analysis. Pancreatin from hog pancrease (Sigma-Aldrich, USA; P1750) with 115 units/mg solid of amylase activity was dissolved in phosphate buffer to the concentration of 25 mg/mL. Rice dough was prepared as digestion substrate. Its basic formulation in weight percentage consisted of 53.5% rice (*Oryza sativa*) flour, 10.5% of tapioca (*Manihot esculenta*) starch, 0.7% of propylene glycol alginate (PGA), 2% of vegetable oil and 33.3% of deionized water. It was prepared to serve as a model system of instant soup-based rice noodle available in the market. The rice flour used contains 80.2% carbohydrate, 6.7% protein, 0.3% ash, 0.3% fat, and 12.5% moisture (w/w). Tapioca starch (T37) with a moisture content of 11.0% (w/w) was provided by National Starch, Singapore. PGA was obtained from ISP Inc. (San Diego, CA). Vegetable oil was purchased from local grocery stores.

PGA was dissolved in deionized water at 60 °C on continuous stirring for 10 min and subsequently cooled to room temperature. It was then added into dry mixed rice flour and tapioca starch. Vegetable oil was added, and the mixture was kneaded into dough. Dough was sheeted by passing through three pairs of rollers with reduction of the gap distance before slitting it into noodle strands. The final noodle strand had the thickness of 2.68 ± 0.09 mm and width of 6.72 ± 0.09 mm. Noodle strands (20 g) were boiled in 112.5 mL of deionized water at 100 °C for 3 min. Upon cooling to room temperature, noodle strands were cut by cork borer into small discs of 61.41 ± 1.24 mg each. Two dough discs were placed into seven separate test tubes, each containing 2.8 mL of LB tea solution prepared with 30 mg of LB tea leaves/mL of phosphate buffer. Two hundred microliters of pancreatin solution was pipetted into each test tube, and digestion was allowed to take place at 37 °C for 1, 30, 60, 90, 120, 150, and 180 min before termination by heating the solution to 100 °C for one min. 1.50 mL of digestion medium was withdrawn from each test tube and centrifuged for 20 min at 20000*g* and 23 °C. Refractive index (RI) of the clear supernatant was measured by a RX-5000α refractometer (Atago Co. Ltd., Japan). A series of seven test tubes were prepared as blanks. They were subjected to the same experimental procedures, except using dead pancreatin obtained by treating the live enzyme solution at 100 °C for 10 min. RI of the sample was corrected by subtracting away the RI values of the respective blank. In doing so, the influence of LB teas to the recorded RI was eliminated, rendering the final value to be solely contributed by the sugars present. The entire experiment was repeated with LB tea of different concentration, namely, 5, 10, and 20 mg/mL (which corresponded to 0.573, 1.14, 2.29 mg of GAE/mL). A control set of experiments was also carried out by replacing the digestion medium of LB tea with phosphate buffer.

Amount of reducing sugars liberated into the digestion medium was quantified based on its RI. To achieve this, five maltose standard curves were constructed by plotting RI (corrected values by subtracting away the RI values of a blank) versus maltose concentration, which covered the range from 2.5 to 20 mg/mL. The first standard curve was obtained by preparing the sugar standards in phosphate buffer. It was utilized to determine the sugar concentration in digestion mediums of the control experiment. An additional four curves were constructed by preparing the maltose standards in 5, 10, 20, or 30 mg/mL LB tea. Amount of sugars (in maltose equivalent) liberated into the digestion medium was calculated according to its respective standard curve.

Correlation Evaluation between Refractive Index and Reducing Sugar Concentration. This experiment was performed to evaluate the suitability of using RI in quantifying the amount of reducing sugars produced during digestion. Amount of reducing sugars liberated into the digestion medium was determined by an assay reported previously (*20*). In short, 150 μL of a centrifuged digestive medium was pipetted into a reaction vial containing 150 μL of deionized water and 200 μL of DNSA reagent. The reaction vial was immersed into a water bath controlled at 100 °C for 5 min. Upon cooling to room temperature, the mixture was further diluted 10 times. An aliquot of 200 μL was pipetted into a well of a

microplate, and absorbance at 540 nm was recorded on the Multiskan Spectrum microplate reader. Reducing sugars present were quantified according to a maltose standard curve constructed with the concentration range from 0.2 to 1.2 mg/mL. The experiment was repeated for digestion mediums of different digestion duration (1, 30, 60, 90, 120, 150, and 180 min). A correlation curve (**Figure 4**) of RI against reducing sugar concentration (in maltose equivalents) determined by this method was plotted using the average data of three triplicates.

RESULTS AND DISCUSSION

HSA and AGH Inhibition Activity. Structural variation among the enzymes of different origins significantly influences their susceptibility to different inhibitors. In view of this, the current investigation was conducted with mammalian glycosidases. Although HSA was employed in this work instead of human pancreatic α-amylase (HPA), the determined inhibitory potencies were expected to be comparable to that of HPA because these proteins share a high degree of amino acid sequence similarity with 97% identical residues overall and 92% in catalytic domains (*22*, *23*).

Teas were brewed at 80 °C for 30 min at their respective concentrations prior to these experiments. A temperature lower than the typical temperature of boiling water was selected to minimize catechin epimerization, and thereby avoid the change in the tea polyphenol profile. Catechins have low stability at neutral pH and undergo epimerization readily at high temperature (*24*, *25*).

Inhibitory potency of catechins, theaflavins and teas against HSA and AGH was determined, and their IC_{50} are listed in **Table 1**. Acarbose was selected as positive control in both assays. **Figures 1a** and **1b** displayed a typical inhibition curve of acarbose, TF3 and LB tea against HSA and AGH respectively. IC_{50} of an inhibitor is very dependent on the assay conditions such as enzyme concentration and origin, substrate type and concentration, reaction duration, temperature and pH. This makes data comparison with the literature a difficult task. However, by utilizing acarbose as a benchmark, comparison of the general inhibitory trend could be achieved. Current findings are in agreement with previous reports (*17*, *19*).

On the whole, theaflavins were better inhibitors of HSA and AGH in comparison to catechins. Among them, TF3 ranked the first having the IC_{50} of 2.9 μM and 165 μM against HSA and AGH respectively. Its inhibitory effect was much more potent against HSA than AGH, outperforming acarbose in inhibiting the former. From the results, it could also be deduced that the galloyl (3,4,5-trihydroxybenzoyl) moiety was critical in determining theaflavin inhibitory activity, as the IC_{50} decreased when the number of galloyl moieties attached increased. As for catechins, significant inhibitory effects were only shown by their epi forms. Similarly, the galloyl moiety played an essential role. Polyphenols interact with enzyme primarily via noncovalent interactions, namely, hydrogen bonding and pi−pi interactions of the aromatic ring with protein in particular (*26*). The galloyl moiety could contribute to these interactions (*27*). It may also be possible that the more acidic gallate group dissociates more completely under the assay pH and becomes anionic and interacts with the protein through charge.

Among the tea samples, black teas (LB and DB) showed substantial inhibitory effects against HSA and AGH. Their IC_{50} values ranged from 0.42 to 0.67 mg of tea leaves/mL for both enzymes. This was then followed by oolong tea (LO and DO) with the IC_{50} values ranging from 0.98 to 1.05 and 3 to 4.4 mg of tea leaves/mL for AGH and HSA respectively. Having the IC_{50} range from 2.8 to 4.4 mg of tea leaves/mL, green tea (LG and DG) showed relatively low inhibitory activity. The strong inhibitory potency of black teas could be mainly contributed by its theaflavin content. However, the theaflavin concentrations in the black teas tested herein did not account for the activity of the black tea. The inhibition effect of black teas is due to collective activity of all the ingredients.

Total Phenolic Content and Enzyme Inhibition Activity. As tabulated in **Table 1**, phenolic content of the tea samples did not vary considerably. Hence, there was no solid relationship between TPC and enzyme inhibition activity. For example, LO had the highest TPC. However, its IC_{50} against both HSA and AGH was much greater than that of LB and DB. This indicated that specific interactions occurred between tea polyphenols and the enzymes and each tea polyphenol displayed a different level of inhibition against HSA and AGH. For example, the IC_{50} of TF3 against HSA was 160 times lower than that of AGH. A unique polypeptide sequence in HSA might have favored the binding of TF3. A similar situation was found in studies performed on other polyphenols. It was previously deduced that porcine pancreatic α-amylase (PPA) was potently inhibited by luteolin, myricetin and quercetin, but little inhibitory effect was observed against AGH (*28*). Meanwhile, fruits rich in anthocyanin effectively inhibited AGH, but an appreciable extent of PPA inhibition was only observed in tannin-rich fruits (*29*). Thus, it can be concluded that structural diversity between α-amylase and α-glucosidase substantially renders them to different degree of polyphenol inhibition. In addition, interactions between the enzymes and polyphenols are particularly specific that different levels of inhibition might occur among glycosidase variants. For instance, polyphenols that typically inhibit yeast α-glucosidase were weak inhibitors of mammalian α-glucosidase (*28*, *30*).

Polyphenols Profiles in Different Teas. A profile of the key polyphenols in teas was presented in **Figure 2**. Results indicated that theaflavins were present only in black tea samples. This was as expected since theaflavins were products of oxidative coupling between EC and EGC from fresh tea leaves (*31*). As enzymatic fermentation proceeded, content of theaflavins increased along with catechin decrement. Hence, black teas had a relatively low amount of catechins as well. As oolong tea was semifermented, it was anticipated that catechin content in LO and DO shall lie between that of black and green teas. However, this trend was only observed in samples from the D series. This could probably be attributed to processing variation between manufacturers.

A considerable amount of theaflavins in black teas explained their potent inhibitory effects against HSA and AGH. Since the major polyphenols in oolong and green tea samples were mainly catechins, it was not unexpected that their IC_{50} were comparatively higher. In contrast to theaflavins, catechins were weaker enzyme inhibitors. Nevertheless, ECg and EGCg indeed exhibited substantial inhibition activity against AGH. Hence, tea from DG in comparison to DO should display greater inhibitory potential against this enzyme. However, results (**Table 1**) disclosed otherwise. This suggested that inhibition activity of the teas could be a summation effect of all the polyphenols present in them.

Effect of Tea in Rice Noodle Digestion by Pancreatin. A majority of the studies executed previously elucidated the inhibitory effects of tea polyphenols or their infusions in a model system, where starch solution or synthetic substrate solution was generally utilized (*28*, *29*). Although the outcomes were all positive, such a conclusion cannot be extended to a real food system, which typically comprises protein, fiber, lipid, and micronutrients. Hence, the inhibitory activity of tea against its digestion may be altered significantly under such circumstances. Rice noodle was selected for our study because it was starchy food and popular particularly in Asia. The noodle sample was prepared based on an industrial instant rice noodle formulation. LB

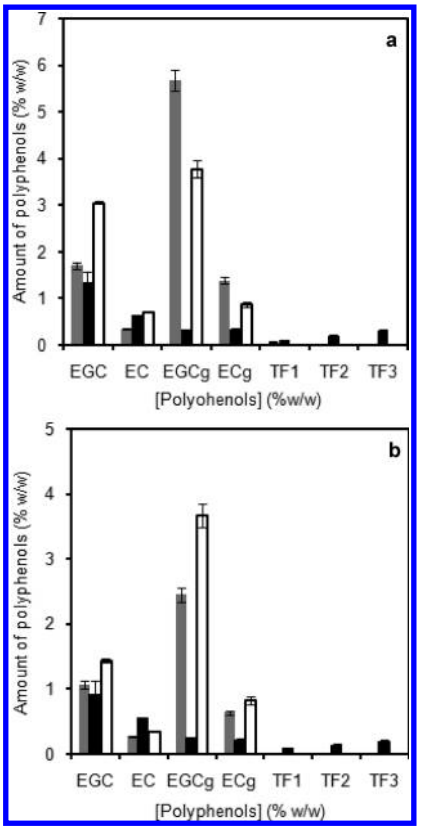

Figure 2. Weight percentage of the main catechins and theaflavins quantified in (**a**) LO (gray bar), LB (■), and LG (□); (**b**) DO (gray bar), DB (■), and DG (□) by HPLC.

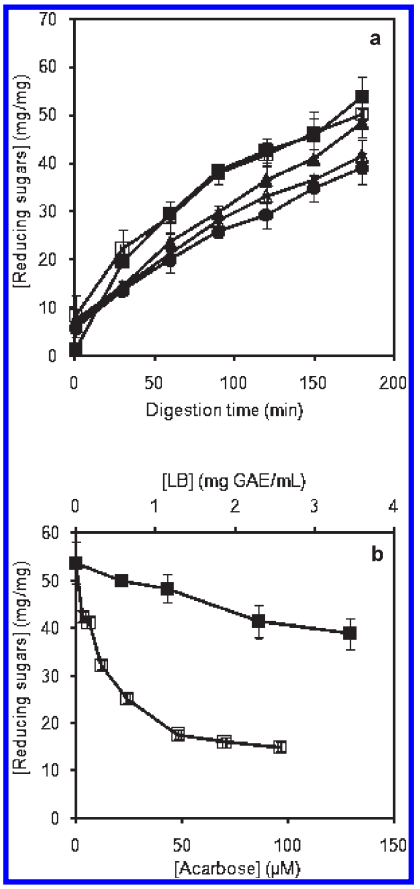

Figure 3. Amount of reducing sugars (mg of maltose equivalent/mg of dough dry matter) liberated into the digestion medium during *in vitro* pancreatin digestion of rice dough (**a**) for a duration of 180 min in the absence of inhibitor (■) and 0.573 (□), 1.145 (▲), 2.291 (△), and 3.346 (●) mg of GAE/mL of LB), and (**b**) at 180 min at various concentrations of acarbose (□); and LB (■).

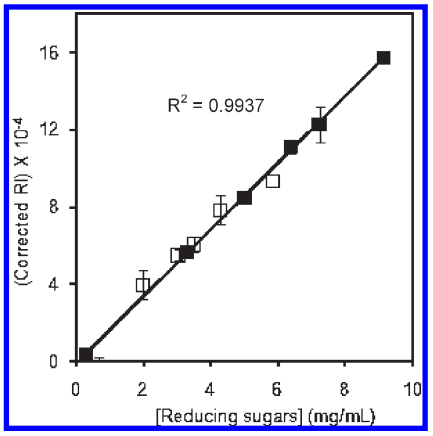

Figure 4. Linear correlation between the corrected RI of the digestion medium (in the absence of inhibitor (■) and in 30 mg of LB tea leaves/mL (□)) and the concentration of reducing sugars present in them.

was selected in this study as it was shown to be the best tea sample in inhibiting HSA and AGH on rice starch digestion.

Cooked rice noodle was subjected to three hours digestion by hog pancreatin in LB. **Figure 3a** illustrates the digestion profile of rice noodle in various concentrations of LB (0.5727 mg of GAE/mL or 5 mg of tea leaves/mL, 1.1454 mg of GAE/mL or 10 mg of tea leaves/mL, 2.2909 mg of GAE/mL or 20 mg of tea leaves/mL and 3.3463 mg of GAE/mL or 30 mg of tea leaves/mL). Based on the manufacturer guidelines, each LB tea bag (2 g of tea leaves) should be brewed in 200 mL of water, rendering to the final concentration of 10 mg of tea leaves/mL. This value fell comfortably within the experimental range. Solid square data points represented the control experimental set, whereby the digestion medium was phosphate buffer in place of LB. Results showed that rice noodle digestion was significantly retarded in the presence of LB, particularly at higher concentrations.

In order to compare the efficiency of tea in detaining starch digestion, a similar experiment was conducted by substituting the LB with acarbose solutions. Amount of reducing sugars liberated at the end of the digestion was plotted against acarbose concentration (**Figure 3b**). It could be deduced from these curves that rice noodle digestion was severely retarded in the present of acarbose. On the other hand, the inhibitory effect of LB tea was much more gradual. For instance, at the highest LB tea (30 mg of tea leaves/mL) and acarbose concentration (96 μM), rice noodle digestion was reduced by 28% and 72% respectively.

Black tea may be inferior to acarbose in terms of delaying starch digestion. However, treatment with the latter often leads to adverse health effects (such as flatulence, diarrhea and abdominal pain) among diabetic patients (*32*). These are primarily due to microbial fermentation on the undigested starch fractions in the large intestine. Hence, such a potent drug is not applicable for the general population. In contrast, black tea could be an option to delay starch digestion upon the ingestion of a carbohydrate rich diet. This may be beneficial among patients that are on weight management program as well as for the weight conscious consumers. Black tea is capable of retarding starch digestion and, at the same time, not leading to carbohydrate malabsorption. In light of our findings, a human clinical trial shall be conducted to evaluate the effect of teas on reducing glycemic index.

Refractive Index as a Convenient Starch Digestion Monitoring Technique. Refractive index (RI) is heavily used in the food industry for monitoring sugar levels of beverages. It is a highly rapid and reliable physical parameter, yet, to our best knowledge, there was no publication to date reporting the use of RI as a technique of monitoring enzymatic starch digestion kinetics and inhibition. We rationalize that, as pancreatin digestion on rice noodle proceeded, starch would be broken down to reducing sugars and oligosaccharides of various chain lengths. Most of them were water-soluble and, therefore, liberated into the digestion medium. Their concentration increased with digestion time. Thus, monitoring of reducing sugar production could be an effective way of estimating starch digestion. A typical maltose standard curve that we had constructed exhibited excellent linearity with the R^2 of 0.9999. Based on the standard curve, the limits of detection and quantitation were calculated as 0.1596 and 0.6312 mg/mL of maltose equivalent respectively. To further validate the RI as a quantitative method for our purpose, we determined the correlation between RI and reducing sugar concentration quantified by DNSA assay. Results are delineated in **Figure 4**, which is a plot of corrected RI (after deducting the blank's reading) versus reducing sugar concentration as determined by DNSA assay. Two sets of experimental data are presented in this figure. Solid symbols represent values of the control set of experiments where the digestion medium was phosphate buffer. Meanwhile, the open symbols represent values of the sample set of experiments where the digestion medium was LB tea of 30 mg of tea leaves/mL. Aliquot of each data point was a fraction of the digestion medium subjected to a definite digestion duration. These data points were averaged over three experiments, and the standard deviation was comparatively small. Based on the curves, RI was indeed linearly correlated with sugar concentration. These curves superimposed with each other, indicating that this method was not significantly affected by the tea as the following factors were deliberated: (i) interference owing to the tea was eliminated by plotting the corrected RI values during standard curve construction, and (ii) sugar quantification in the digestion medium was accomplished by extrapolating the net RI values from the designated standard curve.

Apart from being a source of antioxidant, tea was also able to detain starch digestion via digestive enzyme inhibition. Results demonstrated that theaflavins were far more potent in inhibiting HSA and AGH in comparison to catechins. Their degree of inhibition against HSA and AGH varied, indicating that specific interactions might be involved in the binding. The fully fermented black tea consisted of significant amount of theaflavins. Among other tea samples, black tea had exhibited potent inhibitory activity against HSA and AGH. This inhibitory capability was retained in the digestion of rice noodle, suggesting that black tea could potentially delay starch digestion following carbohydrate dense meal consumption. Hence, habitual drinking of black tea is beneficial for general weight conscious consumers. Nevertheless, proper *in vivo* study is essential to further evaluate the bioefficacy and potential use of black tea for long-term weight maintenance. Refractive index measurement of the digestion medium was demonstrated to be effective in detecting reducing sugars liberation. Hence, it was proposed that this simple method is efficient for monitoring starch digestion and searching for starch hydrolase inhibitors originating from foods.

ABBREVIATIONS USED

GI, glycemic index; CVD, cardiovascular diseases; HSA, human salivary α-amylase; PPA, porcine pancreatic α-amylase; AGH, α-glucosidase; LB, black tea L; LO, oolong tea L; LG, green tea L; DB, black tea D; DO, oolong tea D; DG, green tea D; EGCg, epigallocatechin gallate; EGC, epigallocatechin; ECg, epicatechin gallate; EC, epicatechin, C, catechin hydrate; TF1, theaflavin; TF2, theaflavin monogallate; TF3, theaflavin digallate; DNSA, 3,5-dinitrosalicyclic acid; PNPG, 4-nitrophenyl α-D-glucopyranoside; TPC, total phenolic content; GAE, gallic acid equivalent; PGA, propylene glycol alginate; RI, refractive index.

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