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A High-Throughput Assay for Quantification of Starch Hydrolase Inhibition Based on Turbidity Measurement

Tingting Liu, Lixia Song, Hongyu Wang, and Dejian Huang*

Food Science and Technology Program, Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Republic of Singapore

Supporting Information

ABSTRACT: A high-throughput method for rapid determination of starch hydrolase inhibition was developed using a 96-well microplate UV−vis reader to monitor the turbidity decrease over time. The area under the curve of turbidity measured over time was used to quantify the inhibitory effect of polyphenolic compounds on porcine pancreatic amylase, rat intestine α-glucosidase, and fungal amyloglucosidase. Acarbose equivalence (AE) was introduced for the first time and defined as IC_{50} of acarbose divided by the IC_{50} of the sample measured under the same 96-well plate. This way, the run-to-run variations are canceled out. Among the plant extracts tested, grape seed extracts (1,440 μmolAE/g) and cinnamon bark extracts (1600 μmolAE/g) are the most active in inhibiting rat intestine α-glucosidase. For porcine α-amylase inhibition, grape seed extracts (5710 μmol AE/g) are close to four times more active (equal weight basis) than acarbose (1550 μmolAE/g).

KEYWORDS: α-Amylase, α-glucosidase, amyloglucosidase, starch hydrolysis, proanthocyanidins, area under the curve (AUC), turbidity

INTRODUCTION

Diabetes mellitus is a metabolic disorder in the endocrine system; diabetic patients do not produce enough insulin or are insulin insensitive and thus have a high postprandial blood glucose level after consuming high sugar or easily digestible starchy foods.[1] Worldwide, more than 220 million people have diabetes, and this number is increasing rapidly as the aging population expands (http://www.who.int/mediacentre/factsheets/fs312/en/). Recent warnings on the side effects of antidiabetic drugs, Rosiglitazone and Pioglitazone,[2] highlight the urgent need of alternative and safer means of blood glucose control, ideally through functional foods that contain bioactive ingredients that may regulate blood glucose concentration toward the normal range. Therefore, opportunities and challenges for the food industry are emerging in the area of evidence-based functional foods with a low glycemic index that may decrease starch digestion rates.

The digestive process of starch involves saliva α-amylase, pancreatic α-amylase, and the small intestinal brush border α-glucosidase, that is, maltase-amyloglucosidase and sucrase-isomaltase.[3−5] Determination of the starch hydrolase activity and inhibitor effectiveness is conventionally done by chromogenic assays such as 3,5-dinitrosalicyclic acid (DNSA) assay for reducing sugar content,[6,7] glucose oxidase/peroxidase (GOD/POD) assay,[8,9] and *p*-nitrophenyl-α-D-glucopyranoside (PNPG)[10] or *p*-nitrophenyl-α-D-maltopentaoside (PNPG5) assay.[11] The DNSA assay and GOD/POD method are indirect methods and tedious operationally and are thus not suitable for high-throughput format. The PNPG/PNPG5 assay has been done in 96-well microplate, but they are only synthetic substrates of starch hydrolase. The short detection wavelength (405 nm) used may suffer from food sample background interference from natural pigments such as flavonoids and carotenoids. Moreover, the use of synthetic substrate may give false positive results. For example, resveratrol was initially found to be a sirtuins activator when the synthetic fluorogenic peptide substrate, Fluor de Lys, was applied in the sirtuins activity assay.[12] Kaeberlein and co-workers discovered that resveratrol enhanced binding and deacetylation of peptide substrate that contained Fluor de Lys. However, when nonlabeled peptide with the same sequence as the natural protein substrate was applied as the substrate, resveratrol has no activation effect on Sirtuins.[13] The mechanism of resveratrol activation on human SIRT1 was further studied by comparing fluorescent probe-labeled peptides with that of tritium-labeled acetylated histone, the native substrate of the sirtuins.[14] It was found that the resveratrol only activated sirtuins when the substrates containing fluorophore were used but not when tritium-labeled native substrate was used. The presence of fluorophore decreased the affinity of the labeled substrate to sirtuins. Resveratrol enhanced the binding of and thus behave as the activator. This finding highlights the potential problem of false positive when, for the convenience of measurement, one substitutes the native substrate with a synthetic one that has a different structural motif. Starch is composed of macromolecules and has large structural diversity. The molecular sizes of amylose and amylopectin, the pattern of starch crystallinity, and the chain length distribution vary with the botanical source of the starch. Therefore, it is challenging, if not impossible, to substitute starch with a small molecular synthetic substrate in studying starch hydrolase activity.

Quantification of starch hydrolase activity by measuring the changes of optical density as an indication of turbidity level is a

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rapid and simple method, but it is rarely applied for investigation of starch hydrolase inhibition. Turbidity is the optical density of a sample fluid arising from the interaction of light and insoluble particles. Samples containing insoluble solids will interfere with the light transmittance, and the interference is related to the size, shape, and composition of the particles and to the wavelength of the incident light.[15] Turbidity can be measured by using microplate UV−vis reader, which is mostly applied for monitoring cell growth dynamics.[16,17] The purpose of this study is to fully develop and validate a high-throughput method based on turbidity measurement for investigation of the dynamic of enzymatic hydrolysis of starch particles. We hope our method can be generally applied in research on novel starch hydrolase inhibitors from vastly diverse botanical sources, starch microstructure, and digestibility relationship and in formulations of foods with low glycemic index.

MATERIALS AND METHODS

Reagents and Instruments. Amyloglucosidase (from *Aspergillus niger*, 300 U/mL) (A7095) and α-amylase (A3176, type VI-B, from porcine pancreas), corn starch (S4126), acarbose (A8980), glucose assay kit (glucose oxidase/peroxidase reagent G3660; *o*-dianisidine reagent D2679), and α-glucosidase in the form of rat intestine acetone powder were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Grape seeds extracts (OPC content, 95%) were obtained from Tianjin Jianfeng Chemical Co. (Tianjin, China). Cranberry pomace and cranberry fruit powders were gifts from the Decas Farm (Wareham, MA). Cinnamon barks (Indonesia) were purchased in local market. Plant extracts were prepared using acetone−water−acetic acid (70:29:1) mixture followed by LH-20 column purification as described by Priors and co-workers.[18] The total phenolic contents of the extracts were quantified using Folin−Ciocalteu reagent according to literature.[19] The compositions of cranberry pomace extracts, cranberry fruit extracts, and cinnamon bark extracts were analyzed by high-performance liquid chromatography (HPLC). The polypenolic identities of grape seed extracts and cinnamon barks were further characterized by Finnigan/MAT LCQ ion trap mass spectrometer (San Jose, CA) equipped with an electrospray ionization (ESI) source. The full-scan mass spectra with negative mode from m/z 100 to 1500 were recorded. The microplate reader (Bio-Tek Instruments Inc., Winooski, VT) was used to determine turbidity change.

The enzyme concentrations used for this essay were 3 U/mL porcine pancreatic α-amylase, 0.3 U/mL fungal amyloglucosidase, and 9.6×10^{-3} U/mL rat intestine α-glucosidase. Acarbose was prepared in the sodium phosphate buffer (0.1 M, pH 6.9). Luteolin, cinnamon barks, grape seeds, green tea, cranberry pomace, and cranberry fruits extracts were first dissolved in dimethylsulfoxide and then diluted by sodium phosphate buffer for analysis. As a negative control, 1 mL of dimethyl sulfoxide was diluted with sodium phosphate buffer and measured in parallel with samples. For α-amylase, the final inhibitor concentrations were determined as [grape seeds extracts] = 3.8, 5, 6.3, 7.5, and 8.8 μg/mL; [cinnamon bark extracts] = 15, 20, 25, 30, and 35 μg/mL; [cranberry fruits extracts] = 10, 20, 30, 40, and 50 μg/mL; [cranberry pomace extracts] = 7.5, 15, 22.5, 30, and 37.5 μg/mL; [luteolin] = 25, 50, 75, 100, and 125 μg/mL; [green tea extracts] = 25, 50, 75, 100, and 125 μg/mL. For α-glucosidase, the inhibitor concentrations were determined as [grape seeds extracts] = 1.3, 2.5, 5.0, and 7.5 μg/mL; [cinnamon bark extracts] = 1.3, 2.5, 3.8, 5.0, and 6.3 μg/mL; [cranberry fruits extracts] = 2.5, 5, 7.5, 10, and 12.5 μg/mL; [cranberry pomace extracts] = 5, 7.5, 10, and 12.5 μg/mL; [luteolin] = 25, 50, 75, 100, and 125 μg/mL; [green tea extracts] = 25, 50, 75, 100, and 125 μg/mL. For amyloglucosidase, the final concentrations used were [grape seed extracts] = 0.2, 0.4, 0.6, 0.8, and 1.0 μg/mL; [cinnamon bark extracts] = 12.5, 25, 50, and 62.5 μg/mL; [cranberry fruit extracts] = 10, 25, 32.5, and 40 μg/mL; [cranberry pomace extracts] = 12.5, 20, 25, and 32.5 μg/mL; [luteolin] = 25, 50, 75, and 100 μg/mL; [green tea extracts] = 25, 50, 75, 100, and 125 μg/mL.

Determination of α-Glucosidase Activity. The activity of α-glucosidase in the rat intestine acetone powder was determined before use. The rat intestine acetone powder (0.5 g) was dissolved in 20 mL of sodium phosphate buffer (0.1 M, pH 6.9) and stirred in an ice bath for 30 min before it was centrifuged at 2000*g* and 4 °C for 10 min. The supernatant was kept at −20 °C for further use. The unit of α-glucosidase stock solution was determined by glucose assay kit, which allowed measurement of the glucose liberated from enzymatic hydrolyzation. α-Glucosidase solution (diluted 40×, 150 μL) was preincubated with 150 μL of sodium phosphate buffer (0.1 M, pH 6.9) in the thermo shaker for 5 min at 37 °C. The reaction was started by adding 300 μL of 20 mg/mL maltose solution. Eighty microliters of mixture was taken out every 2 min and immediately placed in a boiling water bath to stop the reaction. Forty microliters of inactivated suspension was incubated with 80 μL of glucose assay reagent in a microplate at 37 °C for 30 min. Eighty microliters of 6 M sulfuric acid was added, and the solution was mixed by shaking. The absorbance was measured at 540 nm. The glucose calibration curve was prepared at a final concentration of 5−25 μg/mL and determined in triplicate by using glucose assay kits. One unit (U) of enzyme activity was defined as the amount of the enzyme that liberates 1 μmol of glucose from the substrate in 1 min under the test conditions.[20] The optical density (y-axis, OD at 540 nm) is increased over time (x-axis) with $y = 0.141x - 0.0042$, $R^2 = 0.99$. The α-glucosidase concentrations, prepared by a series of dilutions for the determination of the dose−response relationship, are 2.6, 1.9, 1.3, 0.8, and 0.6 U/mL as calculated by stock solution with a concentration of 1.5 U/mL.

Determination of Dynamic Range of the Turbidity Measurement. A starch solution (1%) was prepared in sodium phosphate buffer (0.1 M, pH 6.9) by heating it in a hot plate until it was boiling. The solution was then cooled to room temperature in ambient air with continuous stirring. The freshly prepared corn starch solution was diluted with sodium phosphate buffer (0.1 M, pH 6.9) to 1−10 mg/mL. Two hundred microliters of solution for each concentration was dispersed to each well. The absorbance of different starch concentrations was measured at 660, 700, 800, 900, and 970 nm by microplate reader ($n = 3$) to select the best wavelength for the assay. The blank sample was prepared with sodium phosphate buffer.

High-Throughput Assay of Starch Hydrolase Activity. Dose−activity response relationships were determined for α-glucosidase, α-amylase, and amyloglucosidase. The reactions were performed in triplicate by adding 50 μL of α-glucosidase, α-amylase (6, 8, 10, and 12 U/mL), or amyloglucosidase (0.2, 0.4, 0.8, 1.0, 1.2, 1.4, and 1.6 U/mL) to each well. The solution in each well was further diluted to 100 μL by sodium phosphate buffer. The reaction was initiated by injecting 100 μL of 1% gelatinized corn starch to each well using a 12-channel multichannel pipet. The absorbance was immediately measured every 15 s for 60 min at 600 nm in a microplate reader at 37 °C with shaking intensity set at the highest level setting (4) to avoid starch sedimentation. The enzyme activity was inversely proportional to the area under the curve (AUC) for the turbidity readings.

Determination of Inhibitor Activity. Briefly, 50 μL of enzyme solution was mixed with 50 μL of inhibitors with series of different concentrations and preincubated in microplate reader for 15 min at 37 °C. The reaction was started by injecting 100 μL of starch solution using a 12-channel multichannel pipet. The turbidity change was immediately monitored at 660 nm for 1 h with one reading per 15 s. The microplate reader shaking intensity was set at the highest level (4) to ensure sufficient mixing and avoid starch sedimentation. The percentage

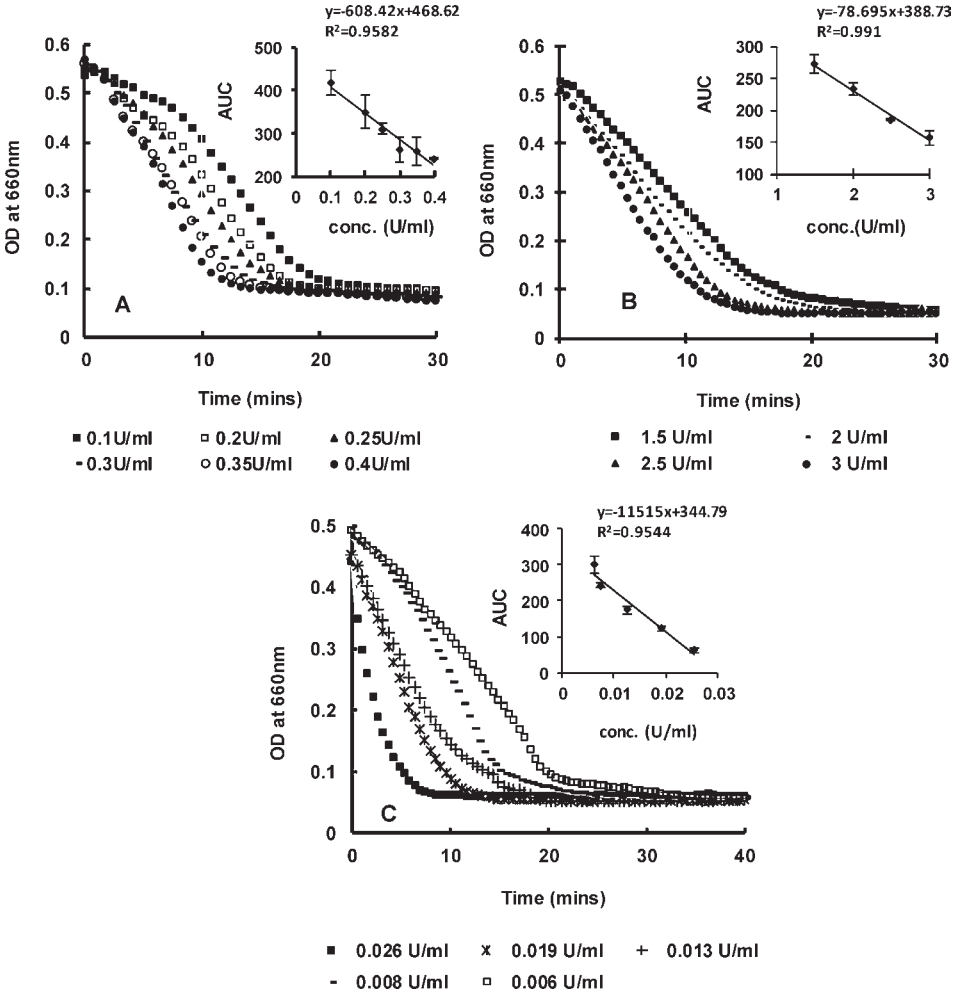

Figure 1. Kinetic curves of starch hydrolysis in the presence of different hydrolases. (A) Amyloglucosidase (*Aspergilllus niger*), (B) α-amylase (porcine pancreatic), and (C) α-glucosidase (rat intestine acetone powder). Assay conditions are 37 °C and starch concentration, 5.0 g/mL. Insets are dose−response relationships of the AUC vs enzyme concentrations.

of inhibition was defined by eq 1.

$$\text{inhibition}\% = \frac{\text{AUC sample} - \text{AUC control}}{\text{AUC sample}} \times 100 \qquad (1)$$

in which AUC_{sample} is area under the curve of inhibitor and $AUC_{control}$ is the area under the curve without inhibitors. The IC_{50} is normally defined as the concentration of inhibitor producing 50% inhibition of enzyme activity under the assay condition specified.[21] It was obtained from interpolation of percentage of inhibition against inhibitor concentration curve. In our definition, the IC_{50} is the inhibitor concentration that gives AUC twice as large as that of the uninhibited reaction.

Determination of Precision and Accuracy. The precision was tested by repeated analysis of the same assay and further by day-to-day variation. Amyloglucosidase (50 μL; 1.2 U/mL) was preincubated with 50 μL of acarbose (2, 4, 6, 10, and 12 μg/mL) in a microplate reader at 37 °C for 15 min. The reaction was started by adding 100 μL of 1% corn starch to each well. The experiments were determined for 5 days under the same experimental conditions. Accuracy, as reflected from recovery test, was also measured by using acarbose. The recovery was calculated from the concentrations obtained by regression analysis versus the known acarbose concentrations added as inhibitor.

Statistical Analysis. The results are given as means ± standard deviations (SDs). The significance of the data was analyzed by *t* test using SPSS statistical software.

RESULTS AND DISCUSSION

Dose Response between Turbidity and the Concentration of Starch. Turbidity can be determined in a wide range of wavelengths of visible light, and the "absorbance" does not have the characteristic peak like the normal UV−vis spectra of chromophores. Turbidity measurements are typically performed between the wavelengths of 620−700 nm. To determine the most suitable wavelength, the linearity range has been compared at five different wavelengths, that is, 660, 700, 800, 900, and 970 nm. The results showed that the turbidity measured at 660 nm gave the lowest limit of detection and limit of quantization (LOQ) values, which indicated that the sensitivity of the measurement at this wavelength was higher as compared to the others.

Quantification of the Enzyme Activity. Using corn starch as the substrate, the measured turbidity decreased with time due to the action of α-glucosidase, amyloglucosidase, and α-amylase. The AUC decreased with increasing enzyme concentration (Figure 1A−C), giving a fairly good linear fit. Because of the complexity of starch hydrolysis kinetics, the AUC as opposed to the initial rate would be a better parameter to reflect the dynamics of the starch hydrolysis. Therefore, we applied the

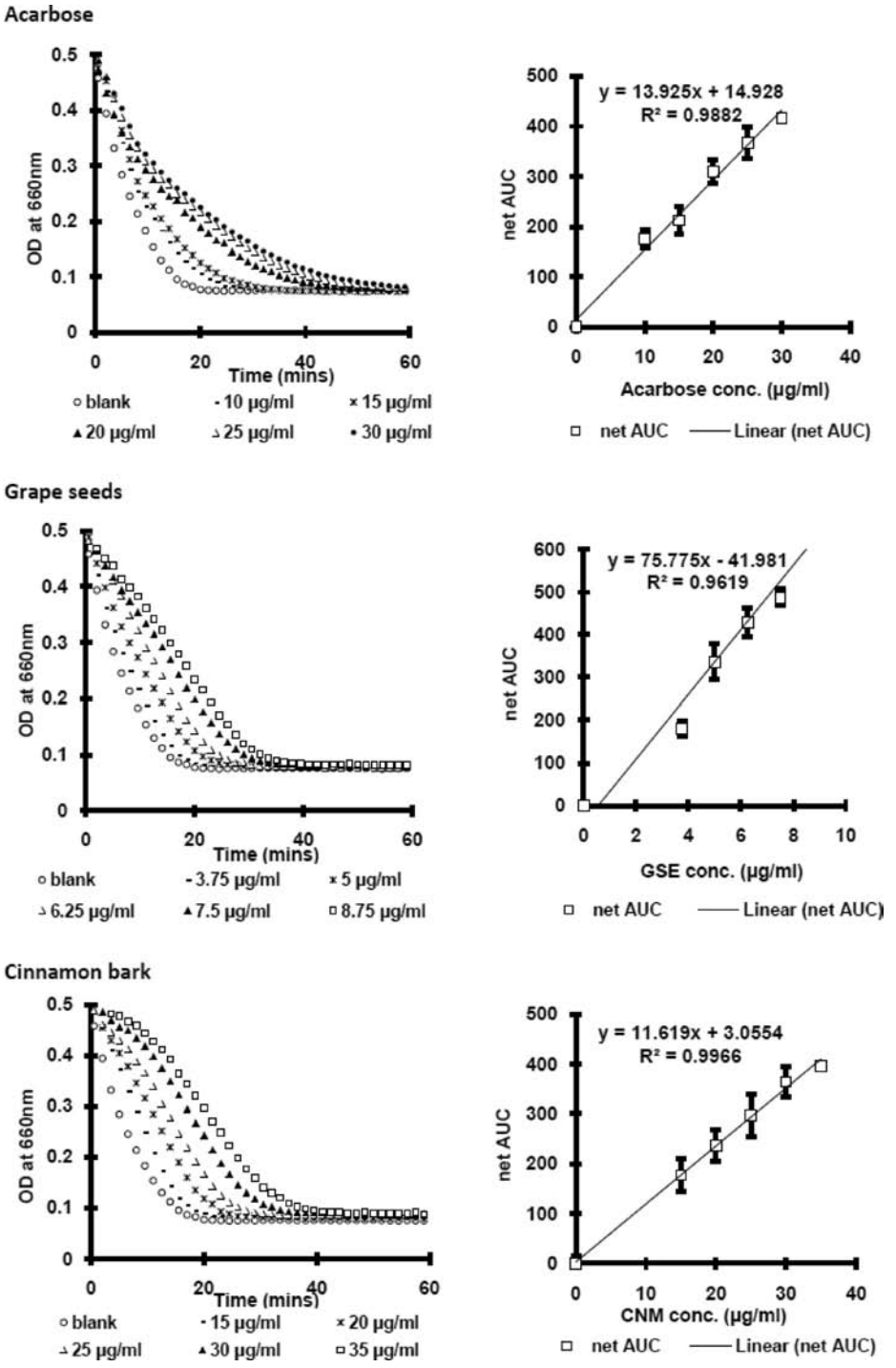

Figure 2. Representative kinetic curves of starch hydrolysis in the presence of inhibitors. Left: turbidity change at different concentrations of acarbose, grape seed extracts, and cinnamon bark extracts. Right: Dose response of net AUC vs the inhibitor concentrations.

AUC of the completed kinetic curves instead of using the initial rate. The AUC approach has been demonstrated to be very effective in a situation when the reaction kinetics is highly complex and extraction of kinetic parameters from the initial rates becomes not feasible. The well-known example is the oxygen radical absorbance capacity assay.[22]

Inhibitor Activity. Acarbose is a prescription drug for hyperglycemia and has been shown to effectively inhibit α-amylase and α-glucosidase;[23,24] thus, it is chosen as a reference standard. The typical inhibition assay plots are displayed in Figure 2. The enzymatic reaction is retarded with increasing acarbose concentration, and this becomes more obvious as the reaction proceeds to more than 10 min. When the AUC and percentage of inhibition are plotted against the inhibitor concentration (acarbose), a dose−response relationship was observed. The representative kinetic curve and the observed dose−response relationship of grape seed extracts and cinnamon bark extracts are shown in Figure 2. As the concentration of the extracts

increases, the reaction slows down, but the kinetics curve patterns are different from those of acarbose. The exact reason behind this is not clear at this moment.

Precision and Accuracy. The precision of the measurement was determined using amyloglucosidase and acarbose with average IC_{50} of 2.1 μM (Table 1). The accuracy was determined by recovery tests at four acarbose concentrations, and three replicates are satisfactory according to the requirement of AOAC guidelines (AOAC, Peer Verified Method Program, Manual on Policies and Procedures, Arlington, VA, November, 1993).

Acarbose Equivalence. The IC_{50} values of tested natural extracts on three starch hydrolase are given in Table 2. IC_{50} is the most commonly used parameter for enzyme inhibitor activity. However, IC_{50} is dependent on many factors such as enzyme origin, concentration of substrates, and assay conditions (pH, temperature, and the type of buffers used). It is thus hard to compare the data reported from different laboratories.[25] Thus, we introduced the concept of acarbose equivalence (AE), which is defined as follows:

$$\text{AE of a sample} = \frac{IC_{50} \text{ of acarbose}}{IC_{50} \text{ of a sample}}$$

In a 96-well format, the samples are measured in parallel with acarbose, and the IC_{50} of both sample and acarbose measured under the same conditions will cancel out the run-to-run variations. Conventionally, a lower IC_{50} value means higher inhibition activity. For a single component sample, the molar concentration is preferentially used, and the inhibition can be expressed as μM (acarbose)/μM (sample). For samples with mixture of compounds, the concentration is normally expressed as g/L. Therefore, the acarbose equivalence units are μmol AE/g. It is apparent that one AE is equivalent to 1 μmol of acarbose. One gram of acarbose has 1550 μmol, which is listed in Table 2 for better comparison with the polyphenolic compounds on equal weight basis.

The IC_{50} values of the samples were converted into AE (Table 2). It is intuitive that higher AE means higher inhibitory activity, and this is in contrast to IC_{50}. The most active inhibitor for rate intestine α-glucosidase is cinnamon bark extracts, which has AE value of 1600 μmol AE/g and comparable to that of acarbose (1550 μmol AE/g). The grape seed extracts also show high activity with AE value of 1400 μmol AE/g. The cranberry pomace extract is a much weaker inhibitor, while the cranberry fruit powder extract does not show significant inhibition. Grape seed extracts are also potent inhibitors for pancreatic α-amylase with AE values over four times higher than that of acarbose on equal weight basis. The rest have comparable activity acarbose.

Both IC_{50} and acarbose equivalence provide a quick assessment of inhibitor activity without taking into consideration the mode of enzyme inhibitions, which could be highly complex and include nonreversible inhibition (e.g., denaturalization of the protein or covalent modification of catalytically active center) and reversible inhibition (e.g., competitive, uncompetitive, noncompetitive, and mixed inhibition). While acarbose is a competitive inhibitor, polyphenolic compounds could act via wide range of mechanisms because of their diverse structural motif.[26] Because of the strong hydrogen-bonding capacity through the phenolic groups, polyphenolic compounds are often believed to interact with enzymes through nonspecific binding. Matsui and co-workers demonstrated that theaflavins, an oxidatively condensed flavanols in black tea, are competitive inhibitors of α-glucosidase, and the inhibition is highly sensitive to the substituent group and stereochemistry of the C-3 position.[27] We have shown that proanthocyanidins isolated from mangosteen peels show some selectivity on binding to proteins including α-amylase.[28] Our assay is suitable for high-throughput screening of the inhibition of a sample, and once the activity is established, the detailed mechanisms of enzyme inhibition can be further investigated to better assess their potential as functional ingredients for lowering the glycemic index of starch.

Our data also revealed that the polyphenolic extracts are much weaker inhibitors of fungal amyloglucosidase as compared

Table 1. Accuracy and Precision of the High-Throughput Assay

accuracy[a]				
	found [acarbose] (μM)			recovery %
spiked [acarbose] (μM)	run 1	run2	run 3	average
0.78	0.72 ± 0.13	0.64 ± 0.07	0.91 ± 0.12	97.0 ± 17.8
2.29	2.53 ± 0.03	1.77 ± 0.12	2.27 ± 0.13	95.6 ± 16.9
3.88	3.81 ± 0.23	4.03 ± 0.16	3.58 ± 0.23	98.1 ± 5.8
4.65	4.81 ± 0.11	4.67 ± 0.25	5.06 ± 0.12	104.2 ± 4.3

precision (acarbose, IC_{50} μM)[b]					
day 1	day 2	day 3	day 4	day 5	mean
1.98 ± 0.12	1.90 ± 0.24	1.98 ± 0.25	2.22 ± 0.27	2.40 ± 0.22	2.10 ± 0.21

[a] Accuracy expressed as the average recovery from three replicates. [b] The IC_{50} results are presented as means ± SDs. The significance of day to day variation was tested by SPSS paired *t* tests, $P < 0.01$.

Table 2. IC_{50} and Acarbose Equivalence of Inhibitors Obtained by Starch Hydrolases

	α-glucosidase		α-amylase		amyloglucosidase	
inhibitors	IC_{50} (μg/mL)	AE[a] (μmol AE/g)	IC_{50} (μg/mL)	AE (μmol AE/g)	IC_{50} (μg/mL)	AE (μmol AE/g)
acarbose	5.8 ± 0.6 μM	1550	33 ± 2 μM	1550	2.1 ± 0.2 μM	1550
luteolin	19 ± 1	310 ± 6	ND[b]	ND	89 ± 4	23.5
GSE[c]	4.0 ± 0.1	1400 ± 100	5.7 ± 0.6	5710 ± 600	860 ± 50	2.4 ± 0.1
CNM[d]	3.6 ± 0.5	1600 ± 200	18.0 ± 2.5	1810 ± 100	46.5 ± 3.0	45 ± 3.0
CBP[e]	10.2 ± 0.7	570 ± 40	20.3 ± 1	1610 ± 100	23.5 ± 0.7	89 ± 2.6
CBF[f]	ND	ND	17.5 ± 0.4	1870 ± 100	27.5 ± 1.0	76 ± 3

[a] AE is calculated by IC_{50} of acarbose divided by IC_{50} of other inhibitors, and the units are expressed as μmol of acarbose equivalence per gram of sample. [b] No detectable activity has been observed. [c] GSE, grape seed extracts. [d] CNM, cinnamon bark extracts. [e] CBP, cranberry pomace extracts. [f] CBF, cranberry fruit extracts.

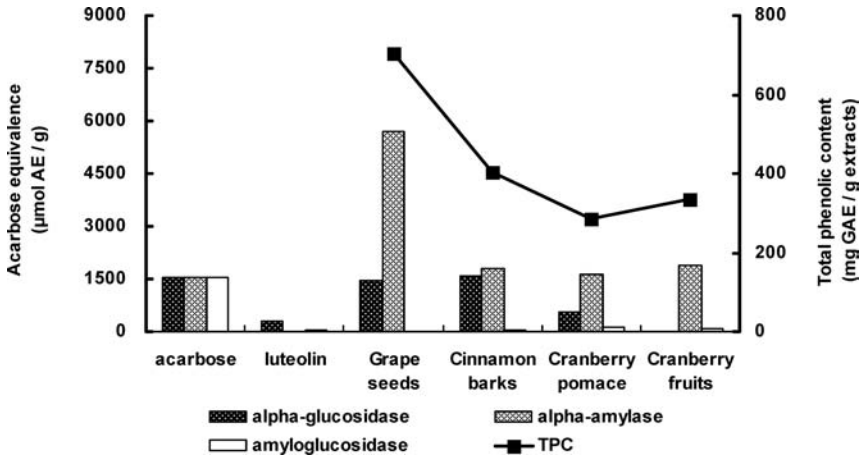

Figure 3. Comparison of acarbose equivalence among different starch hydrolases and total phenolic contents of different samples.

to acarbose. Structural variation among the enzyme of different origins could be the reason for such differences. The fungal amyloglucosidase bears less similarity to human brush-border starch hydrolases.[29] For screening of inhibitors as active ingredients for low glycemic index foods, it is necessary to use enzymes of animal origin that are more comparable to that of human digestion tract.

We measure the inhibition activity of luteolin because it has been reported that luteolin was a relatively potent (comparable to acarbose) α-amylase and α-glucosidase inhibitor revealed by the PNPG assay.[30] In sharp contrast to the reported results, we found that luteolin was five times less active than acarbose in inhibiting rat intestine α-glucosidase and exhibited no measurable activity for α-amylase. The exact reason of such difference needs further investigation. Nonetheless, the discrepancy highlights the importance of the assay used in enzyme inhibition measurements.

The total phenolic contents were not strictly inversely proportional to the inhibition activity of the extracts as shown in Figure 3. It also was noted that no starch hydrolase inhibition activity was detected with green tea extracts, although it has high polyphenolic content of 465 mg GAE/g, as green tea extracts contain mainly monomeric flavanols. We suspected that the oligomeric proanthoyanidins presented in these plant extracts are responsible for the inhibition activity. The amount of oligomeric (dimer or above) OPCs was estimated from the HPLC chromatograms using epicatechin as a reference standard (results shown in Table S1 in the Supporting Information). Grape seed extract has the highest OPC content at 45%, and this seems to explain the reason of its high α-amylase inhibition activity, whereas cinnamon bark extract (16% OPCs) and cranberry fruit extract (18% OPCs) have comparable AE value for α-amylase. The cranberry pomace extracts have only 10% OPCs content, and the AE value for α-amylase is lower. However, for α-glucosidase inhibition, cinnamon bark extract has a much higher activity than that of the cranberry fruit extract. The structural profiles, instead of the total amount of OPCs, could be the reason for the difference. Cinnamon bark extracts contain a significant amount of A type trimer as shown in HPLC chromatogram (Figure S2 in the Supporting Information) and the ESI-MS spectrum (Figure S6 in the Supporting Information). To further test if the OPCs are indeed responsible for the enzyme inhibition activity, we depolymerized the oligomeric proanthocyanidins with mercaptoacetic acid by following a previously reported procedure.[31] We found that the resulting depolymerized mixtures did not show any starch hydrolase inhibition activity. Therefore, we concluded that the OPCs in these botanical extracts are the active components for retarding the activity of starch hydrolases.

In summary, the results obtained herein demonstrate that turbidity measurement is a fast, accurate, and high-throughput method to determine starch hydrolase activity and to screen the effectiveness of inhibitors of botanical extracts. Cinnamon bark extracts and grape seed extracts were demonstrated to be most potent in inhibiting rat intestine α-glucosidase and porcine pancreas α-amylase as compared to acarbose, and its usage as active ingredient for low GI food production is worthy of consideration. In a broader perspective, cinnamon is reported to have antidiabetic effects through insulin stimulation activity as shown by in vitro study,[32] animal model,[33] and limited human trials.[34] The strong starch hydrolase inhibition of the polyphenolic compounds in cinnamon bark may be one of the many possible mechanisms of its antidiabetic activity.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Figures showing results of HPLC. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: chmhdj@nus.edu.sg.

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■ ABBREVIATIONS USED

PNPG, *para*-nitrophenyl-α-D-glucopyranoside; DNSA, 3,5-dinitrosalicyclic acid; AUC, area under the curve; GOD/POD, glucose oxidase/peroxidase; AE, acarbose equivalence.

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